Seasonal variation in maternal and umbilical cord 25(OH) vitamin D and their associations with neonatal adiposity

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Abstract

Design: Neonatal body fat is an important indicator of foetal energy supply and growth with potential importance for long-term health. In this study, we wanted to explore seasonal variation of 25-hydroxy-vitamin D (25(OH)D) in maternal and umbilical cord plasma (UCP) to examine whether maternal and foetal 25(OH)D levels were associated with maternal BMI and neonatal fat mass (FM), and to explore the relationship among maternal and neonatal 25(OH)D levels, maternal glucose/insulin levels and UCP C-peptide.

Methods: An observational, prospective study of determinants of foetal growth and birth weight in healthy pregnant women. Total body composition in 202 newborns was measured by dual-energy X-ray absorptiometry. Circulating levels of biomarkers were assessed in mothers at gestational weeks 14–16 and 30–32 and UCP.

Results: The mean 25(OH)D concentration in UCP was significantly lower than in maternal circulation (31 vs 45 nmol/l, $P < 0.001$). Maternal and UCP 25(OH)D levels varied significantly with season. No significant association between maternal BMI (weeks 14–16) and UCP 25(OH)D concentration was found. We found a strong positive association between maternal 25(OH)D and UCP 25(OH)D ($P < 0.001$). There was no significant linear association between maternal BMI (weeks 14–16) and maternal 25(OH)D. We found no association between maternal 25(OH)D levels and glucose/insulin levels, nor with maternal or UCP 25(OH)D on UCP C-peptide levels. Finally, neonatal total body FM was positively associated with UCP 25(OH)D, $P = 0.02$.

Conclusions: We demonstrated seasonal variation in maternal and neonatal 25(OH)D levels at northern latitudes. UCP, but not maternal, 25(OH)D was a significant predictor of neonatal total FM. Maternal BMI and metabolic parameters such as glucose, insulin and UCP C-peptide levels were not associated with 25(OH)D in mothers or offspring.

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Introduction

The pleiotropic effects of 25-hydroxy-vitamin D (25(OH)D) in relation to foetal development and neonatal body composition have received attention recently (1, 2). This interest has largely been driven by the well-documented relationship between anthropometric characteristics at birth and the future health of the

neonates (3). The growth of the human foetus involves an accretion of adipose tissue during the last trimester (4); neonatal body fat is therefore an important indicator of foetal energy supply and growth conditions (5, 6), which are of potential importance for long-term outcome and health (7).

The foetal levels of 25(OH)D *in utero* and at birth are dependent on the maternal pool of 25(OH)D and trans-placental transfer. Maternal vitamin D status during pregnancy may influence skeletal development (8) and body composition in the offspring (9) by influencing programming and development of muscle and fat tissue. Countries at northern latitudes, such as Norway, where u.v.-B (UVB) is insufficient during almost half of the year, exhibit seasonal variation in 25(OH)D levels in the general population and likely pregnant women, with potential consequences for foetal development (10).

Vitamin D is a fat-soluble prohormone that promotes intestinal calcium absorption. Regardless of their source, the vitamin D precursors ergocalciferol (D₂) and cholecalciferol (D₃) are transported to the liver and metabolised into the prohormone, 25-dihydroxyvitamin D, and finally activated by 1- α hydroxylase primarily in the kidneys to the biologically active form 1,25-dihydroxyvitamin D (1,25(OH)₂D). The major circulating form of the vitamin, 25(OH)D, is considered to be the best measure of vitamin D status (11). As the developing foetus does not produce vitamin D by itself, and the intrauterine and neonatal periods are critical for vitamin D-related effects, the child is dependent on maternal levels and their ability to pass the placental barrier (7). Previous studies have demonstrated not only that maternal vitamin D status is tightly correlated with umbilical cord plasma (UCP) 25(OH)D concentrations (12, 13, 14) but also that trans-placental transport is dependent on maternal BMI.

Vitamin D deficiency and low plasma 25(OH)D levels have been epidemiologically associated with a broad range of conditions, including cancer, diabetes, cardiovascular disease, hypertension and pregnancy complications (15, 16, 17, 18). Excess body weight is associated with decreased serum 25(OH)D concentrations and a high prevalence of vitamin D deficiency (19, 20). The reduced bioavailability of the fat-soluble 25(OH)D, which is related to accumulation in body fat, may be the main reason for this association (21). However, the associations among obesity in pregnancy, vitamin D status and neonatal vitamin D levels have not been studied extensively, nor has the relationship between vitamin D status and neonatal adiposity.

