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Original Research Article

Maternal vitamin D status, fetal growth patterns, and adverse pregnancy outcomes in a multisite prospective pregnancy cohort

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A B S T R A C T

Background: Few studies have examined maternal vitamin D status and fetal growth patterns across gestation. Furthermore, time points in pregnancy at which maternal vitamin D status is most critical for optimal fetal growth and pregnancy outcomes are uncertain.

Objectives: Our objective was to examine whether first and second trimester maternal vitamin D status are associated with fetal growth patterns and pregnancy outcomes.

Methods: We conducted a secondary analysis using data and samples from a multisite prospective cohort study of nulliparous pregnant females in the United States. We measured serum 25-hydroxyvitamin D (25(OH)D) for 351 participants at 6–13 and 16–21 weeks of gestation. Fetal growth was measured by ultrasound at 16–21 and 22–29 weeks of gestation, and neonatal anthropometric measures at birth. We constructed fetal growth curves using length, weight, and head circumference *z*-scores, and calculated risk of preterm birth (<37 wk) and small for gestational age (SGA). We examined outcomes across 25(OH)D concentrations assessed continuously, using Institute of Medicine (IOM) cutoffs (<50 compared with ≥50 nmol/L), and using exploratory cutoffs (<40, 40–59.9, 60–79.9, ≥80 nmol/L).

Results: Vitamin D insufficiency (25(OH)D <50 nmol/L) was prevalent in 20% of participants in the first trimester. Each 10 nmol/L increase in first trimester 25(OH)D was associated with a 0.05 [95% confidence interval (CI): 0.01, 0.10] increase in length-for-age *z*-score but was not associated with weight or head circumference. There were no differences in risk of preterm birth or SGA using IOM cutoffs; participants with first trimester 25(OH)D <40 compared with ≥80 nmol/L had 4.35 (95% CI: 1.14, 16.55) times risk of preterm birth. Second trimester 25(OH)D was not associated with fetal growth patterns or with pregnancy outcomes.

Conclusions: First trimester 25(OH)D is positively associated with linear growth. Low first trimester 25(OH)D (<40 nmol/L) is associated with a higher risk of preterm birth. Second trimester 25(OH)D is not associated with fetal growth or pregnancy outcomes assessed.

Keywords: vitamin D, fetal growth, preterm birth, SGA, adverse pregnancy outcomes

Introduction

Low vitamin D status during pregnancy is common in the United States, with an estimated 28% of females who are pregnant or lactating at risk of inadequacy or deficiency per 2001–2006 national estimates for pregnant populations [1]. This is a major public health concern, as inadequate maternal vitamin D status (<50 nmol/L) has been associated with adverse pregnancy outcomes for both the mother and infant, including preeclampsia, gestational diabetes mellitus, and preterm birth [2]. There is substantial evidence suggesting that maternal vitamin D supplementation may improve maternal and neonatal outcomes, though results from intervention studies have been mixed [3–6], which

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; HEI-2010, Healthy Eating Index-2010; INTERGROWTH-21st, International Fetal and Newborn Growth Consortium for the 21st Century; IOM, Institute of Medicine; IRB, Institutional Review Board; nuMoM2b, Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be; SGA, small for gestational age.

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may be because of the differences in populations studied, supplementation dose and timing, and maternal characteristics including baseline vitamin D status. Thus, the effects of vitamin D supplementation during pregnancy on maternal and infant outcomes remain unsettled.

Vitamin D plays important roles beginning early in pregnancy and is required for immune cell function during the processes of implantation and placental formation [7]. Vitamin D has been positively associated with the production of vascular endothelial growth factor and placental growth factor, both pro-angiogenic factors that regulate early vascularization of the placenta [8–10]. In addition, vitamin D plays essential roles in bone health and formation, and maternal vitamin D status has been associated with fetal skeletal development [11–13] and birth weight [14,15]. However, vitamin D status in relation to fetal growth patterns across gestation has been minimally studied [15]. Also, prior studies have largely examined maternal vitamin D status at mid-gestation or later in relation to birth weight [3,15,16], and associations between first trimester maternal vitamin D status and pregnancy outcomes are poorly understood. Thus, there is a knowledge gap regarding the time points in pregnancy at which maternal vitamin D status is most critical for optimal fetal growth and pregnancy outcomes.

To fill these research gaps, we aimed to examine associations between first and second trimester vitamin D status and fetal growth, gestational age at birth, and risk of preterm birth and small for gestational age (SGA).

