Vitamin D status of pregnant women with obesity in the United Kingdom and its association with pregnancy outcomes: a secondary analysis of the UPBEAT study

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Running title: Vitamin D status of pregnant women with obesity

List of abbreviations

CI	Confidence interval
GDM	Gestational diabetes mellitus
HbA1c	Hemoglobin A1c
IQR	Interquartile range
LGA	Large-for-gestational-age
NHS	The National Health Service
OR	Odds ratio
OGTT	Oral glucose tolerance test
RCT	Randomized controlled trial
SD	Standard deviation
SGA	Small for gestational age
UK	United Kingdom
UPBEAT	UK Pregnancies Better Eating and Activity Trial
USA	United States of America
25(OH)D	25-hydroxyvitamin D

Abstract

Prenatal vitamin D deficiency is widely reported and may affect perinatal outcomes. In this secondary analysis of the UK Pregnancies Better Eating and Activity Trial (UPBEAT), we examined vitamin D status and its relationship with selected pregnancy outcomes in women with obesity (BMI≥30kg/m^2) from multi-ethnic inner-city settings in the UK. Determinants of vitamin D status at a mean of 17±1 weeks' gestation were assessed using multivariable linear regression and reported as percent differences in serum hydroxyvitamin D (25(OH)D). Associations between 25(OH)D and clinical outcomes were examined using logistic regression. Among 1089 participants, 67% had 25(OH)D <50nmol/L and 26% had concentrations <25nmol/L. In fully adjusted models accounting for socio-demographic and anthropometric characteristics, 25(OH)D was lower among women of Black (% difference = -33; 95%CI: -39 to -27), Asian (% difference= -43; 95%CI: -51 to -35) and other non-White (% difference= -26; 95%CI: -35 to -14) ethnicity compared to women of White ethnicity (*n*=1086; *P*<0.001 for all). In unadjusted analysis, risk of gestational diabetes was greater in women with 25(OH)D <25nmol/L compared to ≥50nmol/L (OR=1.58; 95%CI: 1.09 to 2.31), but the magnitude of effect estimates was attenuated in the multivariable model (OR=1.33; 95%CI: 0.88 to 2.00). There were no associations between 25(OH)D and risk of preeclampsia, preterm birth, or SGA or LGA delivery. These findings demonstrate low 25(OH)D among pregnant women with obesity and highlight ethnic disparities in vitamin D status in the UK. However, evidence for a greater risk of adverse perinatal outcomes among women with vitamin D deficiency was limited.

Keywords: 25-hydroxyvitamin D, vitamin D, pregnancy outcomes, perinatal health, high BMI, obesity, gestational diabetes, hyperglycaemia

Introduction

Low vitamin D status, as reflected by a circulating 25-hydroxyvitamin D [25(OH)D] concentration <50nmol/L[1], is a global public health issue that is widely prevalent among pregnant women across all WHO world regions[2; 3]. In the UK, where vitamin D deficiency is defined as a 25(OH)D concentration <25nmol/L[4], the most recently available data from the National Diet and Nutrition Survey (Years 9-11; 2016-2017 and 2018-2019) estimated 15% of women aged 19-64 years have a vitamin D status that falls below this threshold[5]; however there is a lack of recent nationally representative data on vitamin D status among pregnant women in the UK.

Reported relationships between prenatal vitamin D status and maternal and offspring health outcomes are inconsistent, thereby challenging the concept of pregnancy-specific targeted thresholds for 25(OH)D[6]. Recent and pooled data from observational studies suggest an association between low maternal 25(OH)D and increased risk of adverse outcomes including gestational diabetes mellitus (GDM)[7; 8; 9] , preeclampsia[10] and both preterm[11] and small-for-gestational age (SGA) at birth[11]. Although the benefits of routine prenatal vitamin D supplementation remain unclear[6; 12], maternal vitamin D status is a modifiable determinant of neonatal 25(OH)D[13], and hence maternal vitamin D deficiency is a known risk factor for neonatal vitamin D deficiency.

Compared to a BMI within the 'healthy' range, a greater prevalence of vitamin D deficiency has been reported among individuals with obesity[14; 15], including pregnant populations[16; 17]. The inverse relationship between 25(OH)D and BMI has been attributed to both volumetric dilution and sequestration of vitamin D in adipose tissue[18; 19], meaning a greater vitamin D intake may be required among individuals with overweight and obesity to achieve target 25(OH)D thresholds. Limited data from randomised trials suggest an inverse relationship between BMI and achieved 25(OH)D following intervention with vitamin D[20], such that greater BMI attenuates the slope of the vitamin D intake-25(OH)D response relationship during pregnancy[13; 21]. In line with worldwide trends in overweight and obesity, the rising incidence of women who enter pregnancy with a BMI ≥30kg/m^2 is a global concern[22; 23]. In England and Wales, 23% of women with a recorded BMI were classified as having obesity at the first antenatal appointment in the years 2018-19[24]. Earlier audit data (years 2015-2017) found variations in BMI across the main ethnic categories in the UK, with severe obesity (BMI ≥35kg/m^2) reported to be more common among women of White and Black

ethnicity[25]. However, the vitamin D status of pregnant women with obesity in the UK is not well characterised. Data is specifically lacking among ethnically-diverse cohorts, despite previous reports from other European cohorts that clearly highlight lower vitamin D status in women of non-White ethnicity[26; 27; 28; 29]. Understanding of the distribution of 25(OH)D and prevalence of deficiency is therefore required to inform evidenced-based guidelines for vitamin D intake in pregnant women and identify populations who would benefit most from targeted public health campaigns.