Maternal BMI is one of the most consistent determinants of foetal weight, growth and body composition (6, 22). Maternal BMI is positively associated with circulating glucose levels. Furthermore, maternal BMI and glucose have been established as independent determinants of large-for-gestational-age newborns and excessive body fat at birth (23, 24). Recently, attention has

been focused on a potential role for vitamin D in the maintenance of normal glucose homeostasis in pregnancy. In addition, vitamin D deficiency has been found to be associated with pancreatic β -cell dysfunction and insulin resistance in non-pregnant diabetic and non-diabetic populations (25, 26). Glucose is the main energy substrate for intrauterine growth (27). UCP levels of C-peptide are used as an index of foetal β -cell function, rather than insulin levels, because degradation of insulin is increased in the presence of slight haemolysis (28). Thus, foetal size has been correlated with umbilical total insulin, free insulin and C-peptide (29). Moreover, the recent Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study showed a linear relationship between increasing maternal glucose and UCP C-peptide with birth weight (23, 30).

One of the primary roles of vitamin D is the regulation of calcium and phosphorus absorption and metabolism for bone health. This effect is especially important during pregnancy because adequate vitamin D concentrations are necessary to ensure appropriate maternal response to the calcium demands of the foetus and neonatal handling of calcium. The relationship between maternal vitamin D and foetal bone development has recently been reviewed (31), but no association between maternal vitamin D levels and neonatal bone mineral content was demonstrated. This observation is in agreement with our recent findings, where we found that neither foetal nor maternal 25(OH)D seemed to have a major role in bone mass development in the neonates (32).

Our study included samples from a Norwegian prospective cohort study of healthy pregnant women (33). The samples were collected throughout all seasons, and our first aim in this study was to explore the seasonal variations of 25(OH)D in the maternal and UCP sides of the placenta. Secondly, we wanted to test the hypotheses that maternal and foetal 25(OH)D levels are associated with the maternal BMI and neonatal percentage and gram total fat mass (FM). Finally, we wanted to explore this relationship by examining maternal and neonatal 25(OH)D status, maternal glucose and insulin and UCP C-peptide in this cohort.

Subjects and methods

The study sample was based on a sub-cohort of 202 women from the STORK study: an observational, prospective study of determinants of foetal growth and birth weight in healthy pregnancies for which the details have been published previously (32, 33). In short, the STORK cohort

consists of women of Scandinavian heritage ($n=1031$), who registered for obstetric care at Oslo University Hospital Rikshospitalet, from 2001 to 2008. Exclusion criteria were multiple pregnancies, known pre-gestational diabetes and severe chronic diseases (lung, cardiac, gastrointestinal or renal).

The women were scheduled for antenatal visits at gestational weeks 14–16 and 30–32. The maternal height (self-reported) was obtained at the first visit, and the weight was measured at each visit. The BMI was calculated as weight (kg) divided by height (m^2). In the analyses, only the BMI from the first visit was used, as this value was the best estimate for the pre-gestational body composition. Intake of macronutrients, including the total vitamin D intake (from food or supplements), was calculated. However, as it was not possible to distinguish between the intake from food and supplements, and as there was not a significant association between the estimated vitamin D intake and maternal 25(OH)D, these data are not shown in the manuscript.

Biochemical variables

A 75 g oral glucose tolerance test (OGTT) was performed twice, at gestational weeks 14–16 and 30–32. During the OGTT, serum samples were obtained every 30 min for 2 h. Maternal glucose and insulin were measured in the blood and serum samples from the OGTT. Glucose and insulin areas under the curve (AUC) during the OGTT were calculated using the trapezoidal rule. Glucose measurements were performed with an Accu-Chek Sensor glucometer using Accu-Chek glucose test strips (Roche Diagnostics GmbH). The glucose was measured immediately in EDTA blood. Serum insulin was analysed by a RIA (DPC, Los Angeles, CA, USA).

The serum samples were collected in 6 ml Vacutainer tubes, centrifuged at room temperature at 2500 *g* for 10 min, aliquoted and stored at -80°C until analysed: 4 ml Vacutainer tubes containing EDTA were centrifuged (2500 *g* and 4°C for 25 min) and frozen at -80°C , aliquoted and stored at -80°C until analysed. Umbilical cord blood was collected into EDTA tubes by the midwife, centrifuged for plasma separation and placed at -20°C for less than a month and at -80°C for long-term storage.

