F & S ___ style revision

Running Title: Vitamin D and fetal growth

Title page

Title: Pregnancy in dark winters: Implications for fetal bone growth?

Jennifer M WALSH MB[1], Mark KILBANE PhD[2], Ciara A MCGOWAN BSc, [1] Malachi J MCKENNA MD[2], Fionnuala M MCAULIFFE MD[1].

[1]UCD Obstetrics and Gynaecology, School of Medicine and Medical Science, University College Dublin, National Maternity Hospital, Dublin, Ireland.

[2]St Vincent's University Hospital, Dublin, Ireland.

Reprint requests

Professor Fionnuala McAuliffe MD, FRCOG, FRCPI

UCD Obstetrics and Gynaecology, School of Medicine and Medical Science, University College Dublin, National Maternity Hospital, Dublin, Ireland.

+353 (0) 1 6373216

fionnuala.mcauliffe@ucd.ie

Disclosure: None of the authors have a conflict of interest.

The Health Research Board, Ireland, and the National Maternity Hospital Medical Fund funded this research.

Capsule: The high prevalence of maternal hypovitaminosis D in women who are pregnant during winter months in northern latitudes may have detrimental effects on fetal skeletal growth.

Abstract

Objective:

To prospectively examine the prevalence of hypovitaminosis D in pregnancy and to correlate maternal and fetal vitamin D to fetal anthropometry.

Design:

A prospective cohort study.

Setting:

Tertiary referral maternity hospital, Dublin, Ireland.

Patients:

Sixty pregnant women.

Intervention:

Serum 25-hydroxyvitamin D (25OHD) was measured in early pregnancy, at 28 weeks and in cord blood at delivery. Two subgroups were analyzed to examine results in the context of seasonal variation in 25OHD: a winter and a summer cohort. Fetal anthropometry was assessed at 20 and 34 weeks and at delivery neonatal anthropometry recorded.

Main outcome measures:

The prevalence of hypovitaminosis D and the relationship between fetal growth and serum 25OHD concentrations.

Results:

There was a high prevalence of hypovitaminosis D ranging from 33% to 97%, with a marked seasonal variation. Fetal 25OHD concentrations correlated with all biometry at 20 weeks. In the winter cohort, a correlation was found between early pregnancy 25OHD and femur length at 20 weeks($r=0.34, p=0.07$) and between 28-week 25OHD($r=0.43, p=0.02$) and femur length at 34 weeks. Infant length was shorter in those with early pregnancy 25OHD less than the median. (52.1 vs. 53.6cm,$p=0.04$).

Conclusion:

The high prevalence of maternal hypovitaminosis D during winter months in northern latitudes may have detrimental effects on fetal skeletal growth.

Key words: Vitamin D, pregnancy, fetal femur length, and fetal growth.

Introduction

Vitamin D is required for normal calcium homeostasis and bone mineralization, and vitamin D deficiency leads to rickets in childhood or osteomalacia in later adult life (1, 2). Hypovitaminosis D in pregnancy has been linked to a wide variety of additional adverse outcomes, including pre-eclampsia, low birthweight, gestational diabetes and an increased predisposition to autoimmune disease in later life (3), but the evidence is inconsistent and inconclusive as regards causality (4). The developing fetus is entirely dependent upon the maternal pool of calcium; as such, there are growing concerns about the implications of hypovitaminosis D during pregnancy(5, 6). Despite the increasing awareness in the medical literature however, it appears that the public health message to date is inadequate, with many studies reporting a high prevalence of hypovitaminosis D in pregnant populations (7, 8). This is possibly compounded by the lack of consensus in the literature regarding recommendations for antenatal vitamin D supplementation (9). Risk factors for vitamin D deficiency include dark, pigmented skin, regular use of sunscreens, maternal obesity, and living in high latitude regions, especially during winter or spring months (3). In northern countries at latitudes above 42 degrees north, endogenous production of vitamin D essentially ceases from November until March (10, 11).