Methods

Design and study population

We conducted a longitudinal, observational substudy using data and samples from the Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be (nuMoM2b), a multicenter prospective cohort study of 10,038 nulliparous pregnant females conducted in the United States [17]. Enrollment occurred across the following 8 clinical sites or its subsite: Case Western Reserve University, Columbia University, Indiana University, Magee-Women's Hospital, Northwestern University, University of California Irvine, University of Pennsylvania, and the University of Utah. Participants were recruited in the first trimester (visit 1, 6–13 6/7 weeks of gestation) and had follow-up study visits during the early second trimester (visit 2, 16–21 6/7 weeks of gestation) and late second/early third trimester (visit 3, 22 to 29 6/7 weeks of gestation). Pregnancy data and biospecimens were collected at study visits and at delivery and included demographic and lifestyle data assessed via questionnaires, medical record data, and blood samples. Study participant inclusion criteria for the original study were the following: having a viable single gestation, enrolled between 6 and 13 6/7 weeks of gestation, and no prior pregnancy lasting ≥20 wk. Exclusion criteria were: maternal age <13 y; history of 3 or more spontaneous abortions; likely fatal fetal malformation; known fetal aneuploidy; assisted reproduction with a donor oocyte; multifetal reduction; participation in an intervention study anticipated to influence maternal or fetal outcomes; prior enrollment in nuMoM2b; plan to terminate the pregnancy; or inability to provide consent. All study participants provided informed consent before enrollment. A target enrollment of 10,000 participants was estimated to have 80% power to detect both modest and rare effects on preterm birth (1100 expected cases). Study recruitment occurred from October 2010 through September 2013. A detailed description of study methods has been published previously [17].

Because 25-hydroxyvitamin D (25(OH)D) was not measured for the original nuMoM2b cohort, we randomly selected a sample of 351 nuMoM2b participants for 25(OH)D measurement for our study, using methods to achieve a sample representative of the original nuMoM2b cohort. We selected our sample from a subset of participants who had previously been included in a nuMoM2b nested case-control study, in which all participants with an adverse pregnancy outcome and a representative sample of participants without an adverse pregnancy outcome had been included for biospecimen sampling [18]. Before selecting our sample, we applied the following exclusion criteria: <18 y old, missing first trimester body mass index (BMI) measure, underweight (BMI <18.5 kg/m^2), fetal aneuploidy, missing ultrasound measures at visit 2, or missing first or second trimester maternal serum samples. We then applied stratified random sampling methods according to adverse pregnancy outcome status, to achieve a sample of 352 participants representative of the original distribution of participants with (16%) and without (84%) an adverse pregnancy outcome as in the original nuMoM2b cohort, to achieve a sample representative of the main nuMoM2b study cohort. We excluded 1 participant who was identified as having chronic liver disease following random selection, giving a final sample size of 351 for analysis (participant flowchart is displayed in Supplemental Figure 1).

On the basis of the literature for expected mean differences [15], we estimated that a sample size of 351 would have power of 0.99, 0.82, and 0.71 ($\alpha = 0.05$) to identify mean z-score differences between highest and lowest vitamin D categories of 0.40, 0.34, and 0.28 for length, weight, and head circumference, respectively.

Institutional Review Board (IRB) approval was obtained for the original study through each site's local governing IRB (ClinicalTrials.gov identifier: NCT01322529). This research was deemed nonhuman subjects research and IRB exempt by The Pennsylvania State University IRB (IRB #00023120).

Maternal characteristics

Sociodemographic factors including maternal age, race and ethnicity, education, smoking, and poverty level were assessed via questionnaires administered at the first study visit. Race and ethnicity were self-reported using separate categories for each according to 1997 Office of Management and Budget guidelines (race: American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White; ethnicity: Hispanic or Latino, Not Hispanic or Latino) [19], which were developed into 4 categories for analysis (Non-Hispanic white, Non-Hispanic black, Hispanic, and Other). Height and weight measured at the first study visit were used to calculate first trimester maternal BMI. Fetal sex, gravidity, obstetric complications, and pre-existing medical conditions were assessed through medical record abstractions. Preconceptual Healthy Eating Index-2010 (HEI-2010) scores [20] and average daily alcohol consumption, calcium, and energy intake were derived from responses to a food-frequency questionnaire administered at the first study visit. Multivitamin use was assessed at each study visit.

Blood samples and measurement of vitamin D status

After maternal blood was collected in the parent trial, blood tubes were centrifuged and serum was aliquoted into cryovials and stored at –80°C in a biorepository. Serum samples from visits 1 and 2 for the 351 participants in this study were shipped frozen to the Department of Nutritional Sciences at The Pennsylvania State University. We measured 25(OH)D_3 and 25(OH)D_2 concentrations using liquid chromatography-mass spectrometry. National Institute of Standards

and Technology standard reference materials (SRM 1949) were used for calibration of methods and as a control with sample batches. Between batches coefficients of variation and lower limits of quantification were 4% and 0.23 nmol/L for 25(OH)D_3, and 3% and 0.19 nmol/L for 25(OH)D_2, respectively. Total 25(OH)D [25(OH)D_3 + 25(OH)D_2] was used for analysis.

We compared fetal growth patterns in relation to 25(OH)D as a continuous variable and also according to Institute of Medicine (IOM) cutoffs: deficiency [25(OH)D < 30 nmol/L], insufficiency [25(OH)D 30 to <50 nmol/L], and sufficiency [25(OH)D ≥ 50 nmol/L] [21]. Because of the small numbers of participants with first and second trimester 25(OH)D deficiency (n = 7 and n = 3, respectively), we created binary categories (<50 compared with ≥50 nmol/L) for our main analysis. Because optimal vitamin D status in pregnancy is unclear [22,23], we additionally examined 25(OH)D within exploratory cutoffs we developed to capture low, moderate, and higher status: <40, 40–59.9, 60–79.9, and ≥80 nmol/L. We chose these cutoffs according to the range of 25(OH)D concentrations within our study population and to create a reasonable number of observations within each category. Although these cutoffs are not directly comparable to recommended guidelines, they provide additional insight by examining a range of low-to-high 25(OH)D concentrations that are not captured in current recommended cutoffs [21,23].