Maternal circulating levels of 25(OH)D were analysed in samples of gestational weeks 30–32. Measurements of 25(OH)D and C-peptide were performed in the UCP. The plasma levels of 25(OH)D and C-peptide were measured using a RIA from DiaSorin (MN, USA) and Millipore Corporation (Billerica, Stillwater, MA, USA) respectively.

All assays were performed according to the manufacturer's instructions. The intra- and inter-assay coefficients of variation (CV values) were $<10\%$ for all assays.

Dual-energy X-ray absorptiometry measurements

Neonatal body composition was determined by dual-energy X-ray absorptiometry (DXA; GE Lunar Prodigy Densitometer (software version 12.10; GE Medical Systems, Lunar Corp., Madison, WI, USA). All DXA scans were performed within 4 days *postpartum*. The scanning procedure has been described in detail previously (34). DXA measurements provided information on bone mass, total FM and fat-free mass (FFM). Percentage fat was based on DXA-derived fat minus DXA-derived sum of fat, lean and bone mineral content.

According to the manufacturer, the CV values (%) for a Lunar DPX-L instrument (regarded by the manufacturers to be similar to the Lunar Prodigy) are 2.0 and 1.1% for FM and FFM respectively (35).

Ethics

Written informed consent was obtained from the participants. The study was approved by the Regional Ethics Committee, Southern Norway, and performed according to the Declaration of Helsinki.

Statistical analysis

Descriptive statistics are means (s.d.) or medians (quartiles). Comparisons of the women and neonates in the study sample and the women and neonates born at term, who were not elected for this study, were performed by independent sample *t*-tests or Mann–Whitney *U* tests.

The maternal and cord blood seasonal variations in 25(OH)D were estimated by functional data analysis, using Fourier basis functions (36) to account for cyclic variation. The number of basis functions was chosen based on visual inspection of plots of estimated curves combined with calculations of the corresponding generalised cross-validation criterion (37). Pointwise 95% CIs were calculated (37).

Univariate and multivariable linear regression analyses were used to estimate the association of maternal BMI with maternal and UCP 25(OH)D, maternal glucose AUC, insulin AUC and C-peptide in cord blood, and neonatal body fat. Regression analyses were also used to estimate the association of maternal and UCP 25(OH)D levels with maternal glucose AUC, insulin AUC and

C-peptide in cord blood, and neonatal body fat. Linear regression model assumptions were checked for each analysis separately. A P value <0.05 was considered statistically significant.

Except for the analysis of the seasonal variation, which was performed using the *fda* package in R 3.0.0 (38), all analyses were performed using SPSS version 18.0 (SPSS, Inc.).

Results

Demographics for mother and child

The demographic and clinical details in the study sample are presented in Table 1. The neonates in the study population were comparable with the neonates in the main cohort regarding birth weight (3612 g (480) vs 3670 g (487); $P=0.13$) and gestational age at birth (40.2 week (1.2) vs 40.2 week (1.2); $P=0.90$). The women in the study sample had a significantly lower BMI, glucose AUC and fasting insulin at gestational weeks 14–16 ($0.001 \leq P \leq 0.13$) and a lower BMI, fasting glucose and fasting insulin at weeks 30–32 ($P \leq 0.02$) than the other eligible women in the STORK cohort.

Seasonal variation in maternal and UCP 25(OH)D concentrations

The mean 25(OH)D concentration in UCP was significantly lower than in maternal circulation (31 vs 45 nmol/l, $P<0.001$; Table 1). The distribution of maternal and UCP 25(OH)D concentration varied by time of the year is shown in Fig. 1. The pointwise 95% CIs in Fig. 1 show that the concentration of maternal 25(OH)D varied significantly by time of the year, with highest levels in the Norwegian summer months (June–September) and the lowest levels in the winter months (December–March).

The same seasonal variation (relative to month of birth) was found in UCP 25(OH)D, with the highest levels during June–October and the lowest levels during December–April.

Association between maternal BMI and vitamin D levels in mother and child

No significant association between maternal BMI (weeks 14–16) and UCP 25(OH)D concentration was found (standardised regression coefficient, $\beta=0.10$, $P=0.23$),

Table 1 Sample characteristics of the pregnant women and their infants. Numbers are mean (s.d.) or median (Q_1 , Q_3).