Accurate assessment of the prevalence of hypovitaminosis D is limited in many studies to date by the heterogeneity of populations studied, the effects of seasonal variation, and the reliance on a single assessment of 25OHD at just one time point in pregnancy (12, 13). Our objective was to clarify the prevalence of hypovitaminosis D in pregnancy by assessing 25OHD concentrations in early pregnancy, at 28 weeks' gestation and in fetal blood from the umbilical cord at delivery in two cohorts of

healthy Caucasian women pregnant at opposite times of the year in order to account for seasonal variation. We also sought to assess the possible implications of vitamin D deficiency for fetal growth, in particular fetal bone.

Materials and Methods

This was a prospective cohort study of 60 mother and infant pairs at the National Maternity Hospital, Dublin, Ireland with institutional ethics approval and written maternal consent. At our institution routine screening for vitamin D deficiency is not performed. All women were Caucasian and recruited to the study at first antenatal consultation. Women were excluded if they had an underlying medical condition, if they were less than 18 years of age, or if they were unable to give full informed consent.

Two specific cohorts were recruited. The first group (winter cohort) consisted of 30 women who were recruited in early pregnancy in September/October and delivered in March/April. A further 30 women were recruited in March/April and delivered in September/October (summer cohort). All women had 25OHD measured in early pregnancy (mean 14.3±2.6 weeks), at 28 weeks gestation and in fetal blood from the umbilical cord at delivery. At 20 weeks' gestation (range 19+1 to 21+5) a routine fetal anomaly ultrasound was performed and fetal biometry including biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) recorded. Fetal biometry was assessed again ultrasonographically at 34 weeks' gestation (range 33+4 to 34+5 weeks).

Serum 25OHD concentrations were measured by competitive radioimmunoassay (Immunodiagnostic Systems Limited, Boldon, Tyne & Wear, UK). The coefficients of variation (CV) for the 25OHD assay are as follows: inter-assay CV at a

concentration of 29 nmol/L was 6.2% and at a concentration of 106 nmol/L was 7.7%; intra-assay CV at a concentration of 29 nmol/L was 3.0% and at a concentration of 74 nmol/L was 2.7%. In order to ensure a high standard of analysis, we participate in the Vitamin D External Quality Assessment Scheme (14).

At delivery infant birthweight, length and head circumference were recorded. Serum 25OHD concentrations of above 50 nmol/L were classified at sufficient, and concentrations of <30 nmol/L considered at high risk of deficiency as per the recent Institute of Medicine (IOM) report (4).

Dietary vitamin D intake was assessed using a 3-day food diary, which was completed 1–2 weeks following the first antenatal consultation. Women were requested to record their usual food and beverage intake over 3 consecutive days, including a weekend day. Dietary data were entered and analysed using Weighed Intake Software Program (WISP) (Tinuviel Software, Anglesey, UK). WISP uses food composition data from 6th Ed of McCance and Widdowson's 'The Composition of Foods'. (15)

Data was assessed for normality using Shapiro Wilk and P-P plot. Bivariate correlations were assessed using Pearson's correlation coefficient for normally distributed data and Spearman's rho for non-parametric data. Further analysis was performed by comparing outcomes for those patients above and below the median for 25OHD concentrations, namely 41.0 nmol/L in early pregnancy, 45.7 nmol/L at 28 weeks and 30.8nmol/L in cord blood. Comparison of means within groups of patients was accomplished with the independent samples t test. Statistical significance was set at $p<0.05$. Statistical analysis was performed using SPSS Windows's version 18.0 (SPSS, Chicago, IL). Sample size calculation based on a significance level set of 5%

and power set at 90% suggested that 26 in each group would be required to detect one standard deviation difference in vitamin D between the two groups.

Results

Vitamin D status and season

The baseline subject characteristics of the cohort are contained in Table 1. Within the total study population, the mean 25OHD concentration was 45.7±22.6 nmol/L in early pregnancy, 54.4±33.4 nmol/L at 28 weeks gestation, and 31.8±12.6 nmol/L in fetal blood at delivery. All pregnancies resulted in healthy term deliveries with range from 37+1 to 42+1 weeks' gestation.

