Interrelationships Among Vitamin D Status and Body Composition in Mother-Infant Dyads

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Abstract

Background: Vitamin D status of pregnant women is associated with body composition of the offspring. The objective of this study was to assess whether the association between maternal vitamin D status and neonatal adiposity is modified by maternal adiposity preconception.

Methods: Healthy mothers and their term appropriate weight for gestational age (AGA) infants (n=142; 59% male, Greater Montreal, March 2016-2019) were studied at birth and 1 month postpartum (2-6 weeks). Newborn (24-36 hour) serum was collected to measure total 25-hydroxyvitamin D [25(OH)D] (immunoassay); maternal pre-pregnancy BMI was obtained from the medical record. Anthropometry, body composition (dual-energy X-ray absorptiometry) and serum 25(OH)D were measured at 2-6 weeks postpartum in mothers and infants. Mothers were grouped into 4 categories based on their vitamin D status (sufficient 25(OH)D ≥ 50 nmol/L vs. at risk of being insufficient <50 nmol/L) and pre-pregnancy BMI (<25 vs. ≥ 25 kg/m²): insufficient-recommended weight (I-RW, n=24); insufficient-overweight/obese (I-OW/O, n=21); sufficient-recommended weight (S-RW, n=69); and sufficient-overweight/obese (S-OW/O, n=28). Partial correlation and mixed model ANOVA were used while adjusting for covariates.

Results: At birth, infant serum 25(OH)D mean concentrations were below the cut-point for sufficiency of 50 nmol/L for both maternal pre-pregnancy BMI categories; 47.8 [95%CI: 43.8, 51.9] nmol/L if BMI <25 kg/m² and 38.1 [95%CI: 33.5, 42.7] nmol/L if BMI ≥ 25 kg/m². Infant serum 25(OH)D concentrations at birth (r=0.77; p<0.0001) and 1 month (r=0.59, p<0.0001) were positively correlated with maternal postpartum serum 25(OH)D concentrations. Maternal serum 25(OH)D concentration was inversely associated with maternal percent whole body fat mass (r=-0.26, p=0.002). Infants of mothers in I-OW/O had higher fat mass versus those of mothers in S-OW/O (914.0 [95%CI: 766.4, 1061.6] vs. 780.7 [95%CI: 659.3, 902.0] g; effect size [Hedges’ g: 0.42]; p=0.04) with magnitude of difference of 220.4 g or ~28% difference (adjusting for covariates).

Conclusions: Maternal vitamin D status is positively correlated with neonatal vitamin D. In this study, maternal adiposity and serum 25(OH)D <50 nmol/L are dual exposures for neonatal adiposity. These findings reinforce the importance of vitamin D supplementation early in infancy irrespective of vitamin D stores acquired in utero and maternal weight status.

Introduction

Adverse nutritional exposures in utero and the first year of life impair growth (1) and increases the risk of chronic conditions later in life (2, 3). A burgeoning body of evidence suggests that maternal-fetal transfer of vitamin D is associated with body composition and a lean body mass phenotype in childhood (4). This is a very complex physiological phenomenon with multiple factors to consider, some of which are modifiable. Maternal excess adiposity preconception is a modifiable correlate of low vitamin D status in the neonate (5, 6). This is thought to be due to vitamin D sequestration in maternal adipose tissue as well as volumetric dilution due to both larger tissue mass and blood volume (7) and consequently hindered placental transfer of vitamin D (5). Lower cord 25-hydroxyvitamin D [25(OH)D] concentrations are observed in newborns of mothers with BMI over 30 kg/m² compared to those born to mothers with recommended BMI range (18.5 to 24.9
kg/m$^2$), even though the mothers, on average, had sufficient vitamin D status (25(OH)D $\geq$ 50 nmol/L) in the third trimester (5).

Neonates born to mothers with pre-gravid BMI over 25 kg/m$^2$ have considerably higher fat mass and less fat-free mass compared to those born to mothers within the recommended BMI range, yet no difference in birth weight is observed (8). Whereas, according to a large birth cohort of 4321 mother-infant pairs from Ottawa and Kingston, elevated pre-pregnancy BMI is associated with a higher odds of having a large for gestational age infant compared to women within recommended BMI range (9). In addition, gestational weight gain above the Institute of Medicine (IOM) guidelines (10) is associated with higher neonatal whole body fat mass and percentage body fat, particularly in infants born to mothers with pre-pregnancy BMI over 25 kg/m$^2$ (11).

In Canada, not all women are vitamin D sufficient according to the 50 nmol/L 25(OH)D cut-point with as many as 10-15% <50 nmol/L (12-14). According to a pregnancy cohort study in Quebec City, Canada, 44% of mothers in the first to second trimester had 25(OH)D <50 nmol/L (15). This could be mainly due to living in a northern latitude with minimal ultraviolet beta (UVB) solar radiation from mid-fall to mid-spring that limits dermal synthesis of vitamin D (16). Based on reports from a birth cohort study in Singapore, inadequate maternal 25(OH)D at the end of the second and beginning of the third trimester was associated with higher abdominal adiposity in neonates measured within 2 weeks postpartum (17). In addition, in two pregnancy cohort studies (New Zealand and India) maternal vitamin D status above 50 nmol/L of 25(OH)D at the beginning of second or third trimesters was associated with a lower percentage of body fat in children at 5 to 9.5 years of age, (18, 19). Similarly, in a study from the United Kingdom, mothers in the highest quartile of vitamin D status late in the third trimester had children with greater percent lean mass at 4 years of age compared to those of mothers in the lowest quartile (20). These patterns prevailed even after adjusting for sociodemographic factors as well as maternal BMI before (17, 20), during (18, 19) and after pregnancy (20).

The growing body of evidence suggests that fetal exposure to both low maternal vitamin D status and excess adiposity is associated with body composition of the offspring. However, to date, the majority of studies have relied upon BMI as a proxy measure of adiposity and few studies have reported upon lean and fat mass partitioning in both mother and infant. Given the scarcity of data on the associations between vitamin D status and body composition in mother-infant dyads in early postnatal life, the aim of the current study was to explore the correlates of maternal and neonatal vitamin D status and to assess whether the association between maternal vitamin D status and neonatal adiposity is modified by maternal adiposity preconception.

### Materials And Methods

#### Study design and population

Participants included mother-infant pairs (n=142) who were recruited at the Lakeshore General Hospital, located in greater Montréal, Québec, Canada as part of a trial of vitamin D supplementation in breastfed infants, from March 2016 through to March 2019. The present study includes data collected prior to hospital discharge and data from the baseline visit (0.2-1.5 months postpartum) before entering the trial. The inclusion criteria were healthy, singleton, term born infants of appropriate weight for gestational age (AGA) according to a Canadian growth reference (21), and born to mothers intending to breastfeed for at least 3 months.
Exclusion criteria for the present analysis included infants born to mothers with gestational diabetes or hypertension in the present pregnancy, comorbidities (liver, renal, celiac and Crohn's diseases), medications that are known to impact vitamin D metabolism or limit growth as well as smoking or illicit drugs (22-24).