Among a large UK-based cohort, this study aimed to assess the vitamin D status of a multi-ethnic cohort of pregnant women with obesity, and to examine the relationship between vitamin D status and perinatal outcomes.

Methods

Study design and setting

This study was a secondary analysis utilising biological samples and data from the UK Pregnancies Better Eating and Activity Trial (UPBEAT), a complex lifestyle intervention aiming to prevent GDM and reduce risk of large-for-gestational-age (LGA) birth in 1554 pregnant women with obesity[30]. UPBEAT was conducted in eight hospitals in inner-city settings across the UK. Ethical approval was obtained from UK IRAS (reference 09/H0802/5), and the trial was registered prospectively (ISRCTN89971375). The intervention, which encouraged improved dietary and physical activity behaviours, did not reduce risk of GDM or LGA birth[31], and for the purposes of this investigation, the trial was treated as a cohort study as there were also no differences in the 25(OH)D concentration between the intervention and standard care arms (**Supplemental Fig. 1**).

Study participants

In the UPBEAT study, eligible participants were identified in antenatal clinic from general practitioner or midwife referrals. Women aged >16 years with a BMI ≥30kg/m^2, singleton pregnancy and gestational age between 15^{+0} and 18^{+6} weeks were invited to participate. Women were excluded if unwilling or unable to provide informed consent, or if they had pre-existing diabetes, hypertension, renal disease, systemic lupus erythematous, antiphospholipid syndrome, sickle cell disease, thalassemia, celiac disease, thyroid disease, current psychosis or currently prescribed metformin. Verbal and written information was provided to eligible

women and written consent was obtained[30]. Inclusion in the present study was restricted to women for whom a baseline blood sample was available for measurement of serum 25(OH)D.

Demographic, clinical and pregnancy outcome data

Socio-demographic information was recorded at study entry, as collected through interview-administered questionnaires. Ethnicity was self-reported. Socio-economic status was assessed by Index of Multiple Deprivation (IMD), for which scores were calculated for the region of residence, and presented as quintiles. The following anthropometric data were collected using standardized methods[30]: maternal weight (kg) and height (cm); maternal hip, waist and thigh circumferences (cm); and maternal triceps (mm), biceps (mm), suprailiac (mm) and subscapular (mm) thicknesses, which were used to calculate the sum of 4 skinfold thickness (mm). Neonatal length and weight were measured within 72 hours of birth. Customised birthweight centiles were calculated using Gestation Related Optimal Weight (GROW) software version 6.7.5.1 (Gestation Network, Perinatal Institute, Birmingham, UK; www.gestation.net) and LGA and SGA delivery were defined as $\geq 90^{th}$ and $\leq 10^{th}$ percentile, respectively[30].

As per the UPBEAT protocol, diagnosis of GDM was defined according to the International Association of Diabetes and Pregnancy Study Groups criteria as one or more of the following: fasting capillary glucose concentrations of ≥5.1mmol/L and/or 1h venous glucose of ≥10.0mmol/L and/or 2h venous glucose of ≥8.5mmol/L following an oral glucose tolerance test (OGTT). The UPBEAT protocol restricted diagnosis of GDM to participants who had an OGTT conducted between 27^{+0} to 28^{+6} weeks' gestation; however, the present analysis pragmatically extended the timeframe to any OGTT performed between 23^{+2} to 30^{+0} weeks' gestation in order to maximise the sample size for this outcome. Pre-eclampsia was defined as systolic blood pressure ≥140mmHg, diastolic blood pressure ≥90mmHg, or both, on at least two occasions 4h apart, with proteinuria ≥300mg/24h or spot urine protein:creatinine ratio ≥30mg/mmol, or urine dipstick protein ≥2+.

Non-fasting venous blood samples were collected at the first study visit (second trimester), processed to serum within 2 hours and stored at -80°C until analysis. The meteorological season of blood draw was assigned based on the date of the sample collection as follows: Winter – December, January, February; Spring - March, April, May; Summer – June, July, August; Autumn - September, October, November[32].

Laboratory analysis

Maternal HbA1c was measured at the University of Glasgow using a turbidimetric inhibition immunoassay on the Roche, Cobas c311 as previously described[33]; low and high CVs were 1.3% and 1.4%, respectively. Total 25(OH)D (sum of 25(OH)D_2 and 25(OH)D_3) was measured at the Institute of Cardiovascular and Medical Sciences at the University of Glasgow using an electrochemiluminescence-based automated clinical assay (Vitamin D Total assay kit #05894913 190, Roche Diagnostics, Mannheimm Germany) on a Cobas e411 analyser using the manufacturer's standards and quality control material. This automated immunoassay method has been has been standardized against LC-MS/MS, which has been standardised to National Institute of Standardization and Technology (NIST) standard reference material[34; 35]. The assay limit of detection (LoD) was 7.5nmol/L; values below this threshold (*n*=27/1089; 2.5%) were imputed at the level of the LoD. The low and high inter-assay CVs were 11.2% and 9.2%, respectively.

In the present study, vitamin D deficiency and low vitamin D status were defined as a 25(OH)D concentration <25nmol/L and between ≥25 - <50nmol/L, respectively, to facilitate comparison with previous studies exploring maternal vitamin D status[2; 21].