Fetal growth and anthropometric measurements

Fetal ultrasounds were conducted by certified sonographers at participating clinical study sites at visits 1, 2, and 3. At visit 1, the crown rump length was measured to establish gestational age [17]. At visits 2 and 3, fetal head circumference, abdominal circumference, and femur diaphysis length were measured. Estimated fetal weight was calculated at visit 3 using the formula from the International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st), which estimates fetal weight from 22 0/7 to 40 0/7 weeks of gestation [24]. We calculated gestational age-specific z-scores for fetal head circumference, femur length, and estimated fetal weight on the basis of the INTERGROWTH-21st standard growth curves.

Newborn length, weight, and head circumference were obtained from medical records. Gestational age- and sex-specific z-scores for each birth measure were calculated using INTERGROWTH-21st standards [25]. We selected the INTERGROWTH-21st standards because they represent a unified standard by which both fetal and neonatal measurements can be assessed.

Pregnancy outcomes

Small for gestational age was defined as gestational-age- and sex-adjusted birth weight below the 10th percentile using INTERGROWTH-21st standards. Preterm birth was defined as gestational age <37 wk at delivery.

Statistical analyses

We compared maternal and infant characteristics across vitamin D categories using one-way analysis of variance for normally distributed continuous variables, Kruskal–Wallis tests for non-normally distributed continuous variables, and chi-square tests for categorical variables.

To examine associations between maternal 25(OH)D and longitudinal fetal growth patterns, we used mixed-effects linear regression models to construct fetal growth curves in relation to 25(OH)D. In the models, we included participants as random intercepts, maternal characteristics and fetal sex as fixed-effects, and gestational age as the time variable (included both as a fixed and random effect). We used piecewise linear regression splines with a knot at 28 weeks of gestation to accommodate nonlinear growth patterns for length and head circumference, which was determined on the basis of the observations of the raw data using LOWESS regression and model testing. Because estimated fetal weight z-scores were available ≥22 weeks of gestation, growth curves for weight were conducted using estimated fetal weight z-scores from visit 3 and weight z-scores at birth with no spline term. For length, we used z-scores for fetal femur length at visits 2 and 3 and birth length z-scores to construct growth curves. We used z-scores for head circumference for visits 2, 3 and at birth. We adjusted models for the following factors, which were identified as confounders on the basis of the literature: maternal age, height, first trimester BMI, race and ethnicity, multivitamin supplement use, fetal sex, and season of blood draw. Of note, we examined preconceptual HEI-2010 scores, alcohol consumption, and dietary calcium [average daily calcium intake (mg)/1000 kcal] as potential confounders, which characteristics were available for 299 of the 351 participants. We did not include these as covariates in final models as they did not improve model fit (on the basis of R^2 and Akaike information criterion values) or change effect estimates [26].

To examine pregnancy outcomes, we used multivariable Poisson regression models with robust variance estimation to calculate the relative risk of SGA and preterm birth according to vitamin D status. Because of the small numbers of cases, we selected the confounders we expected to be the most influential based on the literature for our adjusted models: season of blood draw, race and ethnicity, maternal BMI, and fetal sex. We used linear regression models to examine vitamin D status and gestational age at birth, adjusted for these same factors to be consistent in our approach.

For all analyses, 25(OH)D was assessed continuously and by using cutoffs. We analyzed first and second trimester 25(OH)D separately in relation to outcomes. We also examined the change in 25(OH)D between first and second trimesters in relation to growth curves, while controlling for first trimester status. We tested for interactions between 25(OH)D and weeks of gestation, race and ethnicity, fetal sex, and first trimester BMI on fetal growth. For sensitivity analyses, we conducted analyses relating 25(OH)D with fetal growth patterns including only term infants (≥37 weeks of gestation).

Results

Our study included participants enrolled across all 8 sites of the original nuMoM2b cohort study. Participant characteristics assessed for our study were similar to those of the original cohort (Table 1). Study participants had a mean age of 27.9 y, a mean BMI of 26.6, and mean length of gestation of 38.8 wk. Two-thirds of participants were non-Hispanic White, and half had a bachelor's degree. We observed differences in maternal age, BMI, race and ethnicity, education, and multivitamin supplementation according to maternal vitamin D status (Supplemental Table 1). Compared with participants with 25(OH)D ≥50 nmol/L, participants with 25(OH)D <50 nmol/L tended to be younger, have higher BMI, and were more likely to be of non-Hispanic Black race and ethnicity. Participants with 25(OH)D <50 nmol/L were less likely to have a bachelor's degree or use multivitamin supplements when compared with participants with 25(OH)D ≥50 nmol/L. We did not observe differences in season of blood draw according to maternal vitamin D status (see Supplemental Table 1).

The mean (standard deviation) 25(OH)D concentration in the first trimester was 68.1 (21.0) nmol/L, with a range of 15.0–151.3 nmol/L. In

TABLE 1
Characteristics of study participants at enrollment (6–13 weeks of gestation) and their infants in the nuMoM2b parent cohort and the substudy sample.