	Women, $n=202^a$ (weeks 14–16)	Women, $n=202^a$ (weeks 30–32)	Infants, $n=202^{a,b}$
Maternal characteristics			
BMI (kg/m^2)	23.9 (3.8)	26.6 (3.8)	
Obese ($\text{BMI} > 30 \text{ kg}/\text{m}^2$)	14 (7%)		
Fasting glucose (mmol/l)	4.0 (0.4)	4.0 (0.4)	
Glucose AUC	9.3 (1.9)	11.9 (2.2)	
Fasting insulin (pmol/l)	24 (15, 34)	37 (25, 58)	
Insulin AUC	408 (280, 582)	770 (565, 1022)	
25(OH)D (nmol/l)		45 (17)	
Infant characteristics			
Gestational age at birth (weeks)			40.2 (1.2)
Birth weight (g)			3612 (480)
C-peptide (ng/ml)			1.1 (0.7)
25(OH)D (nmol/l)			31 (18)
DXA			
Scan day (after birth)			1.8 (1.0)
Scan day weight (g)			3427 (460)
Total body FM (%)			13.5 (2.3)
Total body FM (g)			498 (122)
BMC			93 (12)

BMI, body mass index (kg/m^2 ; self-reported); AUC, area under the curve; DXA, dual-energy X-ray absorptiometry; FM, total body fat mass; BMC, total bone mineral content; 25(OH)D, 25-hydroxy-vitamin D.

^a n may vary due to missing values.

^bBorn at term, defined as birth at gestational week 37 or later.

Figure 1

Relations between time of year and 25(OH)D levels. The black dots represent maternal values and the grey dots represent umbilical cord plasma (UCP) of 25-hydroxy-vitamin D [25(OH)D]. The thick black and grey lines are the smoothed estimates of the maternal and UCP concentration of 25(OH)D respectively. The thin black and grey lines are the corresponding pointwise 95% CIs.

but there was a strong positive association between maternal 25(OH)D and UCP 25(OH)D ($\beta=0.42$, $P<0.001$).

We found no seasonal variation in maternal BMI or birth weight (data not shown). There was no significant linear association between maternal BMI (weeks 14–16) and maternal 25(OH)D ($\beta=-0.03$, $P=0.64$), but a scatter plot with local regression lines indicated a potential non-linear association, with the highest maternal 25(OH)D values for women with low BMI (data not shown). To explore this observation further, BMI was dichotomised by the lower BMI quartile (BMI 21.4 kg/m^2) and the mean 25(OH)D levels were compared in the two groups. The difference between the groups was in accordance with the scatter plot regression lines but non-significant, $P=0.07$. We found no association between 25(OH)D levels related to BMI estimated at the time point corresponding to the vitamin D analyses (weeks 30–32): data not shown.

Association between maternal 25(OH)D levels and maternal glucose and insulin levels, weeks 30–32

Univariate linear regression analyses showed no significant association between maternal 25(OH)D levels

and $\text{AUC}_{\text{glucose}}$ or $\text{AUC}_{\text{insulin}}$ ($\beta=0.06$, $P=0.38$ and $\beta=-0.01$, $P=0.86$ respectively). These results remained non-significant ($\beta=0.09$, $P=0.24$ and $\beta=0.02$, $P=0.83$ respectively) after adjustment for maternal BMI.

Association between maternal 25(OH)D levels and UCP C-peptide concentration

There were no significant associations between maternal or UCP 25(OH)D and UCP C-peptide levels in univariate regression analyses, nor did the result change after adjustment for maternal BMI ($\beta=-0.02$, $P=0.84$ and $\beta=-0.03$, $P=0.75$ for maternal and UCP 25(OH)D respectively).

Association between maternal and UCP 25(OH)D levels and BMI on neonatal FM in gram and percentage

In univariate regression analyses, there was no statistically significant association between maternal 25(OH)D and neonatal total body FM ($\beta=-0.02$, $P=0.75$), but there was a significant association between UCP 25(OH)D and neonatal total body FM ($\beta=0.20$, $P=0.02$). Adjustment for BMI gave only minor changes in the estimates: $\beta=-0.04$, $P=0.67$ and $\beta=0.20$, $P=0.03$ for maternal and UCP 25(OH)D respectively.

In univariate regression analyses, there was no significant association between maternal 25(OH)D and neonatal percentage fat ($\beta=0.04$, $P=0.55$), but a significant association between UCP 25(OH)D and neonatal percentage fat ($\beta=0.20$, $P=0.02$) was found. After adjustment for BMI, the latter estimate was no longer significant. The adjusted estimates were $\beta=0.04$, $P=0.69$ and $\beta=0.17$, $P=0.07$ for maternal and UCP 25(OH)D respectively.