Statistical analysis

Data distributions were assessed using histograms and kernel density plots and summarised as mean ± SD or median (25^{th}, 75^{th} percentile), as appropriate. For categorical data, number (N) and percentage (%) are reported. Variables following a skewed distribution were either natural (ln)-log transformed or transformed to their base 2 log to approximate normality in regression analysis. The association between each maternal socio-demographic, clinical and anthropometric characteristic of interest and serum 25(OH)D in the second trimester was first examined using simple linear regression with $\log_2$-transformed 25(OH)D as the outcome variable given the right-skewed distribution of 25(OH)D. To create a parsimonious multivariable model, variables with $P<0.20$ in the unadjusted analyses were entered into a general linear model and adjusted for IMD, ethnicity, maternal BMI at first study visit, maternal age at first study visit, season at blood sampling, gestational age at sampling and educational attainment, as appropriate to account for confounding of the exposure-vitamin D status relationship. The multivariable model included adjustment for BMI only, to avoid

multicolinearity between BMI and other body size measures (i.e., weight and sum of skinfold thickness), whereby Pearson's $r \geq 0.5$ was used to define collinearity between pairs of continuous variables. Effect estimates were back-transformed and reported as mean percent differences with 95% confidence intervals (CI).

To facilitate meaningful comparison of effect sizes when examining the association between 25(OH)D and blood glucose measurements, both the independent (maternal 25(OH)D in second trimester) and dependent (HbA1c, fasting glucose and glucose measures at 1- and 2-hours post OGTT) variables were standardised using a Fisher-Yates transformation to create a normally distributed variable with a mean of zero and an SD of one[36] before use in regression models; as such, the standardised regression can be interpreted as SD change in the outcome variables per SD change in serum 25(OH)D. Logistic regression was used for categorical outcome data and presented as odds ratios (ORs) with 95%CIs, using 25(OH)D ≥50nmol/L as the reference catergory and 25(OH)D <25nmol/L and between ≥25-<50nmol/L as the comparators. Multivariable models included adjustment for the intervention arm assigned at enrolment to the UPBEAT trial, as well as known demographic characteristics to be associated with 25(OH)D; ethnicity, educational attainment, maternal BMI at first visit, maternal age at first visit, season at blood sampling, gestational age at blood sampling and gestational age at delivery, as appropriate. In post-hoc exploratory analysis, we created similar regression models fitted to data stratified by ethnic group to examine whether the magnitude of the effect estimates for the association between 25(OH)D and pregancy outcomes differed by ethnicity. Stratified analysis was conducted among women of White and Black ethnicity only, given the very low sample size in the other non-White ethnic groups.

Statistical analysis was conducted using Stata v17.0 (StataCorp, College Station, Texas, US), with significance set at $P<0.05$.

Results

Population characteristics

Of 1091 participants with available 25(OH)D data in the UPBEAT cohort, 2 participants were excluded from the present analysis due to an early OGTT (13 weeks' gestation) (*n*=1), and lack of information on the date of blood sample collection (*n*=1). Hence, 1089 participants were included in the present study, representing 70% of the primary UPBEAT study cohort. The population characteristics of the full study cohort and stratified by thresholds of vitamin D status are shown in **Table 1**. The mean age of participants at enrolment was 30.5 ± 5.6 years and median BMI was 35.2 (32.7, 38.7) kg/m^2. Over 75% of participants were classified as having a relatively low socioeconomic status based on the 2 highest quintiles of IMD. One-third of women identified as non-White ethnicity (33%). The majority of participants lived in London (42%), of which 43% came from the most deprived areas, and just under two-thirds did not hold a university degree or equivalent (Table 1).

There was an even distribution of blood samples drawn across all 4 seasons (Table 1). The median serum 25(OH)D concentration was 38.9 (24.5, 56.4) nmol/L. In total, 727 (67%) women were classed as having low a vitamin D status (25(OH)D <50nmol/L) and more than one quarter of women (26%) had a 25(OH)D concentration <25nmol/L. The prevalence of vitamin D deficiency was highest among women of Asian ethnicity and lowest among women who identified as White (Table 1, **Figure 1**).

Association of maternal characteristics with 25-hydroxyvitamin D concentrations

In both unadjusted and multivariable-adjusted linear regression models, maternal age was positively associated with serum 25(OH)D in the second trimester (**Table 2**). Participants who held a university degree had a greater 25(OH)D concentration than those without. Women living in the most deprived area had a lower 25(OH)D concentration compared to women with an IMD score <5, however, effect estimates were attenuated in adjusted analysis such that the percent difference between IMD quintiles only remained significant for comparison between the third and fifth quintiles (Table 2). Compared to White women, 25(OH)D was lower among women of non-White ethnicity, for which the percent difference was greatest for women of Asian ethnicity (percent difference = 41%; 95%CI: -49 to -31; *P*<0.001); inferences were unchanged upon adjustment for covariates. Greater weight, BMI and skinfold thickness was associated with a lower 25(OH)D concentration in both

unadjusted and adjusted models, but effect estimates were minor. Serum 25(OH)D was lower among women whose blood sample was taken in winter and spring compared to the summer months (Table 2).

Association between maternal 25(OH)D and maternal outcomes

Compared to women with 25(OH)D concentrations ≥50nmol/L in the second trimester, the occurrence of preeclampsia, preterm birth, and both SGA and LGA birth was similar in women with low vitamin D status (25(OH)D ≥25-<50nmol/L), and vitamin D deficiency (25(OH)D<25nmol/L) (**Table 3**). In unadjusted analysis, the odds of developing GDM were greater in women with 25(OH)D <25nmol/L versus ≥50nmol/L, but effect estimates were attenuated and rendered non-significant upon adjustment for maternal socio-demographic characteristics (Table 3). There was a minor negative association between 25(OH)D concentration and blood glucose measured at the 2-hour OGTT time point, that was attenuated albeit remained statistically significant in multivariable-adjusted analysis, such that each SD increase in 25(OH)D was associated with a 0.07 SD decrease in blood glucose (95%CI: -0.14 to -0.003; *P*=0.042; *n*=931) (**Table 4**), which is equivalent to -0.11 mmol/L (95%CI: -0.21 to -0.005). Associations between 25(OH)D and either HbA1c or fasting glucose were not observed (Table 4).