**Obstetric history, demographic and lifestyle surveys**

Prior to hospital discharge, the obstetric history including maternal age, pre-pregnancy weight, weight at delivery, parity, and mode of delivery were obtained from the medical record. The mother’s pre-pregnancy BMI was then calculated using mothers’ weight prior to pregnancy and measured height at the postpartum visit. Demographic information surveyed included: maternal age as well as self-reported population group (white/all other groups combined including if unknown) which was used according to the proposed guideline from Canadian Institute for Health Information (25), and in doing so any mixed ancestry were categorized within the group denoted as other groups. In addition, the highest level of education completed (elementary/high school, college/vocational school, or university), household income which was surveyed and collapsed into ≥70,000, <70,000 Canadian dollars annually, or not reported according to the median income for Canadian families with children (26). Lifestyle factors were surveyed including maternal multivitamin supplement use (yes/no) and the frequency (every day, almost every day, 2-3/week or less); and exercise habits (yes/no), typical frequency (none, 1-2 h/wk, ≥3 h/wk), and intensity (low, moderate or high), during the 3 months prior to pregnancy and then separately for across pregnancy. Moreover, whether the infants had received vitamin D supplements containing 400 IU/d prior to the follow-up visit was surveyed. Measurements at the postpartum visit included anthropometry, body composition, and blood samples from each infant and their mother.

**Biochemistry Measurements**

Capillary blood was sampled from the neonates between 24 and 36 h of life. After discharge, mothers and their infants participated in a baseline visit held at the Mary Emily Clinical Nutrition Research Unit, McGill University. Capillary blood samples (0.4-0.5 ml) were collected in the non-fasted state from infants by heel lance within 24-36 h of birth and 1 month (± 0.5 month) after birth. One 5 ml venous sample (non-fasted state) was taken from mothers at the baseline visit to assess maternal vitamin D status which does not vary significantly between delivery and 1 mo postpartum (27, 28). Samples were centrifuged (4000 x g for 20 min at 6°C) to obtain serum to assess vitamin D status by measuring total serum 25(OH)D using an automated chemiluminescence immunoassay (Liaison, Diasorin Inc.). The laboratory maintained a certificate of proficiency from the Vitamin D External Quality Assessment Scheme. Vitamin D control samples from the National Institute of Standards and Technology (NIST) quality assurance program were implemented in routine quality control measures. The inter-assay coefficient of variation for NIST972a (levels 1 to 4) was on average <10% and the accuracy 97.4% of certified values. The inter-assay coefficient of variation for an internal laboratory control (62.8 nmol/L) human serum sample was 8.2% across all assays. Deming regression with the following equation was used to standardize the original measured 25(OH)D values for mothers and infants to NIST reference measurements: standardized concentration=0.9634 (Liaison concentration + 3.122 nmol/L). In a subgroup of mothers and infants (n= 83), total 25(OH)D was in agreement (mean difference= -0.75) with liquid chromatography tandem mass spectroscopy (Queen's University, Kingston, Ontario, Canada) using an assay certified by the Vitamin D Standardization-Certification Program. In the current study, the cut-point for sufficiency of vitamin D status in mother-infant dyads was set at ≥50
nmol/L of serum 25(OH)D in accordance with the IOM (29) and since vitamin D status above this cut-point also positively relates to lean mass at 3-4 y (20, 30). The population cut-point of 40 nmol/L of serum 25(OH)D was not used in the present analysis since the assessment was at the level of individual mother-infant dyads.

Skin pigmentation and UVB exposure

Skin tone of the infant was measured at the research facility by taking the average of three measurements at the inner upper arm for constitutive pigmentation (basal color uninfluenced by UV light or other environmental factors) using a spectrophotometer (CM-700d/600d, Konica Minolta, USA). Individual typological angle ($\text{ITA}^\circ$) was calculated with the $L^*$ and $b^*$ values using published equations (31). Infants were classified into two skin tone groups (F I-II; F IV-VI) based on Fitzpatrick scales (32, 33). Based on the strength of solar UVB, vitamin D synthesizing /vitamin D non-synthesizing periods (April 1$\text{st}$-October 31$\text{st}$/November 1$\text{st}$-March 31$\text{st}$) at birth were used as a proxy for potential vitamin D synthesis (16).

Anthropometric Measurements

At the research facility, infant weight was measured to the nearest gram using an electronic scale with a dynamic weighing program (Mettler-Toledo Inc., Switzerland); weight of standardized and pre-weighed light gown and dry diaper was subtracted. Crown heel length (to the nearest 0.1 cm) was measured using an infantometer (O’Learly Length Boards, Ellard Instrumentation Ltd., US) and the average of two measurements was recorded. Head circumference was measured (to the nearest 0.1 cm) using a non-stretchable tape (Perspective Enterprises, US) from the most prominent point of the frontal bone to the most posterior prominent point of the occipital (34). Weight, length, and BMI for age $z$-scores were calculated using WHO growth standards and software (WHO AnthroPlus, Switzerland). Maternal anthropometric measurements, including weight using a balance-beam scale (Detecto; Webb, US) to the nearest 0.1 kg while wearing light clothing and no shoes, and height to nearest 0.1 cm using a wall-mounted stadiometer (Seca Medical Scales and Measuring Systems, US) were used to calculate BMI (kg/m$^2$). Additionally, total weight gain during pregnancy was estimated by subtracting pre-pregnancy weight from the weight obtained at delivery (35) and classified into 3 categories (inadequate, adequate, and excess weight gain) according to pre-pregnancy BMI and IOM guidelines (10).

Body Composition Measurements

Body composition was assessed using a fan-beam dual-energy X-ray absorptiometer (DXA; APEX version 13.3:3, Hologic 4500A Discovery Series, Bedford, MA) that provides a three-compartment model of body composition. Each infant wore a standardized light gown with no metal or plastic components and a dry diaper and was scanned using the infant whole-body software. To minimize the potential for movement artefact, infants were wrapped in a single receiving blanket, and most were scanned while sleeping. Mothers wore standardized light clothing with no metal or plastic components, glasses and jewelry were removed and scans were captured using whole body software. For quality control and quality assurance purposes, a spine phantom (Hologic phantom; No. 14774) was used at each study visit and the coefficient of variation for bone mineral content, bone mineral density, and bone area were <1%; the radiographic uniformity tests were within established limits across the study. Whole body scans provided lean mass (g) excluding bone mineral content,
fat mass (g and %); from these values, lean mass index (LMI, lean body mass (kg)/stature (m)²), and fat mass index (FMI, fat mass (kg)/stature (m)²) were then calculated using standing height for mothers and crown heel length for infants.

**Power analysis and sample size estimation**

This was a convenience sample and thus a retrospective power was calculated based on changes in the primary outcome (fat mass) between mothers with pre-pregnancy ≥ 25 kg/m² and 25(OH)D <50 (n=21) and those with pre-pregnancy ≥25 kg/m² and 25(OH)D ≥50 (n=28). Power was estimated to be 75% using the procedure described by Kononoff (36) for specific data sets accounting for the fixed effects of gestational weight gain, neonatal sex, gestational age, UVB period at birth, actual age of infant at the postnatal visit, and infant length in a mixed model design.