A marked seasonal variation in 25OHD was observed (Tables 1 & 2). In women in the summer cohort whose early pregnancy sample was taken in March/April the prevalence of vitamin D sufficiency was only 10% and the prevalence of those at high-risk of deficiency was 50%. In the winter cohort whose early pregnancy samples were drawn in September/October the prevalence of sufficiency was 67%, and only 7% were at high risk of vitamin D deficiency ($p<0.05$). As the pregnancies progressed the seasonal effect was maintained, such that by 28 weeks gestation (December/January for the winter cohort and June/July for the summer cohort) just 30% of the winter cohort had sufficient 25OHD concentrations compared to over 53% of the summer cohort ($p<0.05$). In the winter cohort who delivered in March/April the fetal sample showed that only 3 % were sufficient and 47% were at high risk of deficiency (25OHD <30 nmol/L). Though 25OHD concentrations were significantly higher at 28 weeks in the summer cohort who delivered in September-October than at

28 weeks in the winter cohort, only 13% of the summer cohort were sufficient, and 43% were at high risk of deficiency at the time of delivery.

The mean dietary intake of vitamin D as assessed by the 3-day food diary in early pregnancy and including intake from both diet and supplements was low, at just 2.78±1.8 micrograms for the total cohort. There was no significant difference in dietary vitamin D intakes between the summer and winter cohorts. (3.03±2.1μg vs. 2.52±1.5 μg, p=0.3).

Within the cohort, 37 women were taking prenatal vitamin supplementation, which included vitamin D. The mean gestation at commencement of supplements was 7.4±6.5 weeks and at discontinuation 35.9±8.3 weeks. The mean 25OHD concentration of those taking prenatal supplements was significantly higher in early pregnancy compared with those receiving no supplementation, (51.6±23.7 nmol/L vs. 37.1±17.5 nmol/L, p=0.01); however no significant difference persisted at 28 weeks gestation (52.8±24.2 nmol/L vs. 59.3±46.4 nmol/L, p=0.5) or in fetal blood at delivery (33.7±13.8 nmol/L vs. 29.8±9.3 nmol/L, p=0.2).

Vitamin D status and fetal biometry

The correlation of fetal biometry with both maternal and fetal 25OHD concentrations is shown in Table 3. Overall, fetal 25OHD concentrations correlated with fetal biometry at 20 weeks gestation (HC r=0.39, p=0.002; BPD r=0.34, p=0.008; AC r=0.34, p=0.009; and FL r=0.35, p=0.008). In the winter cohort, a positive correlation was found between maternal early pregnancy 25OHD and fetal femur length at 20 weeks gestation (r=0.34, p=0.07). Additionally a positive correlation was noted between both maternal 28-week 25OHD (p=0.02) and fetal 25OHD (p = 0.009) and fetal femur length at 34 weeks gestation. No significant difference was seen in the

fetal biometry at 20 or at 34 weeks between those women taking and those not taking prenatal supplements. No significant association was noted in either cohort between maternal and fetal 25OHD and infant birthweight, length or head circumference.

A comparison of the fetal and neonatal anthropometry of those with serum 25OHD concentrations above and below the median for the cohort at each time point is contained in Table 4. Fetal biometry at 20 weeks gestation was significantly greater for those with cord blood 25OHD above rather than below the median concentration. Additionally, the mean infant length at birth was significantly shorter in those with a 25OHD concentration less than the median in early pregnancy. (52.1 vs. 53.6cm, p=0.04).

Discussion

Our findings, in a cohort of healthy pregnant women have demonstrated a high prevalence of hypovitaminosis D in pregnancy, despite the fact that over half of the cohort (37 women) reported taking prenatal supplementation. Just 23 women (38%) in early pregnancy and 25 (42%) at 28 weeks gestation had sufficient vitamin D concentrations (25OHD ≥50 nmol/L). The prevalence of high-risk for vitamin D deficiency was particularly high in fetal blood at delivery, with just 5 cases (8%) having 25OHD ≥50 nmol/L. Maternal 25OHD concentrations correlated with fetal 25OHD as assessed by cord blood at delivery, reflecting the fact that the developing fetus is entirely dependent upon maternal 25OHD concentrations.