Discussion

This study demonstrates seasonal variation in maternal and neonatal 25(OH)D levels, with neonatal levels being approximately two-thirds of the maternal levels. Maternal 25(OH)D levels did not predict neonatal FM, whereas 25(OH)D in UCP was associated with DXA-assessed neonatal total body FM. Finally, maternal BMI, glucose, insulin and UCP C-peptide were not related to maternal or neonatal 25(OH)D levels. The seasonal variation of 25(OH)D was assessed from maternal and umbilical cord evenly distributed during the year. Thus, vitamin D status exhibits a strong seasonal variation that parallels the seasonal change in the fluence of solar UVB. Our data are in accordance with a study recently published in Germany (10).

During winter, the UVB fluence rate in the Nordic countries (50–71°N) is below the level required for vitamin D synthesis in skin (39). Our measured seasonal variation of the vitamin D status is in agreement with results from non-pregnant subjects from other Scandinavian investigations demonstrating a 20–120% increase in calcidiol levels from winter to summer (40, 41). We found maternal and neonatal 25(OH)D levels with a peak in June through September and with minimum levels during the winter, from December through March. As expected, 25(OH)D levels in maternal and UCP were significantly correlated, but the seasonal peak in UCP seems to have a delay of approximately a month. Furthermore, 25(OH)D levels in the neonates were approximately two-thirds of the maternal levels, which confirms earlier observations (10, 42). Vitamin D-binding protein is known to increase slightly during pregnancy (43); however, the magnitude of this increase is not large enough to explain the maternal–foetal difference. The correlation of UCP and maternal 25(OH)D levels suggests that 25(OH)D may be the predominant metabolite transferred to the foetus.

The foetal 25(OH)D levels at birth are dependent on the maternal concentration of 25(OH)D and the placental ability to transfer 25(OH)D. Cord concentrations of the three major vitamin D metabolites are consistently lower than those measured in maternal serum. UCP 25(OH)D and 24,25-dihydroxyvitamin D concentrations correlate significantly with those found in the maternal circulation, implying that these two secosteroids pass easily across the placental barrier and that the vitamin D pool of the foetus depends entirely on maternal levels (42).

Vitamin D levels are decreased in obesity, possibly because the fat-soluble vitamin is stored in the fat tissue. Obese pregnant women may need larger amounts of vitamin D supplementation to provide their neonates with sufficient levels of vitamin D (21). In this study, we could not confirm a lower 25(OH)D level in the women with the higher BMI, nor was there any significant linear association between maternal BMI (weeks 14–16) and maternal 25(OH)D. Moreover, we could not demonstrate a seasonal variation for maternal BMI. These findings are consistent with a recent study (44) demonstrating similar levels of 25(OH)D when measured at 36–38 weeks of pregnancy, irrespective of BMI. Studies in non-pregnant populations have shown a relationship between vitamin D status and body fat (19, 20). The weakly indicated non-linear association between maternal BMI and 25(OH)D, that is, potentially increased plasma 25(OH)D levels in pregnancy with low BMI, remains to be elucidated, although non-significant in our sample. To some extent,

an inverse relationship between vitamin D and adipocytes may be attributed to the sequestering of vitamin D in fat stores. In our study sample, low BMI is most likely because of lifestyle factors, such as high education, eating regime or more physical activity.

In our study, we found no significant association between maternal BMI (weeks 14–16) and UCP 25(OH)D levels in contrast to a previous study (44), where maternal obesity was associated with UCP 25(OH)D levels. This study included normal-weight and obese mothers, and both groups had similar maternal 25(OH)D levels irrespective of BMI category. Maternal obesity was presented as a category, and the study showed that UCP 25(OH)D levels were significantly different in infants born to normal-weight compared with obese mothers. Thus, infants born to obese mothers had lower levels of 25(OH)D compared with infants born to lean mothers. Our cohort consists predominantly of normal-weight pregnant women (BMI 26.6, weeks 30–32), as only 7% were categorised as obese (BMI > 30 kg/m²). The small amount of obese may explain the inconsistencies between the studies.