**Statistical analysis**

Data analyses were conducted using Statistical Analysis System (SAS; version 9.4, SAS Institute Inc., Cary, NC). Descriptive characteristics for mothers and infants were expressed as mean (95% confidence interval) or n (%). Serum 25(OH)D is used as a continuum of risk for health outcomes, the target of 50 nmol/L is adequate for bone health in almost all individuals (97.5%) which is aligned with the RDA (29). For ease of readability, individuals with serum 25(OH)D concentration <50 nmol/L, were termed as insufficient to reflect the increasing risk of being insufficient as serum 25(OH)D falls below 50 nmol/L. In order to describe the population, mothers were classified into 1 of 4 groups according to serum 25(OH)D concentrations (insufficient: 25(OH)D <50, sufficient: 25(OH)D ≥50 nmol/L) and pre-pregnancy BMI (recommended BMI: <25, overweight/obese: ≥25 kg/m²). The interaction effect of maternal vitamin D status and pre-pregnancy BMI, 2x2 dichotomous variables, formed 4 groups of interest, I-RW: insufficient-recommended weight (25(OH)D <50 and BMI <25 kg/m²), I-OW/O: insufficient-overweight/obese (25(OH)D <50 and BMI ≥25 kg/m²), S-RW: sufficient-recommended weight (25(OH)D ≥50, BMI: <25 kg/m²), and S-OW/O: sufficient-overweight/obese (25(OH)D ≥50 nmol/L, BMI ≥25 kg/m²). Maternal postpartum FMI was classified into three categories (low to normal: <8.9, excess fat: 9-12.9, and obese: ≥13 kg/m²) (37), as well as gestational weight gain which was classified according to pre-pregnancy BMI and IOM guidelines (10) into three groups (inadequate, adequate, and excess weight gain). For maternal and neonatal characteristics at delivery and 1 mo postpartum, data were compared using a mixed model ANOVA for continuous variables as fixed effects followed by post hoc Tukey’s tests with Tukey-Kramer adjustment for multiple comparisons and Chi-square or Fisher’s exact tests (frequency analysis) for categorical variables. According to the product of the interaction effect of maternal vitamin D status and pre-pregnancy BMI, the 4 groups of interests (I-RW, I-OW/O, S-RW, and S-OW/O), frequency analysis creates 3-way crosstabulation tables. Each of the categorical variables was used in the model to stratify the crosstabulation tables followed by the the interaction term (maternal 25(OH)D*pre-pregnancy BMI) creating two 2-way tables of 25(OH)D and pre-pregnancy BMI, for each level of the categorical variables.

The interrelationship among maternal predictors of infant body composition (pre-pregnancy BMI, maternal 25(OH)D, and the interaction effect of these two variables) and neonatal body composition (lean and fat mass, related percentages, and indices) was tested using a mixed model ANOVA and post hoc tests adjusted for multiple comparisons using Tukey-Kramer adjustment. Fixed effects included in the model were...
gestational weight gain, neonatal sex, gestational age (GA) at birth, UVB period at birth, actual age of infant at the postnatal visit, and infant length. Additional variables that were considered in these analyses were: family income, maternal age, self-reported population group, education, multivitamin supplement use and exercise habits during pregnancy. These variables did not improve the model as judged by Bayesian information criterion (BIC) and were not statistically significant (p>0.05), thus these were removed from the final model using the backward elimination method.

In order to determine factors associated with serum 25(OH)D concentrations in mother-infant dyads, maternal and neonatal serum 25(OH)D were separately modelled against maternal indicators of adiposity such as maternal pre-pregnancy BMI (healthy BMI: <25, overweight/obese: ≥25 kg/m²), postpartum BMI (healthy:18.5-24.9, overweight 25.0-29.9, obese ≥30 kg/m²), postpartum FMI (low to normal: <8.9, excess fat: 9-12.9, obese: ≥13 kg/m²), gestational weight gain (inadequate, adequate, and excess), and other important lifestyle or demographic factors including exercise before and during pregnancy (yes/no) as two separate variables, multivitamin supplement use prior to/during pregnancy (yes/no), UVB period at birth/at delivery (vitamin D synthesizing, non-synthesizing period), parity (primiparous, multiparous), maternal education (elementary/high school, college/vocational school, university), and family income (≥70000 , <70000 Canadian dollars, or not reported). In these regression models, the frequency of multivitamin supplements as well as the frequency and intensity of the exercise prior to/during pregnancy did not improve the models and were thus removed. For the regression analysis of neonatal 25(OH)D, neonatal sex (male, female) and skin tone (F I-III, F IV-VI) were additional fixed factors included in the model. Data was tested using Kolmogorov-Smirnov and Shapiro-Wilk for normality and the residuals were normally distributed; and Levene and Bartlett tests of homogeneity of variances to meet the assumptions of the post-hoc testing. For all tests, a p-value of <0.05 was used to guide interpretation of the results.

Results

Maternal characteristics according to vitamin D status and pre-pregnancy BMI categories

Groups categorized based on maternal 25(OH)D and pre-pregnancy BMI were not different in terms of maternal age, whether mothers were born in Canada or outside Canada, self-reported population group and maternal education level (Table 1.). Although the majority of mothers (92.3%) in all groups took a multivitamin supplement during pregnancy, the proportion was lower in I-RW compared to the rest of the groups. Among all mothers, the median dose of vitamin D taken was 600 (95%CI: 400, 600) IU/d during pregnancy. In terms of family income, higher proportion of mothers in I-OW/O had household income <70,000 Canadian dollars yearly compared to other groups (16.7, 61.9, 26.1, and 21.4%; p=0.001, respectively). At the postpartum visit 98.6% of mothers were breastfeeding, 2 mothers discontinued breastfeeding between recruitment at birth and the baseline visit due to milk insufficiency.

Neonatal characteristics at birth and postpartum according to maternal vitamin D status and pre-pregnancy BMI

Newborns (59% male) were 39.6 (95%CI: 39.5, 39.8) weeks of gestational age at birth and 3.4 (95%CI: 3.3, 3.5) kg. There were no differences among groups in terms of GA, age at the postpartum visit, UVB period at birth,
and anthropometric measurements (Table 2). At birth, on average, infant serum 25(OH)D concentrations were below the cut-point for sufficiency of 50 nmol/L for both maternal pre-pregnancy BMI categories of within or above the recommended range (47.8 [95%CI: 43.8, 51.9] vs. 38.1 [95%CI: 33.5, 42.7]). At birth and 1 month of age, infants of mothers in I-RW and I-OW/O had significantly lower serum 25(OH)D concentrations compared to infants born to mothers in S-RW and S-OW/O. In addition, more infants in I-OW/O were male compared to the rest of the groups whereas a higher proportion of infants in S-RW had skin tone F I-III compared to the rest of the groups.