The effect of seasonal variation in 25OHD concentrations is highlighted once again by our findings. Concentrations of 25OHD were lowest at the beginning of spring and highest in early autumn. Our cohort of women was Caucasian and living in Dublin,

which is located at latitude 53 degrees North. Exposure to sunlight is essential for the conversion of provitamin D3 to previtamin D3, and in countries at latitudes above 42 degrees, skin production of vitamin D declines at the end of the summer and ceases during winter months (10).

Our winter cohort had the majority of their antenatal course during a time when exposure to effective sunlight was absent. In these pregnancies, we identified a positive correlation between 25OHD concentrations and fetal femur length at both 20 and 34 weeks' gestation. Though no significant direct correlation was noted between 25OHD and neonatal anthropometry was identified, the mean infant length at birth was significantly shorter in those with an early pregnancy 25OHD concentration less than the median in early pregnancy. Additionally all fetal biometry at 20 weeks gestation was significantly greater for those with cord blood 25OHD above rather than below the median concentration for the cohort.

Our findings clearly demonstrate that the lack of exposure to sunlight in Northern countries has a significant effect on vitamin D status that is not adequately protected against by dietary intake or routine prenatal self-supplementation. This may have potential implications for fetal bone growth. Indeed a number of authors recently have identified similar associations between vitamin D status and fetal bone growth. Mahon et al in 2010[5] speculated that maternal vitamin D insufficiency influenced fetal femoral development from as early as 19 weeks gestation, and recommended that measures to improve maternal vitamin D status should be instituted in early pregnancy. Viljakainen et al[6] in a cohort of 125 women suggested that maternal vitamin D status affects bone mineral accrual during the intrauterine period and affects bone size, despite the fact that the mean total intake of the mothers in their cohort met current recommendations.

Our study does have limitations worthy of consideration. Our cohort consisted of 60 women; however our inclusion criteria were strict including just Caucasian women living in Dublin and our recruitment specifically aimed to assess the prevalence of hypovitaminosis D in the context of known seasonal variation in 25OHD concentrations. In addition, we do not have any information on maternal 25OHD at delivery. The assessment of maternal 25OHD concentrations in both early and later pregnancy, as well as fetal 25OHD in cord blood, and the addition of ultrasonographic data greatly strengthen our findings. The assessment of fetal biometry at both 20 and 34 weeks gestation is essential in order to assess any potential implications of vitamin D depletion on intrauterine growth; our results would suggest that there might indeed be a link that is worthy of further interrogation.

What is clear from our findings is the significant seasonal effect on vitamin D status. For women living in Ireland, and those in countries of similarly high latitude during winter months, vitamin D supply depends entirely on oral intake from natural foods stuffs and fortified food. It is becoming increasingly evident that vitamin D is involved in a wide variety of biological processes, and that vitamin D deficiency may have a number of potential adverse consequences (3, 9). Vitamin D supplementation during pregnancy during winter is therefore essential.

Optimal doses of vitamin D supplementation however, and, indeed, an acceptable definition of vitamin D deficiency, particularly in pregnancy, remains an elusive and often debated topic. We used the recent IOM report definition of vitamin D sufficiency, insufficiency and deficiency in this study (4). The 2011 Endocrine Society Clinical Guideline however defined vitamin D deficiency as a 25OHD below 20 ng/ml (50 nmol/liter) and vitamin D insufficiency as a 25OHD of 21–29 ng/ml (52.5– 72.5) nmol/liter (16). Using this definition in our cohort, just 5 women (8.3%)

sufficient 25OHD in early pregnancy, 10 women (16.7%) were sufficient at 28 weeks and just one fetus with a cord blood 25OHD concentration of greater than 72.5nmol/L. Regardless of definitions used, there is no doubt that there is a significant prevalence of vitamin D deficiency, even despite supplementation in many.