Characteristic	nuMoM2b parent study (n = 10,038)	Substudy (n = 351)
Maternal	Mean ± SD or n (%)	
Age (y)	26.9 ± 5.7	27.9 ± 5.6
BMI (kg/m^2)	26.4 ± 6.3	26.6 ± 6.4
Race and ethnicity		
Non-Hispanic White	5989 (59.7)	229 (65.2)
Non-Hispanic Black	1418 (14.1)	48 (13.7)
Hispanic	1700 (17.0)	45 (12.8)
Other	921 (9.2)	29 (8.3)
Education		
<Bachelor's degree	4940 (49.2)	171 (48.7)
≥Bachelor's degree	5098 (50.8)	180 (51.3)
Gravidity		
1	7438 (74.2)	246 (70.1)
≥2	2590 (25.8)	105 (29.9)
Pregestational diabetes mellitus	151 (1.6)	6 (1.7)
Smoked[1]	1782 (17.8)	58 (16.5)
Pre-pregnancy alcohol use (g/d), median (IQR)[2]	2.8 (0.0,11.4)	3.9 (0.0,12.8)
HEI score[2]	63 ± 13	63 ± 14
Infant		
Gestational age at birth (wk)	38.7 ± 2.5	38.8 ± 2.3
Sex (male)	4848 (51.5)	177 (50.7)
Birth weight (g)	3270 ± 564	3286 ± 588

Abbreviation: HEI, Healthy Eating Index 2010 score.
[1] Smoked during the 3 mo before becoming pregnant.
[2] Alcohol consumption and HEI scores were calculated from food-frequency questionnaires pertaining to the 3 mo before becoming pregnant. Data were available for 8259 parent study participants and for 299 substudy participants.

the second trimester, the mean was slightly higher [78.0 (23.9) nmol/L] but the range was similar (13.0–148.7 nmol/L) to the first trimester. In the first trimester, according to our binary IOM cutoffs 20% of females (n = 70) had 25(OH)D concentrations <50 nmol/L and 80% (n = 281) had concentrations ≥50 nmol/L; in the second trimester, 13% of females (n = 45) had concentrations <50 nmol/L and 87% (n = 306) had concentrations ≥50 nmol/L (Supplemental Table 1). Of note, 2% of participants (n = 7) met criteria for deficiency (<30 nmol/L) in the first trimester and <1% of participants (n = 3) met criteria for deficiency in the second trimester. We observed the following distributions using vitamin D cutoffs we developed: <40 nmol/L (first trimester n = 30 and second trimester n = 24), 40–59.9 nmol/L (first trimester n = 94 and second trimester n = 50), 60–79.9 nmol/L (first trimester n = 130 and second trimester n = 122), and ≥80 nmol/L (first trimester n = 97 and second trimester n = 155).

Fetal growth

Of the 351 participants in our analysis, 96% (n = 336) had fetal growth measures for all 3 time points (visits 2, 3, and at birth), and 4% (n = 15) had measures for only 2 time points. Fetal growth characteristics across gestation and at birth are shown in Table 2.

When we examined first trimester 25(OH)D continuously in relation to longitudinal fetal growth patterns, we observed associations between 25(OH)D and linear growth, but not with weight or head circumference in adjusted models (Supplemental Table 2). Each 10 nmol/L increase in first trimester 25(OH)D was associated with a 0.05 [95% confidence interval (CI): 0.01, 0.10] increase in length z-score after adjustment for confounders. Using the IOM insufficiency cutoffs, we observed no

TABLE 2
Fetal growth characteristics at study visits and at birth.

Growth characteristic	Mean ± SD
Study visit 2 (16–21 wk; n = 351)	
Gestational age (wk)	19.1 ± 1.4
Femur length (mm)[1]	29.5 ± 4.6
Head circumference (cm)[1]	16.4 ± 1.9
Study visit 3 (22–29 wk; n = 340)	
Gestational age (wk)	27.6 ± 1.7
Femur length (mm)[1]	51.9 ± 4.8
Estimated fetal weight (g)[2]	1122 ± 266
Head circumference (cm)[1]	26.0 ± 2.1
Birth (22–42 wk; n = 351)	
Gestational age (wk)	38.8 ± 2.3
Body length (cm)	50.7 ± 3.6
Weight (g)	3294 ± 585
Head circumference (cm)	34.0 ± 2.0

Abbreviations: INTERGROWTH-21st, International Fetal and Newborn Growth Consortium for the 21st Century; SD, standard deviation.
[1] Fetal growth measurement was conducted by ultrasound.
[2] Estimated fetal weight was calculated using INTERGROWTH-21st standards.

differences in fetal growth trajectories for length, weight, or head circumference for first trimester 25(OH)D <50 compared with ≥50 nmol/L (Figure 1; mean z-score differences in Supplemental Table 2). When we examined associations between first trimester 25(OH)D and fetal growth trajectories using our defined cutoffs and 25(OH)D ≥80 nmol/L as the reference, we observed patterns of lower growth trajectories for length and weight as 25(OH)D decreased, but these were not statistically significant (Supplemental Figure 2 and Supplemental Table 3). We observed no pattern when examining first trimester 25(OH)D and head circumference using exploratory cutoffs.