In subgroup analysis stratified by ethnicity, the magnitude and direction of effect estimates differed between White and Black women for most outcomes, however, the odds of having an adverse outcome at 25(OH)D <50nmol/L were not statistically significant in either ethnic group such that inferences from primary analysis were unchanged (**Supplemental Table 1**). The relatively small sample size for Black women resulted in a low absolute number of diagnosed adverse outcomes which resulted in wide confidence intervals surrounding effect estimates and precluded estimation of the odds of preeclampsia and SGA using 25(OH)D ≥50nmol/L as the reference category. The negative association between 25(OH)D and blood glucose measured at the 2-hour OGTT time point was not statistically significant in multivariable models in either White (mean difference= -0.07 SD/SD; 95%CI: -0.16 to 0.02; *P*=0.14; *n*=631) or Black (mean difference= -0.13 SD/SD; 95%CI: -0.29 to 0.02; *P*=0.10; *n*=187) women.

Discussion

Among an ethnically diverse cohort of pregnant women with obesity in the UK, our findings highlight an overall low vitamin D status, with more than two-thirds of women presenting with a 25(OH)D concentration <50nmol/L in the second trimester. While our findings do not support an association between maternal 25(OH)D in early-mid pregnancy and later clinical outcomes, we report a lower 25(OH)D among women of ethnic minority, highlighting a wide disparity in vitamin D status among population sub-groups within the UK. Of particular concern is the high prevalence of deficiency among women of Asian ethnicity, of whom >50% had a 25(OH)D concentration <25nmol/L, a threshold at which risk of nutritional rickets and osteomalacia is increased[1; 4]. Given the resurgence in vitamin D-dependent rickets in recent decades, particularly among children of ethnic minority[37; 38], prevention of maternal vitamin D deficiency is an important consideration for ensuring adequate neonatal vitamin D status and protection against rickets in early infancy[39].

Current Dietary Reference Values (DRVs) for vitamin D are not pregnancy-specific but rather based on 25(OH)D targets for the maintenance of bone health, and as such, there has been little change in dietary vitamin D intake recommendations for pregnant women in the UK[4; 40; 41]. Furthermore, there is no specific national guidance for pregnant women with overweight or obesity. Within a cohort limited to women with obesity (BMI ≥30 kg/m^2), we report an inverse association of 25(OH)D with BMI that remained significant after adjustment for selected maternal socio-demographic determinants of vitamin D status. The present study clearly shows a lower 25(OH)D among women at higher ends of the BMI distribution; only 23% of women with severe obesity had a 25(OH)D concentration above the 50nmol/L threshold. As neonatal 25(OH)D concentrations are dependent on maternal values in late gestation[13], and human breastmilk is typically low in vitamin D[42; 43], the high prevalence of vitamin D deficiency among pregnant women with obesity is a concern, particularly in an era where nutritional rickets remains a public health issue, both in the UK[37; 38] and globally[44].

The prevalence of vitamin D deficiency in our study population was higher in comparison to a previous UK pregnant cohort of BMI heterogenous women from Southampton[45], yet comparable to baseline trial data among women with overweight and obesity in Northern Ireland[21]. Our findings suggest current national guidelines in the UK, which recommend a vitamin D intake of 400IU/d[4], may not be adhered to, and/or may not be adequate for

preventing vitamin D deficiency. While pregnancy-specific thresholds for 25(OH)D may be required, trial-derived dose-response data is needed to determine whether the nutritional requirement for vitamin D to meet existing target thresholds is the same for pregnant women at both ends of the BMI distribution. Data from Ireland[46], New Zealand[47] and Canada[48] suggest a maternal 25(OH)D concentration of 50nmol/L in late gestation is required to prevent neonatal vitamin D deficiency at the 25nmol/L threshold. However, data from Northern Ireland has shown supplementation with 400IU vitamin D_3/d is not sufficient to raise 25(OH)D concentration >50nmol/L in women with overweight and obesity[21]. A greater vitamin D dose than currently recommended is therefore likely required in this population, particularly among women who enter pregnancy with a low vitamin D status.

In line with previously-published data[26; 27; 28; 29], we report a greater prevalence of vitamin D deficiency among women of Black, Asian and non-White ethnicity compared to women who identify as White. As melanin hinders dermal synthesis of pre-vitamin D_3[49], darker skin pigmentation is a well-recognised risk factor for vitamin D deficiency for individuals living at northern latitudes[50; 51]. While ethnic differences in vitamin D status are unlikely to be explained by variations in cutaneous production alone[52], our findings reiterate the need for targeted public health messaging to prevent vitamin D deficiency among the populations who are most at risk. Given the limited sample size in the Asian and non-White ethnic groups, our post-hoc analysis stratified by ethnicity was limited to White and Black women only. While 25(OH)D <50nmol/L was not associated with a greater odds of adverse outcomes in either ethnic group, we acknowledge imprecision of the effect estimates owing to the reduced sample size, and hence such findings should be considered as exploratory only.

We report a greater 25(OH)D among women with a university degree, yet the impact of socio-economic status on vitamin D status was less clear once additional socio-demographic factors were considered in the multivariable model. Despite the availability of funding schemes for low-income households, it was previously estimated that <10% of those eligible obtained free vouchers for vitamin D supplements for children and pregnant women in the UK[53]. Barriers such as complex ordering and reimbursement systems, and limited locations from which supplements can be acquired, have been reported[54]. Despite efforts to increase micronutrient intake among pregnant women of lower socio-economic status, such barriers to supplement use may therefore have contributed to a lower uptake and continued low vitamin

D status among certain subgroups at the time blood samples were drawn for the present study.