Overall, the majority (95.0%) of infants received daily vitamin D supplements (containing 400 IU vitamin D) between discharge from hospital and the follow-up visit. Infant mean serum 25(OH)D concentrations significantly increased during the postnatal period (birth: 44.5 [95%CI: 41.3, 47.6] vs. 1 month: 54.7 [95%CI: 51.9, 57.5] nmol/L; p<0.0001). Infants born with serum 25(OH)D ≥50 nmol/L had significantly lower (p<0.0001) mean change in serum 25(OH)D concentration (1.39, 95%CI: -3.0, 5.8 nmol/L) compared to neonates born with 25(OH)D 30-49.9 nmol/L (12.9, 95%CI: 10.2, 15.7 nmol/L), and those deficient <30 nmol/L (17.7, 95%CI: 12.5, 22.8 nmol/L). Infant serum 25(OH)D concentrations at birth (r=0.77; p<0.0001) and at one month (r=0.59, p<0.0001) were positively correlated with maternal serum 25(OH)D concentrations. These correlations remained evident after adjusting for parity, maternal multivitamin supplement use, gestational age at birth, infant sex, UVB period at birth, infant skin tone.

**Maternal vitamin D status, indicators of adiposity and whole-body lean mass**

In mothers, serum 25(OH)D concentrations were positively associated with whole body lean mass (r=0.23, p=0.006) and inversely associated with percent whole body fat mass (r=-0.26, p=0.002). Maternal serum 25(OH)D was on average higher in mothers with pre-pregnancy BMI (Figure 1.A) <25 kg/m² compared to ≥25 kg/m² or postpartum BMI (Figure 1.B) <25 kg/m² or 25-29.9 kg/m² compared to mothers with BMI ≥30 kg/m². However, on average, maternal serum 25(OH)D concentrations were ≥50 nmol/L in all BMI categories (68.3% and 57.1% of mothers with BMI <25 and ≥25 kg/m² had 25(OH)D concentrations ≥50 nmol/L, respectively). Likewise, serum 25(OH)D of mothers with low to normal or excess FMI categories was higher compared to mothers with FMI in the obese range (Figure 1.C). In a mixed model analysis, appropriateness of gestational weight gain based on pre-pregnancy BMI was not related to maternal serum 25(OH)D (Supplementary Table 1.).

Among other covariates included in the mixed model, mothers who self-reported being physically active (indoor and outdoor combined) 3 months prior to conception or during pregnancy (tested as two separate variables) had higher 25(OH)D concentrations postpartum versus mothers who were not active. In this analysis, self-reported population group (white/all other groups) was a prominent factor related to maternal serum 25(OH)D, however other covariates such as parity, multivitamin supplement use, education level and family income were not related (Supplementary Table 1.).

**Neonatal vitamin D status and maternal indicators of adiposity**

The mean serum 25(OH)D was on average higher in infants of mothers with pre-pregnancy BMI (Figure 1.D) <25 kg/m² compared to ≥25 kg/m² or postpartum BMI (Figure 1.E) <25 kg/m² or 25-29.9 kg/m² compared to
mothers with BMI ≥ 30 kg/m². Similarly, serum 25(OH)D concentrations of infants born to mothers in the low to normal category of FMI were higher compared to infants born to mothers in the obese category (Figure 1.F). However, on average, infant serum 25(OH)D concentrations were below the cut-point for sufficiency of 50 nmol/L for all maternal BMI (38.7 and 20.4% of infants of mothers with <25 and ≥25 kg/m² had vitamin D sufficiency) or FMI categories (39.2, 30.8, and 0% of infants of mothers with all FMI categories of low to normal: <8.9, excess fat: 9-12.9, and obese: ≥13 kg/m² had vitamin D sufficiency respectively). The relationship among neonatal vitamin D status and maternal indicators of adiposity and other maternal factors is shown in detail (Supplementary Table 2).

A significant interaction effect of maternal pre-pregnancy BMI and 25(OH)D concentration was observed for neonatal indicators of adiposity, including neonatal fat mass (Table 3.), fat percentage and FMI adjusting for multiple covariates (Supplementary Table 3.). After pairwise comparison tests, infants of mothers in the I-OV/O group with elevated pre-pregnancy BMI (≥25 kg/m²) and vitamin D insufficiency (25(OH)D <50 nmol/L) had significantly higher fat mass (Figure 2.A), fat percentage (Figure 2.B), and FMI (Figure 2.C) compared to infants born to mothers in the S-OV/O group with BMI >25 kg/m² but vitamin D sufficiency (25(OH)D ≥50 nmol/L). The magnitude of difference in whole body fat mass observed between I-OV/O and S-OV/O groups was 220.4 g (95% CI: 56.4, 384.3) representing ~28% difference with effect size of 0.42. In these adjusted mixed models, gestational weight gain was not associated with neonatal fat mass. A significant interaction effect of maternal pre-pregnancy BMI and 25(OH)D concentration was also observed for neonatal lean mass (Table 4 & Figure 2.D) and lean percentage (Figure 2.E) but not on LMI (Figure 2.F). However, after pairwise comparison tests, only infants of mothers in I-OV/O with BMI ≥25 kg/m² before pregnancy and vitamin D insufficiency (25(OH)D <50 nmol/L) had significantly lower lean percentage compared to infants born to mothers in S-OV/O with BMI ≥25 kg/m² but with vitamin D sufficiency 25(OH)D ≥50 nmol/L (Supplementary Table 4.). In the majority of these mixed models, sex effects were noted. Sex differences manifested as higher whole body lean mass observed in male infants as well as when adjusted for weight compared to females (Supplementary Figure 1.A, B & C); the opposite was observed in terms of adiposity indicators with female infants presenting higher values (Supplementary Figure 1.C, D & E), these observations remained evident after adjustment for covariates.

Discussion

According to our data, maternal adiposity appears to be an effect modifier in the association between maternal vitamin D status and offspring body composition. Healthy term born infants dually exposed to insuffcient maternal vitamin D status and elevated pre-pregnancy BMI had higher whole body fat mass (Δ 220.4 g, ~28% difference) in the early postnatal period compared to infants of mothers with elevated pre-pregnancy BMI yet vitamin D sufficient. Although the majority of mothers took multivitamin supplements containing vitamin D during pregnancy, 64.3% of infants of mothers with elevated pre-pregnancy BMI had serum 25(OH)D <50 nmol/L; and if maternal serum 25(OH)D was < 50 nmol/L all infants had 25(OH)D <50 nmol/L and the majority (71.4%) were vitamin D deficient. This reinforces the importance of building sufficient vitamin D stores in early infancy through vitamin D supplementation.
In the northern hemisphere, most national guidelines for a healthy pregnancy (38, 39) do not have a specific recommendation on vitamin D supplementation for pregnant women with an elevated BMI; nor their newborn. Our observations reinforce the importance of encouraging overweight/obese women to seek nutrition counselling prior to conception (40). This implies that pregnant women who commence pregnancy with $\text{BMI} \geq 25 \text{ kg/m}^2$ should be advised to initiate the consultation and to begin using a multivitamin supplement as soon as possible to help establish vitamin D stores in the developing fetus and ultimately in the newborn (5). This should be followed by early neonatal vitamin D supplementation (29).

Our results are in line with other reports linking maternal vitamin D status to adiposity in the neonatal period (17) or in childhood (18, 19) as well as studies on the association of maternal overweight/obese pre-gravid BMI with neonatal adiposity (8, 41) and underscores the influence of maternal factors in fetal programming of body composition. This study adds that maternal 25(OH)D concentration is an influential factor in neonatal vitamin D status at birth and within neonatal period. The research design enabled us to better understand the significant relationship between maternal excess adiposity preconception and continued through postpartum with neonatal adiposity and vitamin D stores.