When assessed continuously, using the IOM insufficiency cutoffs, or using the additional cutoffs we developed, second trimester 25(OH)D was not associated with growth trajectories for length, weight, or head circumference following adjustment for confounders [Figure 2 and Supplemental Table 2 (results using continuous 25(OH)D and IOM binary cutoffs]; Supplemental Figure 3 and Supplemental Table 3 (results using additional 25(OH)D cutoffs). Of note, when we examined associations between second trimester 25(OH)D and fetal growth patterns for length and weight using our additional cutoffs (reference ≥80 nmol/L), we did not observe the same patterns of lower growth trajectories with decreasing 25(OH)D that we had observed for first trimester 25(OH)D.

Most participants (74%) had an increase in 25(OH)D concentrations between first and second trimesters [median intra-person change was 9.75 (range - 31.51–84.88) nmol/L]. The change in maternal 25(OH)D was not associated with fetal growth patterns for length, weight, or head circumference, after controlling for confounders and first trimester maternal 25(OH)D (P > 0.05 for all associations). No interactions were observed in any of the models examining 25(OH)D and growth.

Pregnancy outcomes

In our study, 21 infants (6%) were born SGA, and 29 infants (8%) were born preterm. First trimester 25(OH)D when assessed continuously or using IOM binary cutoffs (<50 compared with ≥50 nmol/L) was not associated with risk for SGA, preterm birth, or with gestational age at birth following adjustment for confounders (Table 3). Using exploratory cutoffs we developed for 25(OH)D, participants with first

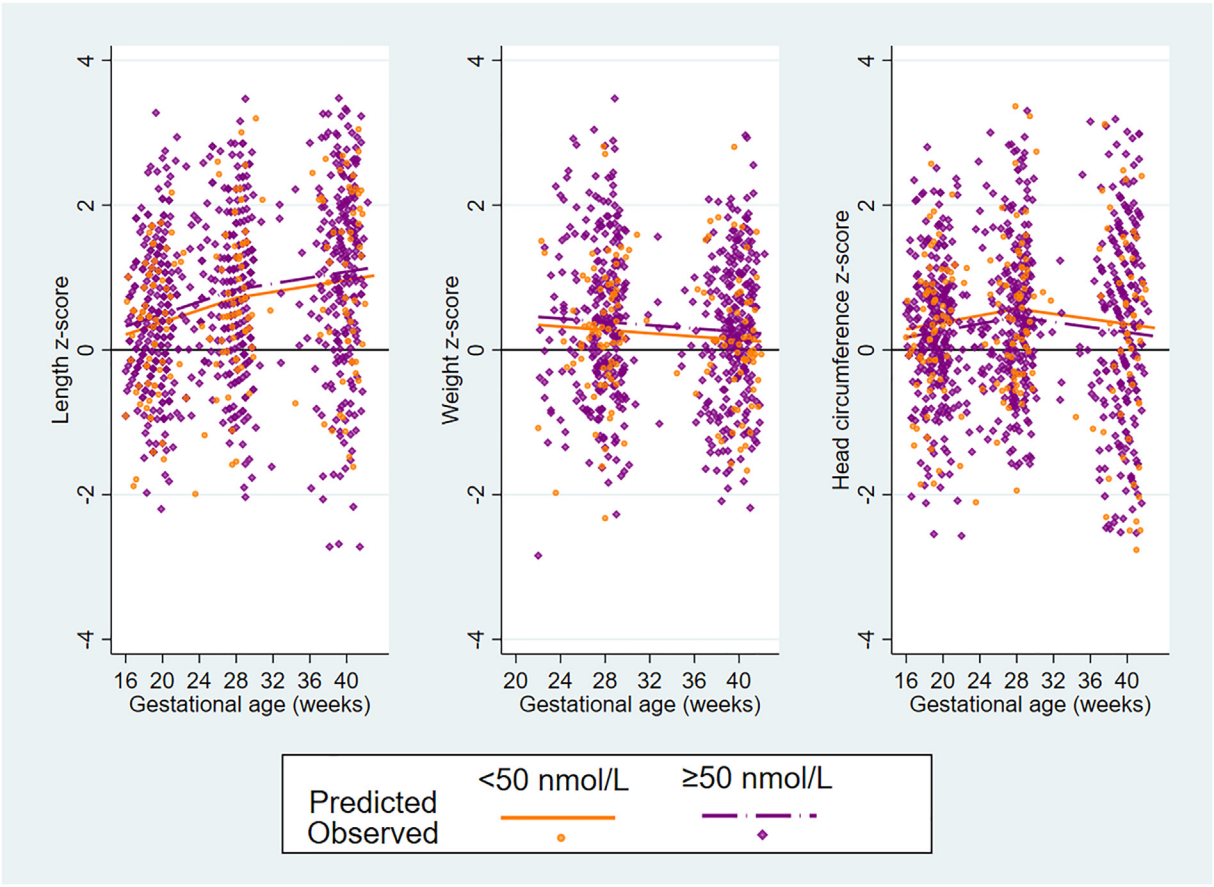

FIGURE 1. Fetal growth patterns between 16 and 42 weeks of gestation, according to first trimester maternal vitamin D status using Institute of Medicine cutoffs for sufficiency. Estimates for length and head circumference were derived from mixed-effects piecewise linear regression spline models with a knot at 28 weeks of gestation [$n = 349$ (1005 observations) for length and $n = 349$ (1003 observations) for head circumference]. Estimates for weight were derived from mixed-effects linear regression models without a spline term ($n = 348$; 689 observations). Adjusted models included covariates for race and ethnicity, first trimester BMI, multivitamin supplement use, season of blood draw, maternal height, maternal age, fetal sex, and weeks of gestation.

trimester 25(OH)D <40 nmol/L had 4.35 (95% CI: 1.14, 16.55) times the adjusted risk of delivering a preterm infant, and delivered on average 1.17 (95% CI: 0.14, 2.19) wk earlier, when compared with participants with 25(OH)D >80 nmol/L (Supplemental Table 4). We observed no differences in the risk for SGA when using these exploratory cutoffs.