The potential impact of vitamin D intervention on blood glucose regulation and its role in prevention of diabetes has been discussed in recent years[55; 56; 57]. At present, pooled trial data show promising albeit conflicting evidence for an effect of vitamin D supplementation on prevention of GDM among generally healthy pregnancies[58; 59]. However, limited evidence from populations with obesity suggest little benefit of vitamin D supplementation. In the multicentre DALI vitamin D study[60], the authors report a reduction in fasting plasma glucose in late gestation following a daily dose of 1600IU vitamin D, but this did not translate to a reduction in GDM. The high frequency of personal micronutrient supplement use and high mean 25(OH)D concentration (>50nmol/L) at baseline limits generalisability of these findings to women with vitamin D deficiency[60]. Expression of the vitamin D receptor in pancreatic islet cells suggest a direct role for $1,25(OH)_2D$ in glucose regulation. In vitro studies of mouse and human tissues provide mechanistic evidence linking vitamin D-mediated gene transcription to insulin secretion in response to glucose exposure. Specifically, $1,25(OH)_2D$ upregulates the expression of voltage-gated calcium channels causing increased calcium influx to the cell, in turn stimulating insulin secretion from pancreatic β-cells[61]. As with others, we did not find strong evidence for an association between 25(OH)D and glucose regulation[62]; compared to women with 25(OH)D ≥50nmol/L, lower vitamin D status was not associated with an increased odds of developing GDM in the present study after adjusting for relevant confounders. Furthermore, while higher serum 25(OH)D was associated with a lower blood glucose concentration measured at the OGTT 2-h timepoint, the effect estimate was minor and unlikely to be clinically meaningful, and we acknowledge the possibility of type 1 errors owing to multiple testing. Given the lack of an association between 25(OH)D and fasting glucose, as well as glucose at the OGTT 1-h timepoint, we caution interpretation of these findings.

Strengths of this study include the large well-characterised and ethnically-diverse cohort of pregnant women with obesity and high levels of socioeconomic deprivation, who are a high-risk group for adverse pregnancy outcomes. However, several limitations should be acknowledged. As the present study utilised data and biological samples from a previously reported intervention trial[31], the sample size was limited to participants with existing data and we recognise that the study population may not be representative of the general UK population within the UK. We assessed 25(OH)D at a single time point during the second

trimester and did not specifically collect data on dietary vitamin D intake or personal vitamin D supplementation use; 25(OH)D reflects habitual vitamin D intake from both cutaneous synthesis and dietary intake, however, 25(OH)D followed a skewed distribution in all ethnic groups and we therefore expect some participants to have taken supplemental vitamin D either alone or as part of a prenatal multiple micronutrient supplementation regimen. It is possible that the 25(OH)D measured at this timepoint is not reflective of changes in vitamin D intake later in pregnancy, which may impact pregnancy outcomes. Variability in the quality of 25(OH)D data across analytical methods has been discussed at length in the literature. We used a well-recognised automated immunoassay that was available at the time of 25(OH)D assessment. However, we acknowledge potential bias of this method due to cross-reactivity with other vitamin D metabolites, including the C3-epimer of 25(OH)D[(63)]. While only 2.5% of samples were at or below the assay LoD of 7.5nmol/L, this possible bias and relatively high LoD may have influenced precision of the effect estimates when modelling determinants of 25(OH)D. We used a statistical-based approach to select variables for covariate adjustment in multivariable models. While empirical methods are valid approaches for covariate selection, we acknowledge the advantages and increasing movement towards illustrative-based approaches (e.g., direct acyclic graphs) to identify confounding relationships[(64)]. Lastly, while parathyroid hormone (PTH) concentrations were not available for this cohort, the interactive effects of low 25(OH)D and elevated PTH, representing functional vitamin D deficiency, may be a more meaningful indicator to explore rather than 25(OH)D alone[(65)]. As the limited evidence relating functional vitamin D deficiency to an increased risk of hypertensive disorders and restricted fetal growth is mixed[(65; 66; 67; 68)], the possibility of a greater risk of adverse perinatal outcomes is worth further exploration in diverse cohorts.

Conclusion

In this cohort of women at high risk of pregnancy complications, our findings do not support a greater risk of GDM, pre-eclampsia, preterm birth or abnormal fetal growth among women with 25(OH)D concentrations below the conventional threshold of 50nmol/L. However, our findings add to the increasing evidence that low vitamin D status is widespread among pregnant women with obesity in the UK, for which women of ethnic minority are most at risk. To meet current recommended thresholds for 25(OH)D, future dose-response trials are required to inform guidance for vitamin D intakes among pregnant women with obesity.

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Declaration of Interests

None to declare.

Author contributions

ACF, SLW, KGN conceptualised and designed this study. KGN, OFQ, KMOC and KVD analysed the data. ACF, KGN, KMOC, KVD, LP, JRF and SLW interpreted the findings. KMOC, KGN and ACF wrote the manuscript. ACF and SLW have primary responsibility for final content. All authors have read and approved the manuscript.

Data sharing plan

The UPBEAT Scientific Advisory Committee accepts applications for use of data from this study upon request (www.medscinet.net/upbeat/).

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Table 1. Maternal characteristics by vitamin D status assessed in the second trimester of pregnancy.