This high prevalence of vitamin D insufficiency and deficiency requires clear guidance on vitamin D supplementation during pregnancy; yet again there is a distinct lack of consensus in the literature. In the United Kingdom and Ireland there are no clear recommendations for routine antenatal supplementation with vitamin D. The NICE guideline for antenatal care in 2008 (17) recommended informing women of the importance of maintaining adequate vitamin D stores in pregnancy, particularly for those at greatest risk of vitamin D deficiency who may chose to take vitamin D at a rate of 400 IU daily, a stance which was endorsed by the Scientific Advisory Committee on Nutrition (18). To achieve sufficiency as defined as 50 nmol/L, the IOM recommends 400–600 IU/day, which it states can be obtained through dietary means without supplementation, and indeed cautioned that higher intakes of Vitamin D may be harmful. Contrary to this, a number of authors have suggested that during pregnancy, supplementation with 400IU vitamin D/day has minimal effect on circulating 25OHD concentrations in either mother or baby (19, 20). In contrast, the Endocrine Society recommends an intake of 1,500–2,000 IU/day to achieve a circulating 25OHD level of more than 75 nmol/L. Doses of up to 4000 IU daily have even been advocated by some (21). This study, published in 2011 by Hollis et al randomized pregnant women to receive 400, 2000 or 4000 IU of vitamin D_3 daily. They found that supplementation with 4000 IU daily was safe and most effective in achieving sufficiency. These authors defend the safely of such high doses, pointing out that pregnant women from different native tribes in Africa have been shown to

have an average circulating 25OHD level of 150 nmol/L throughout pregnancy from the natural environment (22). In their RCT, pregnant women receiving 4,000 IU/day vitamin D3 attained an average circulating 25OHD level of 111 nmol, well below the 150 nmol/ L in a natural environment.

In conclusion, our findings indicate that in an otherwise healthy population, with a higher than 50% rate of supplementation, the prevalence of hypovitaminosis D is higher than expected. This may have implications for intrauterine growth, in particular bone growth. We would advocate that all women living in areas of limited sunshine should be regarded as high risk of vitamin D deficiency and advised accordingly, even in the absence of other risk factors. Further work is clearly warranted to establish the optimal and importantly safe dose of vitamin D supplementation in pregnancy, and the potential implications of inadequate concentrations on fetal, and later childhood and adult bone growth.

Acknowledgements

The Health Research Board Ireland and The National Maternity Hospital Medical Fund supported this research.

References

1. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008;87:1080S-6S

2. Welch TR, Bergstrom WH, Tsang RC.Vitamin D-deficient rickets: the re-emergence of a once-conquered disease. J Pediatr. 2000;137:143-5

3. Mulligan ML, Felton SK, Riek AE, Bernal-Mizrachi C. Implications of vitamin D deficiency in pregnancy and lactation. Am J Obstet Gynecol. 2010;202:429e1-9.

4. Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Food and Nutrition Board, Institute of Medicine of the National Academies. Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academic Press; 2011.

5. Mahon P, Harvey N, Crozier S, Inskip H, Robinson S, Arden N, Swaminathan R, Cooper C, Godfrey K; SWS Study Group. Low maternal vitamin D status and fetal bone development: cohort study. J Bone Miner Res. 2010;25:14-9.

6. Viljakainen HT, Saarnio E, Hytinantti T, Miettinen M, Surcel H, Mäkitie O, Andersson S, Laitinen K, Lamberg-Allardt C. Maternal vitamin D status determines bone variables in the newborn. J Clin Endocrinol Metab. 2010;95:1749-57.

7. McGowan CA, Byrne J, Walsh J, McAuliffe FM. Insufficient vitamin D intakes among pregnant women.Eur J Clin Nutr. 2011; 65:1076-8 .

8. Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM.
High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates.J Nutr. 2007;137:447-52.

9. Hollis BW, Wagner CL. Vitamin D and Pregnancy: Skeletal Effects, Nonskeletal Effects, and Birth Outcomes. Calcif Tissue Int. 2012 May 24. [Epub ahead of print].