Second trimester 25(OH)D was not associated with SGA, preterm birth, or with gestational age at birth when assessed continuously, when using IOM binary cutoffs (Table 3), or cutoffs we developed (Supplemental Table 4).

Sensitivity analyses

Analyses of fetal growth for term only infants ($n = 321$) yielded results similar to primary analyses. Specifically, we continued to observe a mean increase in length *z*-score of 0.05 (95% CI: 0.00, 0.10; $P = 0.036$) for every 10 nmol/L increase in first trimester maternal 25(OH)D. Similar to main analyses, we did not observe associations between first trimester 25(OH)D and weight or head circumference, and observed no association between second trimester 25(OH)D and growth measures. We observed similar, nonsignificant patterns as those for main analyses when we examined 25(OH)D using IOM binary cutoffs (<50 compared with ≥50 nmol/L) and our exploratory cutoffs for term only infants.

Discussion

First trimester maternal vitamin D status was positively associated with fetal linear growth patterns from the second trimester to birth, in a random sample from a large pregnancy cohort in the United States. First trimester maternal vitamin D status was not associated with weight or head circumference fetal growth patterns. We did not observe differences in risk for SGA or preterm birth when assessing 25(OH)D continuously, or in females with insufficient compared with sufficient vitamin D status (<50 compared with ≥50 nmol/L). However, we observed a higher risk for preterm birth and a shorter mean length of gestation when making comparisons across a wider range of first trimester 25(OH)D cutoffs (<40 compared with ≥80 nmol/L). Second trimester vitamin D status was not associated with fetal growth patterns or with pregnancy outcomes assessed. Of note, these findings were observed in a study population in which 80% of pregnant females met criteria for vitamin D sufficiency in the first trimester.

Few studies have examined fetal growth patterns in relation to early pregnancy maternal vitamin D status, and ours is the first study we are aware of to examine multiple time points of early pregnancy maternal 25(OH)D in association with fetal growth. Our findings suggest that the