	Whole cohort	**25(OH)D <25nmol/L**	**25(OH)D ≥25 - <50nmol/L**	**25(OH)D ≥50nmol/L**
	N=1089	**n=286**	**n=441**	**n=362**
25(OH)D, median (25th, 75th percentile) nmol/L	38.9 (24.5, 56.4)	17.6 (12.0, 21.8)	37.0 (31.1, 43.4)	65.6 (56.4, 81.9)
Age, mean (SD) years	30.5 (5.6)	30.1 (5.7)	30.3 (5.7)	30.9 (5.2)
Gestational age, mean (SD) weeks	17 (1.1)	17.1 (1.1)	17.0 (1.0)	16.9 (1.1)
Deprivation status[1], N (%) or n (%)				
1 (Least deprived)	57 (5.3)	11 (19.3)	23 (40.4)	23 (40.4)
2	83 (7.6)	18 (21.7)	35 (42.2)	30 (36.1)
3	121 (11.1)	20 (16.5)	49 (40.5)	52 (43.0)
4	359 (33.1)	88 (24.5)	148 (41.2)	123 (34.3)
5 (Most deprived)	466 (42.9)	148 (31.8)	186 (39.9)	132 (28.3)
Educational attainment, N (%) or n (%)				
University degree	435 (39.9)	102 (23.5)	180 (41.4)	153 (35.2)
Centre[2], N (%) or n (%)				
St Thomas', London	361 (33.1)	101 (28.0)	165 (45.7)	95 (26.3)
Newcastle	225 (20.7)	63 (28.0)	85 (37.8)	77 (34.2)
Glasgow	252 (23.1)	39 (15.5)	95 (37.7)	118 (46.8)
Manchester	117 (10.7)	33 (28.2)	48 (41.0)	36 (30.8)
Bradford	40 (3.7)	25 (62.5)	11 (27.5)	4 (10.0)
St Georges, London	94 (8.6)	25 (26.6)	37 (39.4)	32 (34.0)
Ethnicity, N (%) or n (%)				

	Whole cohort	**25(OH)D <25nmol/L**	**25(OH)D ≥25 - <50nmol/L**	**25(OH)D ≥50nmol/L**
	N=1089	**n=286**	**n=441**	**n=362**
Asian	73 (6.7)	38 (52.1)	23 (31.5)	12 (16.4)
Black	228 (20.9)	85 (37.3)	111 (48.7)	32 (14.0)
White	732 (67.2)	144 (19.7)	285 (38.9)	303 (41.4)
Other	56 (5.1)	19 (6.6)	22 (5.0)	15 (4.1)
Season				
Winter	244 (22.4)	76 (31.2)	96 (39.3)	72 (29.5)
Spring	292 (26.8)	91 (31.2)	122 (41.8)	79 (27.0)
Summer	265 (24.3)	45 (17.0)	113 (42.6)	107 (40.4)
Autumn	288 (26.5)	74 (25.7)	110 (38.2)	104 (36.1)
Anthropometry				
Weight. mean (SD) kg	98.3 (15.5)	99.2 (16.2)	98.9 (16.2)	96.7 (14.0)
Hip circumference, mean (SD)[3] cm	122.8 (10.7)	123.8 (11.6)	123.1 (10.9)	121.7 (9.7)
Waist circumference, mean (SD)[3] cm	108.1 (10.8)	109.1 (11.5)	108.4 (10.7)	107.0 (10.1)
Thigh circumference, mean (SD)[3] cm	69.0 (6.8)	69.4 (7.4)	69.6 (7.0)	68.1 (6.6)
Triceps skinfold, mean (SD)[4] mm	32.3 (8.6)	31.8 (8.7)	32.8 (8.9)	32.1 (8.2)
Biceps skinfold, mean (SD)[5] mm	21.4 (7.6)	22.2 (8.0)	21.6 (7.8)	20.6 (7.1)
Suprailiac skinfold, mean (SD)[5] mm	33.1 (10.9)	34.6 (11.8)	33.4 (11.1)	31.6 (9.7)
Subscapular skinfold, mean (SD)[5] mm	35.8 (10.5)	37.5 (11.6)	36.0 (10.7)	34.4 (9.1)
Sum of skinfolds, mean (SD)[6,7] mm	122.7 (27.6)	126.1 (29.7)	123.8 (28.5)	118.7 (24.0)
BMI, median (25^{th}, 75^{th} percentile) kg/m^2	35.2 (32.7, 38.7)	36.1 (32.8, 39.9)	35.0 (32.9, 38.8)	34.8 (32.5, 37.8)
WHO BMI classification[8], N (%) or n (%)				
Obesity class I	528 (49)	123 (23.3)	217 (41.1)	188 (35.6)

	Whole cohort	**25(OH)D <25nmol/L**	**25(OH)D ≥25 - <50nmol/L**	**25(OH)D ≥50nmol/L**
	N=1089	**n=286**	**n=441**	**n=362**
Obesity class II	359 (33)	92 (25.6)	140 (39.0)	127 (35.4)
Obesity class III	202 (19)	71 (35.1)	84 (41.6)	47 (23.3)
GDM[9,10], N (%) or n (%)	272 (29)	79 (29.0)	120 (44.1)	73 (26.8)

25(OH)D, 25-hydroxyvitamin D; GDM, gestational diabetes mellitus.

[1]N=1086 due to missing data

[2]Serum 25(OH)D measurements were unavailable for UPBEAT study participants recruited from community clinics or Sunderland City Hospitals Foundation Trust, and hence were not included in the present analysis.

[3]N=1084 due to missing data

[4]N=1080 due to missing data

[5]N=1079 due to missing data

[6]N=1077 due to missing data

[7]Calculated by sum of biceps, triceps, suprailiac, and subscapular skinfold thicknesses.