We observed a positive correlation between maternal and neonatal vitamin D status at birth, which decreases 23.4% through the neonatal period in line with other reports (42, 43). This is attributed to the fact that the developing fetus is fully reliant on maternal-fetal transfer of vitamin D (44), however, shortly after birth the majority of infants commenced routine supplements containing 400 IU/d vitamin D and were breastfed. Overall, the majority of infants at birth (67.6%) had serum 25(OH)D <50 nmol/L, whereas at the postpartum visit, this declined to 40%. Even though 4~6 week is not long enough to see a plateau in the response to vitamin D supplementation (45-47), the increments in vitamin D status of infants born with 25(OH)D <50 nmol/L were in agreement with another study (48). The response of infants to vitamin D supplementation inversely related to basal status resulting in a greater increment in serum 25(OH)D in neonates with vitamin D deficiency. Thus, 400 IU/d of vitamin D is suitable for infants born with vitamin D stores ranging from vitamin D deficiency to sufficient status.

Vitamin D status of mothers could be a proxy for other healthy behaviors, reflect quality of diet, or time spent outside. Interestingly, in our study exercise in pregnancy was an influential covariate associated with 14.8 nmol/L higher 25(OH)D concentration in mothers of all BMI ranges. This is of relevance since exercise tends to take place outdoors (49) with a positive association between exercise and time spent outside (50, 51). Outdoor activity and being more exposed to sunlight promote vitamin D synthesis as well as vitamin D mobilization from adipose tissue (52) and consequently support achievement and maintenance of higher vitamin D status.

The genes that regulate fat distribution, adiposity (53), and skeletal muscle phenotypes (54) are responsive to environmental and lifestyle exposures. Achieving appropriate body weight and being physically active preconception and during pregnancy may determine body composition in the offspring (55). Regular physical activity shifts the increased energy demands to maternal muscle mass and away from the adipocytes of the fetus leading to proportionately increased lean mass and decreased adipose tissue (55). This pattern is consistent with the observations in our study and is likely due to mobilization of vitamin D from fat tissue into
circulation (52). Therefore, it can be inferred that genetic, behavioral and environmental interactions contribute to variations in fat-muscle partitioning in early development.

In this study, we did not observe an association between vitamin D status and infant lean body mass, likely due to all infants being born term and AGA. In comparison to infants born AGA, body composition partitioning differs among infants at the extremes of birth weight such as those born small or large for gestational age (56). Consistently, the response to vitamin D supplementation can be different. Infants born large for gestational age tend to have vitamin D insufficiency at birth (57, 58), possibly due to entrapment of vitamin D in fat tissue compared to AGA. In addition, by including only non-smoking mothers we eliminated smoking as a confounder as neonates born to smoking mothers have lower vitamin D status (59) as well as lower lean body mass (60, 61). Lack of association between vitamin D and infant lean body mass might also reflect the rapid growth spurt in the first month of life. Furthermore, sex differences in infant body composition emerged early postnatally. This effect was independent of the UVB period at birth, GA, maternal supplement use, and maternal and neonatal vitamin D status. Male infants have more muscle mass due to the anabolic effect of testosterone which temporarily surges postnatally within 1~3 months postpartum (62, 63). In contrast, in our study and others, females have greater stores of fat mass irrespective of vitamin D status (64).

Limitations and further research

There are some limitations in our study. Pre-pregnancy weight was obtained from medical records, thus some of the values for pre-pregnancy BMI could be self-reported. We relied upon pre-pregnancy BMI as a proxy for overweight and obesity which may have underestimated or misclassified adiposity (65), however, the three-compartmental model of DXA confirmed excess adipose tissue. Infants included in this analysis were all AGA, body composition partitioning is likely to be different from infants born small or large for gestational age, which requires further investigation. Given the design of the study, maternal 25(OH)D concentrations were only measured at the postpartum visit and not at delivery. This may not be a major limitation as maternal 25(OH)D concentrations are not significantly different between 36 weeks of GA and 1 month postpartum (27, 28). Additionally, our analysis might have been statistically underpowered to detect relationships such as the association between gestational weight gain and maternal or neonatal vitamin D status, future larger studies are needed. Lastly, whether the body composition pattern extends later into childhood requires a longitudinal study.

Conclusion

In otherwise healthy mother-infant dyads, maternal overweight/obesity and serum 25(OH)D <50 nmol/L are dual exposures that associate with neonatal serum 25(OH)D <50 nmol/L as well as higher adiposity. More concerning, 71.4% of neonates in this cohort were vitamin D deficient. These results reinforce the importance of postnatal vitamin D supplementation in infants born to mothers with BMI $\geq 25$ kg/m$^2$. In the event of low maternal-fetal transfer of vitamin D, postnatal supplementation with 400 IU/d of vitamin D readily builds vitamin D stores and in doing so may limit the impact of fetal exposures.

Abbreviations
25(OH)D: 25-hydroxyvitamin D; AGA: appropriate for gestational age; BMI: body mass index; UVB: ultraviolet beta.

Declarations

Ethics approval and consent to participate

Prior to collection of data and blood samples, parents provided written informed consent. The study was approved by the St. Mary's Hospital Research Ethics Committee (Montréal, Québec, Canada) that oversees research at the Lakeshore General Hospital. Both ethics approval (REB# 15-34) and trial registration with clinicaltrials.gov (NCT02563015) were completed prior to the beginning of the recruitment. This study was also reviewed and approved by Health Canada Research Ethics Board (REB 2019-033H) and Privacy Management Division (HC-PR-2019-000024). All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable. There are no details, images, or videos related to an individual participant.

Availability of data and materials

The dataset used and/or analysed during the current study will not be made publicly available because permission to share data was not requested at the time of obtaining participant consent but are available from the corresponding author on reasonable request.

Competing interests

The authors state no conflicts of interest.

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Authors’ contributions

HAW, SW, DM, FR, and GJ designed the study. HAW and CAV supervised the study. HAW, CAV, MR, NG, OFS conducted the study; HAW, CAV, MR, NG, OFS collected the data; MR, NG and OFS performed the laboratory analyses. MR performed statistical analysis; MR wrote the final manuscript with the intellectual aid and comments of HAW. All authors (MR, NG, CAV, OFS, SW, DM, FR, GJ, SK, and HAW) have read and approved the manuscript.