As described previously (32), the mean 25(OH)D concentration in UCP was significantly lower than in maternal circulation. The only source of vitamin D in the neonates is through maternal placental transfer of the nutrient hormone, 25(OH)D. The results indicate that different BMIs in pregnant women result in varying efficiency of the transfer of 25(OH)D to the neonates, consistent with the theory of reduced bioavailability of vitamin D in obesity (21). The UCP 25(OH)D differences between neonates born to obese and normal-weight women are consistent with a previous paper (45). This study explored the association between prepregnancy BMI and maternal and infant vitamin D status and found significant differences between prepregnancy obese vs lean mothers. The overweight and obese women when compared with lean women were more likely to have vitamin D deficiency and to deliver newborns with vitamin D deficiency. Our results without significant association between maternal BMI and UCP 25(OH)D levels can be explained by a more normal-weight distribution.

The relationship among maternal vitamin D status, glucose metabolism and foetal growth is likely to be complex. In our study, maternal BMI and metabolic parameters such as glucose, insulin or UCP C-peptide were not related with maternal or neonatal 25(OH)D levels. The concentration of 25(OH)D has been shown to have a positive relationship with insulin sensitivity and a negative effect on β -cell function (25). However, to our knowledge no report of these associations has been

shown in the same study. Separate reports have shown the association of decreased vitamin D with insulin resistance (46) and β -cell dysfunction (47). In our study, UCP C-peptide, which is secreted in equimolar concentrations with insulin, was used as an index of foetal β -cell function. Our data, when compared with previously published data, indicate that the effect of vitamin D on β cells is more complex. Studies that show correlations among vitamin D, glucose and insulin often include subjects with diabetes, impaired glucose tolerance or impaired fasting glucose. Our cohort was healthy, and the women were normotensive and glucose tolerant.

Neonatal adiposity has been found to be a good indicator of excess energy supply to the foetus. We demonstrated a positive association between UCP 25(OH)D and neonatal total FM. In contrast, an inverse relationship between vitamin D levels and adiposity has been demonstrated at later age in children, adolescents and adults (48). The fact that total body percentage FM was significantly associated with UCP 25(OH)D may indicate an association independent of birth weight.

Our finding of a relationship between UCP 25(OH)D and neonatal adiposity is in agreement with a study of DXA-assessed FM in 977 neonates, which concluded that lower maternal vitamin D status may be linked to programmed differences in offspring FM (9). The study (44) also showed that UCP vitamin D was positively associated with neonatal FM. However, in our study, maternal vitamin D levels were not related to neonatal total body FM or percentage fat. The diverging study results might be related to methods; in the latter study, a non-specific paediatric software for neonatal measurements of body composition was used. We measured with a specific infant software programme (34) that increases the sensitivity and specificity of the measured FM and FFM. Thus, our results should give more valid measurements of FM and FFM. Our results are consistent with a recent study, in which maternal vitamin D status in late pregnancy was not related to neonatal DXA-assessed FM (49).

The few randomised controlled trials of vitamin D supplements in pregnant women suggest that supplementation may lead to an increase in birth weight (50); however, these results are controversial (51).

The strengths of the STORK study are the well-characterised cohort and the prospective design. Secondly, DXA-assessed neonatal total body FM was analysed using infant-specific software.

Potential limitations of our report are the nature of UCP samples, which are a mixture of venous and arterial blood, which makes any conclusion about the direct sources of

C-peptide or 25(OH)D at the foetal site difficult. The present work included several research questions, and consequently several analyses of relations between variables within the same study sample. Because of correlations between variables, it is difficult to apply a formal adjustment for multiple comparisons. *P* value would be non-significant after any adjustment, and the only result that might be affected by this is the association between UCP 25(OH)D and neonatal total body FM ($\beta=0.20$, $P=0.02$). However, because of the considerations mentioned earlier, we have presented unadjusted results. Our vitamin D assay could not distinguish between D_2 and D_3 molecular form and measures total 25(OH)D (D_2+D_3) and report a total 25(OH)D concentration. Liquid chromatography–tandem mass spectrometry measures all vitamin D metabolites separately and can be selected as the nominal ‘gold standard method’ for 25(OH)D analysis for the future. Pregnancy is associated with increased levels of vitamin-D-binding protein, as well as 1,25(OH) $_2$ D concentration, so this can have an influence on our results. Furthermore, maternal levels obtained in weeks 30–32 could have changed during the following weeks of pregnancy.

In conclusion

We demonstrated seasonal variation in maternal and neonatal 25(OH)D levels at northern latitudes. The neonatal 25(OH)D levels were consistently lower than maternal levels. UCP 25(OH)D levels were a significant predictor of neonatal adiposity, whereas maternal 25(OH)D levels did not predict neonatal FM. No associations between maternal BMI and metabolic parameters such as glucose, insulin or UCP C-peptide and maternal or neonatal 25(OH)D levels were found.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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