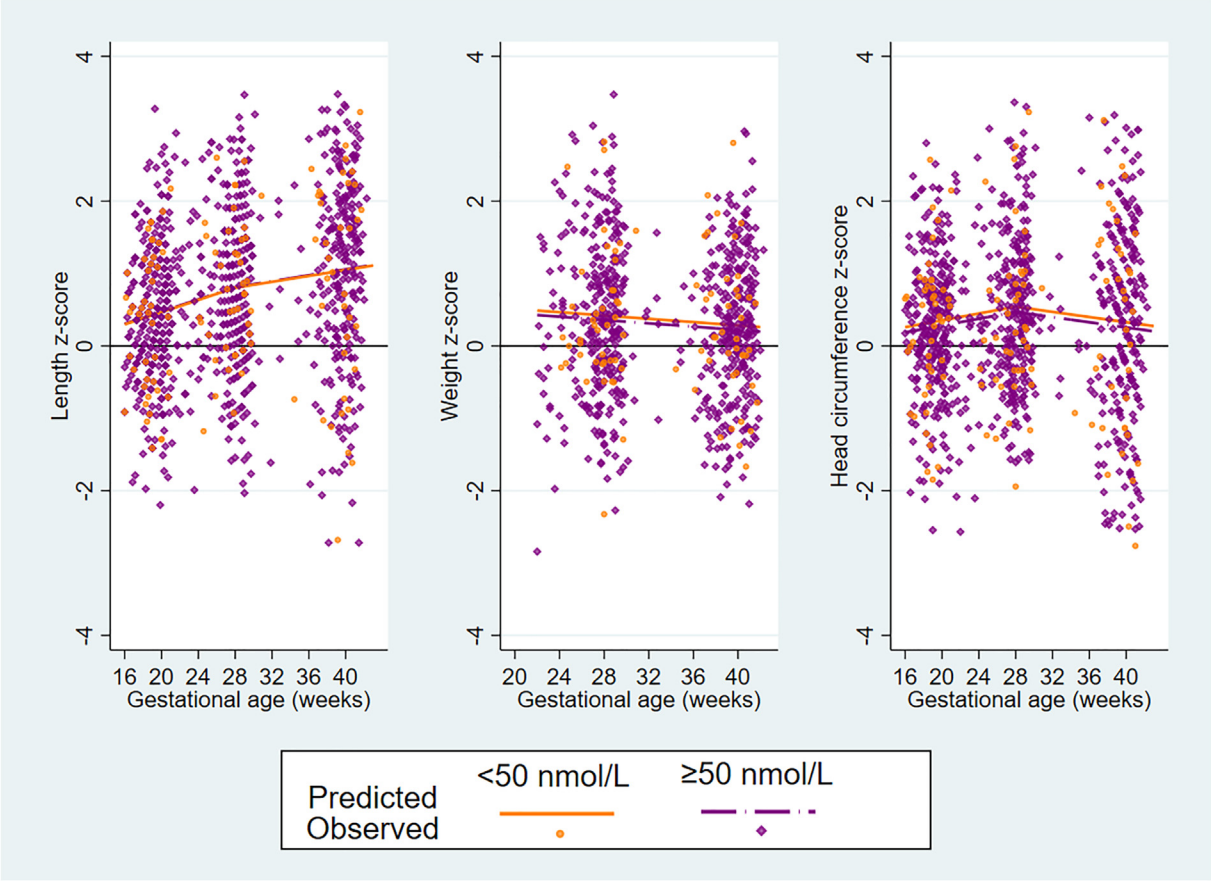

FIGURE 2. Fetal growth patterns between 16 and 42 weeks of gestation, according to second trimester maternal vitamin D status using Institute of Medicine cutoffs for sufficiency. Estimates for length and head circumference were derived from mixed-effects piecewise linear regression spline models with a knot at 28 weeks of gestation [$n = 349$ (1005 observations) for length and $n = 349$ (1003 observations) for head circumference]. Estimates for weight were derived from mixed-effects linear regression models without a spline term ($n = 348$; 689 observations). Adjusted models included covariates for race and ethnicity, first trimester BMI, multivitamin supplement use, season of blood draw, maternal height, maternal age, fetal sex, and weeks of gestation.

first trimester (or preconception to increase first trimester vitamin D status) may represent a critical time point for intervention in females with deficiency. Our findings are supported by those of Zhang et al. [27], in which first trimester 25(OH)D <50 nmol/L was associated with an increased risk for early fetal growth restriction as measured by first trimester crown rump length in 15,651 Chinese females. Additionally, in a randomized controlled trial of 140 pregnant females, first trimester vitamin D supplementation of 1000 international units per day was associated with a longer femur length in individual second and third trimesters [28]. However, longitudinal fetal growth patterns and birth outcomes were not assessed in these comparative studies.

In contrast to our study, Miliku et al. [15] observed associations between second trimester maternal vitamin D status and growth patterns for fetal weight and head circumference in a population-based prospective cohort study of 7098 pregnant females in the Netherlands. Differences in our findings may be because of the use of a lower 25(OH)D cutoff category (<24.1 nmol/L) in the comparison study, which was the vitamin D group for which significant differences in fetal growth were primarily observed, in contrast with our lowest categories (<50 nmol/L using IOM cutoffs; <40 nmol/L using exploratory cutoffs). However, we could not replicate Miliku et al. [15]'s analysis due to the low prevalence of participants with 25(OH)D <24.1 nmol/L in our study.

Findings have been mixed from studies of maternal vitamin D status in relation to preterm birth [16,29] and SGA [2,16,30]. Previous studies have generally measured 25(OH)D or supplementation in the second trimester or later in relation to preterm birth and SGA, and there is a paucity of evidence associating first trimester 25(OH)D with birth outcomes. Schneuer et al. [31] examined 25(OH)D at 10–14 wk of pregnancy in relation to preterm birth <37 wk and SGA <10th percentile in a nested case-control study of 5109 females with singleton pregnancies, using 25(OH)D cutoffs <25, <37.5, 37.5–49.9, 50–75 (reference category), and >75 nmol/L. Similar to our primary analyses using IOM cutoffs and ≥50 nmol/L as the reference group, these authors observed no association between first trimester 25(OH)D and preterm birth. However, we did observe an association between first trimester 25(OH)D and preterm birth when using exploratory cutoffs with a higher reference group of ≥80 nmol/L. Similar to our study, Schneur et al. [31] did not observe increased risk for SGA in females with 25(OH)D <37.5 nmol/L, which closely approximates our <40 nmol/L category, though we used a higher reference group of >80 nmol/L in our analysis. Bodnar et al. [32] noted a U-shaped relationship between 25(OH)D measured at <22 weeks of gestation and risk of SGA, observing a downward trend in the probability of SGA from 20 to 70 nmol/L and then a gradual increase in risk at concentrations >70 nmol/L. In contrast, we did not observe higher risk for SGA in the reference group ≥80 nmol/L compared with middle vitamin D categories (40–59.9 and 60–79.9 nmol/L) when examining first or second trimester 25(OH)D. Differences in findings may be because of the differences in study design or because of a small number

TABLE 3
Birth outcomes in association with maternal first and second trimester 25(OH)D, using Institute of Medicine binary cutoffs for sufficiency[1].