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Tables
**Table 1.** Maternal characteristics according to maternal pre-pregnancy BMI and postpartum vitamin D status

| Characteristic¹ | All | 25(OH)D < 50 nmol/L | 25(OH)D ≥ 50 nmol/L | P-value² | 25(OH)D BMI <25 | BMI ≥25 | 25(OH)D* BMI <25 | BMI ≥25 |
|----------------|-----|---------------------|---------------------|----------|-----------------|---------|-----------------|---------|
|                |     | (n=24)              | (n=21)              |          | (n=69)          | (n=28)  | (n=69)          | (n=28)  |
| Age at delivery, y | 32.2 (31.4, 32.9) | 31.0 (28.9, 33.0) | 32.7 (30.1, 35.2) | 31.9 (31.0, 32.8) | 33.5 (31.7, 35.2) | 0.29 | 0.05 | 0.93 |
| Parity, n (%) | | | | | | | | |
| Primiparous (1) | 44 (31.0) | 12 (50.0) | 6 (28.6) | 21 (30.4) | 5 (17.9) | 0.23 | 0.0009 | 0.28 |
| Multiparous (≥2) | 98 (69.0) | 12 (50.0) | 15 (71.4) | 48 (69.6) | 23 (82.1) | <0.0001 | 0.03 | 0.04 |
| Pre-pregnancy BMI, kg/m² | 24.6 (23.8, 25.3) | 22.4 (21.6, 23.2) | 30.0 (28.1, 31.8) | 21.8 (21.4, 22.2) | 29.2 (27.3, 31.0) | 0.22 | <0.0001 | 0.85 |
| Gestational weight gain, kg | 13.6 (12.6, 14.6) | 13.3 (10.2, 16.5) | 12.0 (9.3, 14.6) | 14.8 (13.5, 16.1) | 12.0 (9.7, 14.4) | 0.49 | 0.07 | 0.53 |
| Serum 25(OH)D, nmol/L | 67.4 (63.1, 71.7) | 40.0 (36.2, 43.9) | 40.4 (36.8, 43.9) | 83.3 (78.1, 88.6) | 71.8 (64.8, 78.8) | <0.0001 | 0.10 | 0.08 |
| Maternal birthplace, n (%) | | | | | | | | |
| Canada | 90 (63.4) | 13 (54.2) | 8 (38.1) | 50 (72.5) | 19 (67.9) | <0.0001 | 0.0001 | 0.35 |
| Elsewhere | 52 (36.6) | 11 (45.8) | 13 (61.9) | 19 (27.5) | 9 (32.1) | 0.58 | 0.27 | 0.11 |
| Self-reported population group, n (%) | | | | | | | | |
| White | 79 (55.6) | 8 (37.5) | 7 (33.3) | 47 (71.0) | 17 (60.7) | <0.0001 | 0.0002 | 0.16 |
| All other groups³ | 63 (44.4) | 16 (62.5) | 14 (66.7) | 22 (29.0) | 11 (39.3) | 0.80 | 0.20 | 0.32 |
| Supplement use⁴, n (%) | | | | | | | | |
| Yes | 131 (92.3) | 20 (83.3) | 20 (95.2) | 65 (94.2) | 26 (92.9) | <0.0001 | 0.0007 | 0.02 |
Table 1. Maternal characteristics according to maternal pre-pregnancy BMI and postpartum vitamin D status

| No | Vitamin D dosage, IU/d | Education, n (%) | Family yearly income, n (%) |
|----|------------------------|------------------|-----------------------------|
|    |                        |                  |                             |
| No |                        |                  |                             |
|    | 11 (7.7)               |                  |                             |
|    | 4 (16.7)               |                  |                             |
|    | 1 (4.8)                |                  |                             |
|    | 4 (5.8)                |                  |                             |
|    | 2 (7.1)                |                  |                             |
|    | 0.76                   |                  |                             |
|    | 0.13                   |                  |                             |
|    | 0.62                   |                  |                             |
|    | 501.5 (478.6, 524.3)   |                  |                             |
|    | 470 (402.8, 537.2)     |                  |                             |
|    | 513.2 (458.8, 567.5)   |                  |                             |
|    | 504.6 (472, 537.1)     |                  |                             |
|    | 510.9 (453.7, 568.1)   |                  |                             |
|    | 0.53                   |                  |                             |
|    | 0.34                   |                  |                             |
|    | 0.48                   |                  |                             |
|    | 0.41                   |                  |                             |
|    | 0.41                   |                  |                             |
|    | 0.28                   |                  |                             |
|    | 13 (9.2)               |                  |                             |
|    | 4 (17.8)               |                  |                             |
|    | 4 (19.0)               |                  |                             |
|    | 4 (5.8)                |                  |                             |
|    | 1 (3.6)                |                  |                             |
|    | 0.001                  |                  |                             |
|    | 0.07                   |                  |                             |
|    | 0.33                   |                  |                             |
|    | 30 (21.1)              |                  |                             |
|    | 5 (13.3)               |                  |                             |
|    | 1 (4.8)                |                  |                             |
|    | 15 (21.7)              |                  |                             |
|    | 9 (32.1)               |                  |                             |
|    | 0.0002                 |                  |                             |
|    | 0.002                  |                  |                             |
|    | 0.01                   |                  |                             |
|    | 99 (69.7)              |                  |                             |
|    | 15 (68.9)              |                  |                             |
|    | 16 (76.2)              |                  |                             |
|    | 50 (72.5)              |                  |                             |
|    | 18 (64.3)              |                  |                             |
|    | 0.0001                 |                  |                             |
|    | 0.0001                 |                  |                             |
|    | 0.67                   |                  |                             |
|    | ≥ $70 000 CAD          |                  |                             |
|    | 80 (56.3)              |                  |                             |
|    | 15 (62.5)              |                  |                             |
|    | 5 (23.8)               |                  |                             |
|    | 42 (60.9)              |                  |                             |
|    | 18 (64.3)              |                  |                             |
|    | <0.0001                |                  |                             |
|    | 0.0001                 |                  |                             |
|    | 0.67                   |                  |                             |
|    | < $70 000 CAD          |                  |                             |
|    | 41 (28.9)              |                  |                             |
|    | 4 (16.7)               |                  |                             |
|    | 13 (61.9)              |                  |                             |
|    | 18 (26.1)              |                  |                             |
|    | 6 (21.4)               |                  |                             |
|    | 0.27                   |                  |                             |
|    | 0.64                   |                  |                             |
|    | 0.001                  |                  |                             |
|    | Not reported           |                  |                             |
|    | 21 (14.8)              |                  |                             |
|    | 5 (20.8)               |                  |                             |
|    | 3 (14.3)               |                  |                             |
|    | 9 (13.0)               |                  |                             |
|    | 4 (14.3)               |                  |                             |
|    | 0.28                   |                  |                             |
|    | 0.13                   |                  |                             |
|    | 1.00                   |                  |                             |