[8]Obesity class I - BMI 30.0–34.9 kg/m^2, Obesity class II - BMI 35.0–39.9 kg/m^2, Obesity class III – BMI ≥40.0 kg/m^2.

[9]N=993 due to missing data

[10]GDM diagnosis at 22^{+0}-30^{+0} weeks' gestation by oral glucose tolerance test.

Table 2. Determinants of maternal serum 25-hydroxyvitamin D assessed in the second trimester of pregnancy (15–18^{+6} weeks' gestation).

Maternal characteristics	*n*	Unadjusted % difference (95% CI)[1]	*P*	*n*	Adjusted[2] % difference (95% CI)[1]	*P*
Age (years)	1089	0.74 (0.07 to 1.42)	0.03	1086	0.88 (0.21 to 1.54)	0.01
Deprivation status						
5 (Most deprived)	466	Reference	--	466	Reference	--
4	359	9.5 (0.5 to 19)	0.039	259	2.9 (-5.2 to 12)	0.50
3	121	27 (12 to 44)	<0.001	121	15 (2.6 to 30)	0.017
2	83	22 (5.1 to 41)	0.009	83	5.9 (-7.9 to 22)	0.42
1 (Least deprived)	57	24 (4.8 to 48)	0.013	57	1.8 (-14 to 20)	0.84
Education						
No university degree	654	Reference	--	651	Reference	--
University degree	435	8.4 (0.5 to 17)	0.037	435	8.3 (0.40 to 17)	0.039
Ethnicity						
White	732	Reference	--	730	Reference	--
Asian	73	-41 (-49 to -32)	<0.001	73	-43 (-51 to -35)	<0.001
Black	228	-33 (-39 to -27)	<0.001	227	-33 (-39 to -27)	<0.001
Other	56	-23 (-34 to -9.0)	0.002	56	-26 (-37 to -13)	<0.001
Season						
Summer	265	Reference	--	265	Reference	--
Winter	244	-16 (-25 to -6.4)	0.002	243	-15 (-23 to -5.6)	0.002
Spring	292	-18 (-26 to -8.7)	<0.001	290	-15 (-23 to -6.0)	0.001
Autumn	288	-7.0 (-16 to 3.3)	0.18	288	-6.1 (-15 to 3.6)	0.21
Anthropometry						
Weight (kg)	1089	-0.29 (-0.53 to -0.05)	0.02	1086	-0.38 (-0.61 to -0.15)	0.001
BMI (kg/m^2)	1089	-33 (-46 to -18)	<0.001	1086	-1.4 (-2.1 to -0.74)	<0.001
Sum of skinfolds (mm)	1077	-0.26 (-0.39 to -0.12)	<0.001	1074	-0.16 (-0.29 to 0.03)	0.017

[1]Effect estimates represents the percent difference in serum 25-hydroxyvitamin D per 1-unit increase in the predictor variable (continuous variables) or in comparison to the reference category (categorical variables).

[2]Adjusted for: Index of Multiple Deprivation, ethnicity, season of blood sampling, BMI, age, gestational age at blood sampling, educational attainment (university degree obtained or not). Adjustment for BMI was not included for anthropometric outcomes due to multicollinearity with weight and sum of skinfold thickness.

Table 3. Occurrence of selected perinatal outcomes by categories of maternal vitamin D status in the second trimester[1].

		Unadjusted model		Adjusted model[3]	
	N (%)[2]	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
GDM					
25(OH)D ≥50nmol/L	73/304 (24)	Reference		Reference	
25(OH)D >25 - <50nmol/L	120/390 (31)	1.4 (1.00 to 1.98)	0.049	1.28 (0.89 to 1.82)	0.18
25(OH)D <25nmol/L	79/273 (33)	1.58 (1.09 to 2.31)	0.02	1.33 (0.88 to 2.00)	0.18
Pre-eclampsia					
25(OH)D ≥50nmol/L	22/347 (6.3)	Reference		Reference	
25(OH)D >25 - <50nmol/L	31/424 (7.3)	1.17 (0.66 to 2.05)	0.60	1.10 (0.61 to 1.99)	0.75
25(OH)D <25nmol/L	15/278 (5.4)	0.84 (0.43 to 1.66)	0.62	0.81 (0.39 to 1.68)	0.57
Preterm birth					
25(OH)D ≥50nmol/L	24/353 (6.8)	Reference		Reference	
25(OH)D >25 - <50nmol/L	23/429 (5.4)	0.78 (0.43 to 1.40)	0.40	0.64 (0.34 to 1.18)	0.15
25(OH)D <25nmol/L	22/281 (7.8)	1.16 (0.64 to 2.12)	0.62	0.86 (0.44 to 1.67)	0.66
SGA					
25(OH)D ≥50nmol/L	35/353 (9.9)	Reference		Reference	
25(OH)D >25 - <50nmol/L	50/429 (12)	1.20 (0.76 to 1.89)	0.44	1.23 (0.77 to 1.96)	0.40
25(OH)D <25nmol/L	40/281 (14)	1.51 (0.93 to 2.45)	0.10	1.63 (0.97 to 2.73)	0.07
LGA					
25(OH)D ≥50nmol/L	33/353 (9.4)	Reference		Reference	
25(OH)D >25 - <50nmol/L	35/429 (8.2)	0.86 (0.52 to 1.42)	0.56	0.81 (0.48 to 1.36)	0.43
25(OH)D <25nmol/L	30/281 (11)	1.16 (0.69 to 1.95)	0.58	1.13 (0.64 to 2.00)	0.68

[1] OR represents the probability of occurrence of the event in each category of vitamin D status compared to 25(OH)D ≥50nmol/L. 25(OH)D, 25-hydroxyvitamin D; GDM, gestational diabetes mellitus; LGA, large for gestational age; OR, odds ratio; SGA, small for gestational age.