10. Webb AR, Kline L, Holick MF.Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin .J Clin Endocrinol Metab. 1988;67:373-8

11. McKenna M, McCarthy R, Kilbane M, Molloy E. Vitamin D nutrient intake for all life stages. Ir Med J. 2011;104:102.

12. Scholl TO, Chen X. Vitamin D intake during pregnancy: association with maternal characteristics and infant birth weight. Early Hum Dev. 2009;85:231-4.

13. Ginde AA, Sullivan AF, Mansbach JM, Camargo CA Jr. Vitamin D insufficiency in pregnant and nonpregnant women of childbearing age in the United States. Am J Obstet Gynecol. 2010;202:436.e1-8

14. Carter GD, Berry JL, Gunter E, Jones G, Jones JC, Makin HL, et al. Proficiency testing of 25-hydroxyvitamin D (25-OHD) assays. J Steroid Biochem Mol Biol. 2010; 121:176-9

15. McCance and Widdowson's The Composition of Foods, sixth summary edition. Food Standards Agency (2002). Cambridge: Royal Society of Chemistry.

16. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911-30.

17. National Collaborating Centre for Women's and Children's Health. Antenatal Care: Routine care for the healthy pregnant woman. London

18. Scientific Advisory Committee on Nutrition. Update on Vitamin D. London; The Stationary Office;2007.

19. Lee JM, Smith JR, Philipp BL, Chen TC, Mathieu J, Holick MF. Vitamin D deficiency in a healthy group of mothers and newborn infants. Clin Pediatr (Phila). 2007;46:42-4.

20. Cockburn F, Belton N.R, Purvis R.J, Giles M.M, Brown J.K, Turner T.L, Wilkinson E.M et al. Maternal vitamin D intake and mineral metabolism in mothers and their newborn infants. Br. Med. J.1980; 5:11–14.

21. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: Double blind, randomized clinical trial of safety and effectiveness. J Bone Miner Res. 2011; (10):2341-57.

22. Luxwolda MF, Kuipers RS, Kema IP, van der Veer E, Dijck-Brouwer DA, Muskiet FA. Vitamin D status indicators in indigenous populations in East Africa. Eur J Nutr. 2012 Aug 10. [Epub ahead of print].

Table 1. Baseline subject characteristics

	Overall	Winter cohort	Summer cohort	p value
Age	31.7 (6.2)	31.2 (7.3)	32.4 (4.8)	0.4
Height cm	166.8 (5.8)	166.2 (5.5)	167.5 (6.1)	0.3
Weight kg	73.6 (11.5)	74.5 (11.1)	72.7 (12.1)	0.5
BMI kg/m^2	26.6 (4.2)	27.1 (4.1)	26.2 (4.4)	0.4
Supplemented	37	23	14	0.05
Smokers	2	2	0	0.5
Early pregnancy 25OHD nmol/L	45.6 (22.6)	57.5 (19.8)	33.8 (18.8)	0.000
28 weeks 25OHD nmol/L	54.38 (33.4)	43.6 (14.8)	65.1 (42.)	0.012
Cord blood 25OHD nmol/L	31.8 (12.6)	31.7 (10.6)	31.8 (14.4)	0.98

Baseline subject characteristics including the mean and SD of the age, weight, height, BMI, serum 25OHD concentrations and the number of smokers and those taking supplements in the entire group, with a comparison of the winter and summer cohorts.

Table 2. Vitamin D status throughout pregnancy in the Winter and Summer Cohorts.

		Sufficient (≥50nmol/L)	Insufficient (30-49.9nmol/L)	Deficient (<30nmol/L)
First trimester	Winter cohort (September / October)	20/30 (66.7%)	8/30 (26.7%)	2/30 (6.7%)
	Summer cohort (March /April)	3/30 (10%)	12/30 (40%)	15/30 (50%)
28 weeks gestation	Winter cohort (December / January)	9/30 (30%)	15/30 (50%)	6/30 (20%)
	Summer cohort (June / July)	16/30 (53.3%)	11/30 (36.7%)	3/30 (10%)
Cord blood	Winter cohort (March / April)	1/30 (3.3%)	15/30 (50%)	14/30 (46.7%)
	Summer cohort (September / October)	4/30 (13.3%)	13/30 (43.3%)	13/30 (43.3%)

Table 3. Correlations between maternal and fetal 25OHD concentrations and fetal biometry.