1 Data are mean (lower and upper 95% confidence limits) or n (%); 2 Data were compared using a mixed model ANOVA for continuous variables followed by post hoc Tukey’s tests with Tukey-Kramer adjustment for multiple comparisons and Chi-square or Fisher exact tests for categorical variables (using frequency procedure to create 3-way crosstabulation tables; categorical variables were used in the model to stratify the crosstabulation tables followed by the last two variables: maternal 25(OH)D*pre-pregnancy BMI, creating two 2-way tables of 25(OH)D and pre-pregnancy BMI, for each level of the categorical variables); 3 other groups included: South Asian, Chinese, Black, Filipino, Latin American, Arab, Southeast Asian, West Asian, Korean, Japanese, or other; 4 use of prenatal supplement containing vitamin D during pregnancy; 5 the median income (in Canadian dollars) for Canadian families with children. Abbreviations: 25(OH)D: 25-hydroxyvitamin D; BMI: body mass index; CAD: Canadian dollar.
Table 2. Neonatal characteristics according to maternal pre-pregnancy BMI and postpartum vitamin D status

| Characteristic | All | \(25(OH)D < 50\) nmol/L | \(25(OH)D \geq 50\) nmol/L | P-value<sup>2</sup> | \(25(OH)D*BMIm</p>
|----------------|-----|------------------------|-----------------------------|-------------------|-------------------|
|                | \(n=24\) | \(n=21\) | \(n=69\) | \(n=28\) | 25(OH)D | BMI | 25(OH)D*BMI |
| Birth          |     |                        |                             |       |      |                 |        |
| Gestational age, (wk) | 39.64 | 39.9 | 39.7 | 39.7 | 39.3 | 0.13 | 0.13 | 0.90 |
| (39.5, 39.8) | (39.4, 40.4) | (39.2, 40.1) | (39.4, 39.9) | (38.9, 39.8) |       |      |                 |        |
| Sex, n (%)    |     |                        |                             |       |      |                 |        |
| Male          | 83 (58.5) | 13 (54.2) | 14 (66.7) | 40 (58.0) | 16 (57.1) | 0.002 | 0.01 | 0.04 |
| Female        | 59 (41.5) | 11 (45.8) | 7 (33.3) | 29 (42.0) | 12 (42.9) | 0.003 | 0.01 | 0.47 |
| UVB period<sup>3</sup>, n (%) |     |                        |                             |       |      |                 |        |
| Synthesizing  | 83 (58.5) | 14 (58.3) | 9 (42.9) | 43 (62.3) | 17 (60.7) | <0.0001 | 0.001 | 0.34 |
| Non-synthesizing | 59 (41.5) | 10 (41.7) | 12 (57.1) | 26 (37.7) | 11 (39.3) | 0.05 | 0.09 | 0.06 |
| Weight (kg)   | 3.4 | 3.4 | 3.5 | 3.3 | 3.4 | 0.12 | 0.44 | 0.69 |
| (3.3, 3.5) | (3.3, 3.6) | (3.3, 3.7) | (3.3, 3.4) | (3.2, 3.6) |       |      |                 |        |
| Weight z score | 0.2 | 0.3 | 0.3 | 0.1 | 0.2 | 0.20 | 0.68 | 0.54 |
| (0.0, 0.3) | (-0.1, 0.7) | (-0.1, 0.7) | (-0.1, 0.2) | (-0.1, 0.5) |       |      |                 |        |
| Serum 25(OH)D, nmol/L | 44.5 | 29.3 | 26.6 | 54.3 | 46.7 | <0.0001 | 0.07 | 0.38 |
| (41.3, 47.6) | (24.8, 33.7) | (23.0, 30.2) | (50.0, 58.6) | (40.8, 52.5) |       |      |                 |        |
| Postnatal visit |     |                        |                             |       |      |                 |        |
| Age, mo       | 0.7 | 0.7 | 0.6 | 0.7 | 0.7 | 0.37 | 0.25 | 0.51 |
| (0.6, 0.8) | (0.6, 0.8) | (0.5, 0.8) | (0.7, 0.8) | (0.6, 0.8) |       |      |                 |        |
| Weight, kg    | 4.0 | 4.1 | 3.9 | 3.9 | 4.0 | 0.61 | 0.68 | 0.29 |
| (3.9, 4.1) | (3.8, 4.3) | (3.7, 4.2) | (3.8, 4.1) | (3.8, 4.2) |       |      |                 |        |
| Weight z score | -0.1 | 0.1 | -0.01 | -0.2 | -0.02 | 0.23 | 0.86 | 0.28 |
|---------------|------|-----|--------|------|-------|------|------|------|
|               | (-0.2, 0.0) | (-2.0, 0.5) | (-0.4, 0.4) | (-0.4, -0.03) | (-0.4, 0.3) |       |      |      |
| Length, cm    | 53.1 | 53.8 | 53.0   | 52.9 | 53.0  | 0.22 | 0.34 | 0.19 |
|               | (52.7, 53.4) | (52.9, 54.8) | (52.2, 53.8) | (52.4, 53.3) | (52.1, 53.9) |       |      |      |
| Length z score| -0.01 | 0.4  | 0.1    | -0.2 | -0.03 | 0.06 | 0.57 | 0.20 |
|               | (-0.2, 0.1) | (-0.1, 0.8) | (-0.3, 0.4) | (-0.4, 0.1) | (-0.4, 0.3) |       |      |      |
| Head circumference, cm | 36.4 | 36.5 | 36.5   | 36.3 | 36.7  | 0.95 | 0.40 | 0.43 |
|               | (36.2, 36.6) | (35.9, 37.0) | (35.9, 37.0) | (36.1, 36.6) | (36.2, 37.2) |       |      |      |
| Head circumference z score | 0.1 | 0.2  | 0.3    | -0.03 | 0.3   | 0.71 | 0.13 | 0.43 |
|               | (-0.02, 0.3) | (-0.3, 0.6) | (-0.1, 0.7) | (-0.2, 0.2) | (0.01, 0.7) |       |      |      |
| Serum 25(OH)D, nmol/L | 54.7 | 45.1 | 42.3   | 61.9 | 54.5  | <0.0001 | 0.08 | 0.42 |
|               | (51.9, 57.5) | (40.3, 49.9) | (34.5, 50.1) | (57.9, 65.9) | (49.3, 59.7) |       |      |      |
| Skin tone4, n (%) |       |      |       |      |      |      |      |      |
| F I-III       | 110  | 17  | 14     | 59   | 20   | <0.0001 | <0.0001 | 0.04 |
|               | (77.5) | (70.8) | (66.7) | (85.5) | (71.4) |       |      |      |
| F IV-VI       | 32   | 7   | 7      | 10   | 8    | 0.48  | 0.72 | 0.75 |
|               | (22.5) | (29.2) | (33.3) | (14.5) | (28.6) |       |      |      |

1Data are mean (lower and upper 95% confidence limits) or n (%); 2Data were compared using a mixed model ANOVA for continuous variables followed by post hoc Tukey’s tests with Tukey-Kramer adjustment for multiple comparisons; and Chi-square or Fisher exact tests for categorical variables (using frequency procedure to create 3-way crosstabulation tables; categorical variables were used in the model to stratify the crosstabulation tables followed by the last two variables: maternal 25(OH)D*pre-pregnancy BMI, creating two 2-way tables of 25(OH)D and pre-pregnancy BMI, for each level of the categorical variables); 3vitamin D synthesizing: April 1st-October 31st or vitamin D non-synthesizing: November 1st-March 31st; 4classified based on Fitzpatrick descriptions: F I-III (light) and F IV-VI (dark) (32, 33). Abbreviations: 25(OH)D: 25-hydroxyvitamin D; F: Fitzpatrick; UVB: ultraviolet beta.
Table 3. Correlates of neonatal body fat mass