[2] Represents total number and percentage of women with outcome of interest.

[3]Adjusted for: ethnicity, season of blood sampling, BMI, maternal age, assigned UPBEAT intervention arm, gestational age at blood sampling and educational attainment (university degree obtained or not).

Table 4. Association between maternal second trimester (15–18^{+6} weeks' gestation) serum 25-hydroxyvitamin D and blood glucose measurements[1].

	Unadjusted model			Adjusted model[2]		
	n	**Difference in SD/SD (95%CI)[1]**	***P***	***n***	**Difference in SD/SD (95%CI)[1]**	***P***
Maternal measures						
HbA1c	1,014	-0.02 (-0.09 to 0.04)	0.44	1,014	0.06 (0.001 to 0.13)	0.045
Fasting glucose[3]	931	-0.05 (-0.12 to 0.01)	0.10	931	-0.03 (-0.10 to 0.03)	0.33
1h glucose[3]	882	-0.02 (-0.09 to 0.05)	0.57	882	-0.04 (-0.11 to 0.04)	0.33
2h glucose[3]	930	-0.08 (-0.15 to -0.02)	0.01	930	-0.07 (-0.14 to -0.003)	0.04

[1] The dependent and independent variables have been Fisher-Yates transformed to a normally distributed variables with a mean of zero and a standard deviation of one[(36)]. The regression coefficients have been standardised and can be interpreted as SD change in the outcome variables per SD change in serum 25(OH)D. 25(OH)D, 25-hydroxyvitamin D; HbA1c, Haemoglobin A1c

[2]Adjusted for: ethnicity, season, BMI, maternal age, assigned UPBEAT intervention arm, gestational age at sampling and educational attainment (university degree obtained or not).

[3]Measurements taken as part of standard oral glucose tolerance test between 23^{+2} to 30^{+0} weeks' gestation.

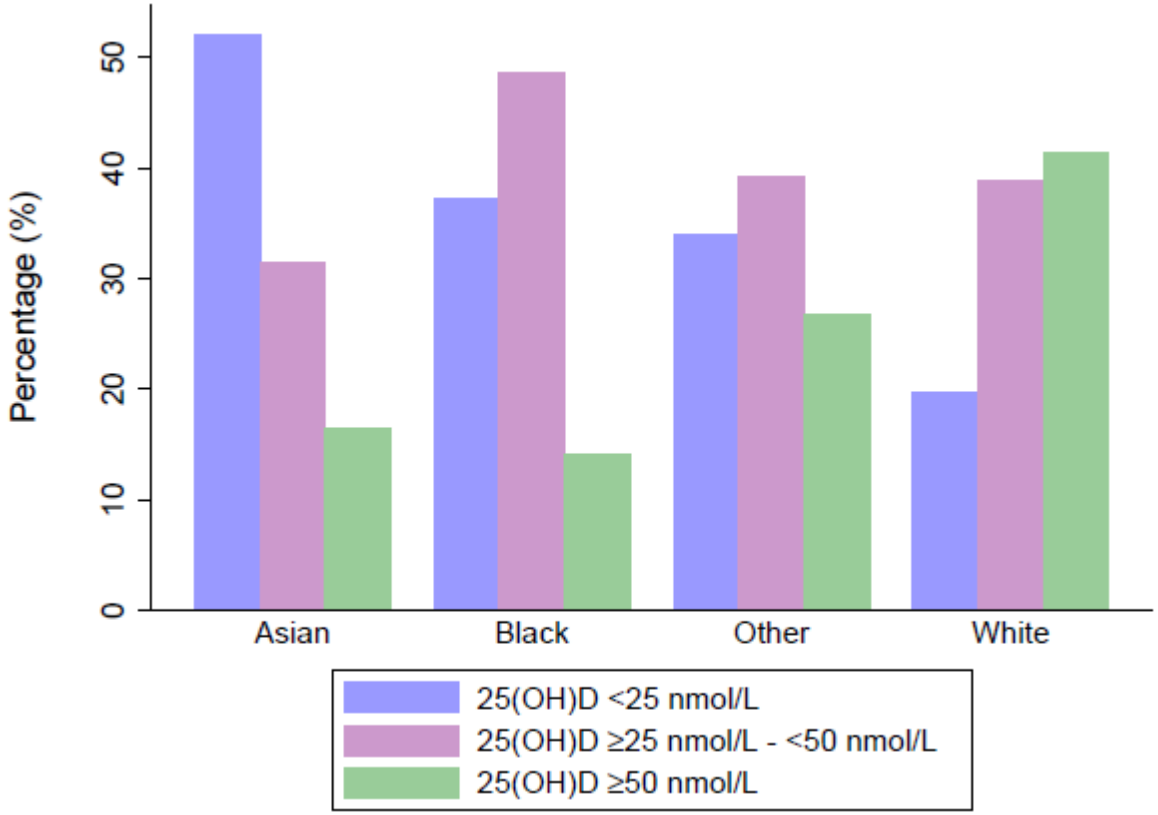

Figure 1. Maternal 25(OH)D concentration (nmol/L) in second trimester (mean: 17 ± 1 weeks' gestation). 25(OH)D; 25-hydroxyvitamin D. N = 73, 228, 56 and 732 for participants of Asian, Black, other non-White and White ethnicity, respectively.