25OHD groups		20 week HC	20 week BPD	20 week AC	20 week FL	34 week EFW	34 week HC	34 week BPD	34 week AC	34 week FL
Overall n = 60	Early pregnancy	0.02	-0.05	0.01	0.04	0.06	-0.2	-0.2	0.01	0.15
	28 weeks	0.2	0.2	0.15	0.15	0.13	0.06	0.03	0.19	0.12
	Cord blood	0.39*	0.34*	0.34*	0.35*	0.02	0.03	0.09	0.04	0.12
Summer cohort	Early pregnancy	0.04	-0.05	-0.05	0.1	0.03	0.03	0.03	0.04	0.03
	28 weeks	0.2	0.01	0.2	0.07	0.04	-0.1	-0.1	0.15	0.23
	Cord blood	0.43*	0.32	0.38	0.28	0.14	0.01	0.01	-0.1	0.12
Winter cohort	Early pregnancy	0.19	0.2	0.2	0.34*	0.03	-0.2	0.38	0.06	0.17
	28 weeks	0.12	0.25	0.12	0.26	0.26	0.14	0.01	0.2	0.43*
	Cord blood	0.39*	0.31	0.31	0.49*	0.15	0.02	0.26	0.05	0.48*

HC Head circumference, BPD Biparietal diameter, AC Abdominal circumference, FL Femur length, EFW Estimated fetal weight, * $p<0.05$

Table 4. Comparison of fetal and neonatal anthropometry of those with 25OHD concentrations above and below the median for the cohort at each time point.

		BPD at 20	HC at 20	AC at 20	FL at 20	BPD at 34	HC at 34	AC at 34	FL at 34	Birthweight	Length at birth	Head Circumference at birth
Early pregnancy	Less than median	49.9 (5.5)	180.3 (29.1)	159.5 (16.2)	34.01 (3.6)	88.6 (3.3)	319.3 (13.2)	315.3 (16.6)	66.1 (3.2)	4011 (495)	52.07 (3.0)	35.4 (1.8)
	Greater than median	49.5 (3.8)	184.6 (15.2)	158.2 (15.6)	34.2 (3.2)	88.1 (3.6)	316.7 (10.5)	318.7 (15.6)	67.3 (3.8)	4106 (462)	53.6* (2.2)	35.9 (1.1)
28 weeks	Less than median	49.2 (5.4)	177.9 (28.7)	155.8 (13.3)	33.4 (3.2)	88.8 (3.4)	319.1 (13.4)	314.8 (15.4)	66.8 (3.2)	4083 (550)	52.4 (2.3)	35.7 (1.9)
	Greater than median	50.2 (3.8)	187.2 (14.7)	161.9 (17.5)	34.7 (3.5)	87.9 (3.5)	317.5 (10.4)	319.1 (16.6)	66.6 (3.9)	4035 (399)	53.4 (2.5)	35.7 (1.0)
Cord blood	Less than median	48.2 (5.1)	173.8 (27.2)	152.1 (12.9)	32.6 (2.8)	88.6 (3.1)	318.4 (10.5)	317.7 (18.1)	66.3 (3.4)	4014 (458)	52.6 (3.2)	35.4 (1.7)
	Greater than median	51.2* (3.8)	191.2* (13.7)	165.4* (15.7)	35.5* (3.3)	88.2 (3.7)	318.2 (13.5)	316.2 (13.9)	67.1 (3.7)	4103 (499)	53.2 (2.0)	36 (1.2)

Mean and SD of fetal biometry at 20 and at 34 weeks' gestation for those above and below the median 25OHD concentrations for the cohort.

HC Head circumference, BPD Biparietal diameter, AC Abdominal circumference, FL Femur length, EFW Estimated fetal weight,
* $p<0.05$