| Mixed model ANOVA | Regression coefficients | 95% Confidence intervals | P-value | Adjusted P-value |
|-------------------|-------------------------|--------------------------|---------|-----------------|
| Neonatal whole-body fat mass g (\(R^2 0.38, R^2_{adj} 0.37\))<sup>1</sup> | | | | |
| Sex of infant (Ref: female) | -119.85 | -219.24, -20.45 | 0.02 | |
| Gestational age at birth, wk | -15.38 | -67.13, 36.38 | 0.56 | |
| Infant age, mo | 435.35 | 187.62, 683.09 | 0.001 | |
| Infant length, cm | 58.80 | 29.99, 87.57 | <0.0001 | |
| UVB period at birth<sup>3</sup> (Ref: non-synthesizing period) | 105.28 | 8.94, 201.63 | 0.03 | |
| Gestational weight gain, kg | 6.57 | -1.61, 14.76 | 0.11 | |
| Maternal pre-pregnancy BMI<sup>4</sup> (Ref: <25 kg/m\(^2\)) | -45.26 | -173.54, 83.02 | 0.12 | |
| Maternal 25(OH)D<sup>5</sup> (Ref: ≥ 50 nmol/L) | -40.57 | -176.04, 94.91 | 0.09 | |
| BMI*25(OH)D interaction (pairwise comparisons) | | | 0.02 | |
| BMI≥25, 25(OH)D<50 vs BMI≥25, 25(OH)D≥50 | 220.40 | 56.19, 384.60 | 0.009 | 0.04 |
| BMI≥25, 25(OH)D<50 vs BMI<25, 25(OH)D<50 | 215.70 | 44.73, 386.68 | 0.01 | 0.07 |
| BMI≥25, 25(OH)D<50 vs BMI<25, 25(OH)D≥50 | 175.14 | 28.38, 321.89 | 0.02 | 0.09 |
| BMI≥25, 25(OH)D≥50 vs BMI<25, 25(OH)D<50 | -4.70 | -161.54, 152.15 | 0.95 | 0.99 |
| BMI≥25, 25(OH)D≥50 vs BMI<25, 25(OH)D≥50 | -45.26 | -173.54, 83.02 | 0.49 | 0.90 |
| BMI<25, 25(OH)D<50 vs BMI<25, 25(OH)D≥50 | -40.57 | -176.04, 94.91 | 0.55 | 0.93 |

<sup>1</sup>Data were compared using a mixed model ANOVA for continuous variables followed by post hoc Tukey’s tests with Tukey-Kramer adjustment for multiple comparisons; <sup>2</sup>Sex of infant (male vs. female); <sup>3</sup>UVB period (April 1<sup>st</sup>-October 31<sup>st</sup> or November 1<sup>st</sup>-March 31<sup>st</sup>); <sup>4</sup>Maternal pre-pregnancy BMI (BMI <25 kg/m\(^2\) or BMI ≥25 kg/m\(^2\)); <sup>5</sup>Maternal serum 25(OH)D (≥ or <50 nmol/L). Abbreviations: 25(OH)D: 25-hydroxyvitamin D; BMI: body mass index; UVB: ultraviolet beta.
Table 4. Correlates of neonatal body lean mass

| Mixed model ANOVA                                      | Regression coefficients | 95% Confidence intervals | P-value | Adjusted P-value |
|--------------------------------------------------------|-------------------------|---------------------------|---------|------------------|
| **Neonatal whole-body lean mass g (R² 0.53, R²_adj 0.52)¹** |                         |                           |         |                  |
| Sex² of infant (Ref: female)                           | 174.52                  | 73.41, 275.64             | 0.0009  |                  |
| Gestational age at birth, wk                           | 32.29                   | -20.36, 84.94             | 0.23    |                  |
| Infant age, mo                                         | 294.98                  | 42.95, 547.00             | **0.02**|                  |
| Infant length, cm                                      | 102.26                  | 72.97, 131.55             | <0.0001 |                  |
| UVB period³ (Ref: non-synthesizing period)             | -68.86                  | -166.88, 29.16            | 0.17    |                  |
| Gestational weight gain, kg                            | -4.56                   | -12.89, 3.76              | 0.28    |                  |
| Maternal pre-pregnancy BMI⁴ (Ref: <25 kg/m²)           | 124.67                  | -5.83, 255.17             | 0.06    |                  |
| Maternal 25(OH)D⁵ (Ref: ≥ 50 nmol/L)                   | 55.67                   | -82.15, 193.49            | 0.43    |                  |
| Maternal pre-pregnancy BMI*25(OH)D                     |                         |                           | **0.04**|                  |
| BMI≥25, 25(OH)D<50 vs BMI≥25, 25(OH)D≥50              | -164.58                 | -384.34, 55.19            | 0.05    | 0.21             |
| BMI≥25, 25(OH)D<50 vs BMI<25, 25(OH)D<50              | -95.57                  | -324.39, 133.24           | 0.28    | 0.70             |
| BMI≥25, 25(OH)D<50 vs BMI<25, 25(OH)D≥50              | -39.91                  | -236.31, 156.50           | 0.60    | 0.95             |
| BMI≥25, 25(OH)D≥50 vs BMI<25, 25(OH)D<50              | 69.00                   | -140.90, 278.91           | 0.39    | 0.83             |
| BMI≥25, 25(OH)D≥50 vs BMI<25, 25(OH)D≥50              | 124.67                  | -47.01, 296.35            | 0.06    | 0.24             |
| BMI<25, 25(OH)D<50 vs BMI<25, 25(OH)D≥50              | 55.67                   | -125.64, 236.97           | 0.43    | 0.85             |

¹Mixed model ANOVA; ²Sex of infant (male vs. female); ³UVB period (April 1st-October 31st or November 1st-March 31st); ⁴Maternal pre-pregnancy BMI (BMI<25 or BMI>25 kg/m²); ⁵maternal serum 25(OH)D (≥ or <50 nmol/L. Abbreviations: 25(OH)D: 25-hydroxyvitamin D; BMI: body mass index; UVB: ultraviolet beta.

Figures
Figure 1

Serum 25(OH)D concentrations of A) mothers according to pre-pregnancy BMI categories (healthy: BMI <25 or overweight/obese: BMI ≥25 kg/m2), B) Serum 25(OH)D concentrations of mothers according to postpartum BMI categories (healthy: BMI <24.9, overweight: BMI 25-29.9, obese: BMI ≥30 kg/m2, C) mothers according to their postpartum fat mass index (FMI) categories (low to normal: 1.1-8.9, excess fat: 9-12.9, and obese: ≥13 kg/m2). Serum 25(OH)D concentrations of infants at birth according to mothers, D) pre-pregnancy BMI categories, E) postnatal BMI categories and, F) FMI categories. Data were compared using a mixed model ANOVA, maternal pre-pregnancy, postpartum BMI and FMI as categorical fixed effects followed by post hoc Tukey’s tests with Tukey-Kramer adjustment for multiple comparisons. Data are mean ± SD.
The interaction effect of maternal pre-pregnancy BMI and maternal 25(OH)D status with neonatal: A) whole-body fat mass, B) whole-body fat percentage, C) fat mass index (FMI), D) whole-body lean mass, E) whole-body lean percentage, and F) lean mass index (LMI). Data were compared using a mixed model ANOVA, maternal pre-pregnancy BMI and 25(OH)D interaction as a categorical fixed effect followed by post hoc Tukey's tests with Tukey-Kramer adjustment for multiple comparisons. Data are mean ± SD.

Supplementary Files

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