25(OH)D (nmol/L)	*n*	No. cases	SGA[1]				No. cases	Preterm birth[1,2]				Gestational age at birth (wk)[3]			
			Unadjusted		Adjusted			Unadjusted		Adjusted		Unadjusted		Adjusted	
			RR	95% CI	RR	95% CI		RR	95% CI	RR	95% CI	Mean change	95% CI	Mean change	95% CI
First trimester															
Per 10 nmol/L increase	351	21	0.97	(0.75, 1.24)	1.02	(0.77, 1.35)	29	0.85	(0.72, 1.00)	0.87	(0.72, 1.05)	0.10	(–0.02, 0.21)	0.05	(–0.08, 0.17)
<50	70	4	0.94	(0.33, 2.72)	0.78	(0.23, 2.66)	8	1.53	(0.71, 3.31)	1.35	(0.60, 3.06)	–0.30	(–0.90, 0.31)	–0.02	(–0.68, 0.64)
≥50	281	17	ref	ref	ref	ref	21	ref	ref	ref	ref	ref	ref	ref	ref
Second trimester															
Per 10 nmol/L increase	351	21	1.06	(0.89, 1.30)	1.15	(0.94, 1.40)	29	0.85	(0.76, 0.96)	0.88	(0.77, 1.01)	0.07	(–0.03, 0.17)	0.02	(–0.09, 0.14)
<50	45	2	0.72	(0.17, 2.98)	0.52	(0.12, 2.31)	7	2.16	(0.98, 4.78)	1.71	(0.71, 4.16)	–0.74	(–1.47, –0.02)	–0.54	(–1.31, 0.23)
≥50	306	19	ref	ref	ref	ref	22	ref	ref	ref	ref	ref	ref	ref	ref

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; INTERGROWTH-21st, International Fetal and Newborn Growth Consortium for the 21st Century; SGA, small for gestational age (<10th percentile using INTERGROWTH-21st standards).

[1] Estimates were derived from Poisson regression models with robust variance estimation and linear regression models ($n = 351$). Adjusted models included covariates for season of blood draw, maternal race and ethnicity, BMI, and fetal sex.

[2] <37 weeks of gestation.

[3] Estimates were derived from multivariable linear regression models adjusted for season of blood draw, maternal race and ethnicity, BMI, and fetal sex.

of SGA cases in our study, limiting our power to detect these same differences.

In a recent systematic review supporting Endocrine Society vitamin D guidelines for pregnant women, the authors concluded that there may be "important potential benefit" of vitamin D supplementation on risk for SGA and preterm birth [33]. Although our findings do not provide evidence of benefits to reduce SGA, they do support potential benefits of supplementation during preconception or early pregnancy to reduce the risk of preterm birth. Given our findings and the wide recognition of season of the year as a major determinant of vitamin D status worldwide [21,34–36], early supplementation may be especially important for pregnancies that begin in late fall or winter months. However, it should be noted that associations between vitamin D status and preterm birth differed in our study according to vitamin D cutoffs and reference categories used, which are important factors to consider in the context of pregnancy and supplementation.

The relationship between early pregnancy vitamin D status and fetal growth patterns for length and birth outcomes may be explained by multiple mechanisms. Vitamin D has been associated with placental angiogenesis, in which higher 25(OH)D has been related to higher production of pro-angiogenic proteins and lower production of anti-angiogenic proteins regulating the formation of the vascular network within the placenta early in pregnancy [8–10]. The disruption of these processes through insufficient vitamin D bioavailability could adversely impact implantation and placental development early in gestation, in turn impacting later fetal growth. Fetal 25(OH)D concentrations closely mirror maternal 25(OH)D [37], and thus, maternal deficiency will be reflected in suboptimal bioavailability of 25(OH)D in the fetus to support skeletal growth, which begins between the sixth and seventh weeks of embryonic development [38]. Vitamin D plays an important immunomodulatory role in placental-decidual function [7,39], and its role in anti-inflammatory pathways may help to decrease the incidence of infections, one of the most common causes for preterm delivery [40]. Future studies are warranted to verify these potential mechanisms.

Strengths of our study included the use of a randomly selected sample representative of pregnant females from a large multisite cohort study with standardized procedures for data collection and outcome ascertainment. Vitamin D and fetal growth measures were collected across multiple time points in pregnancy, enabling us to assess fetal growth patterns across continuous weeks of gestation. Although the INTERGROWTH-21st fetal and newborn standards may not be generalizable to all populations, they allowed us to maximize the validity of prenatal-birth growth curves, which would not be possible for other references where prenatal and birth size standards were developed in separate populations using different methodologies [41–44]. Limitations included a small number of participants overall, and few with vitamin D concentrations <30 nmol/L (the standard definition of deficiency), limiting our ability to make comparisons of fetal growth and birth outcomes for participants with very low vitamin D status. We also had small numbers of cases of SGA and preterm birth, limiting the number of covariates adjusted for in those statistical models.

In conclusion, first trimester maternal vitamin D status was positively associated with fetal linear growth patterns. Low first trimester vitamin D status (<40 nmol/L) was associated with a higher risk for preterm birth and with a shorter mean length of gestation. We did not find evidence of an association between first trimester maternal vitamin D status and fetal growth patterns for weight or head circumference. Second trimester vitamin D status was not associated with fetal growth patterns or pregnancy outcomes. Future research should clarify the influence of timing of vitamin D supplementation, including in the preconception period, and interrogate the mechanisms by which vitamin D may contribute to fetal growth and gestational duration.

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Author contributions

The authors' responsibilities were as follows – CB, ADG: contributed to conceptualization and design of the study, acquisition of data, analysis and interpretation of the data, and drafting of the article; NRB, RMS, WAG, SP: contributed to the conceptualization and design of the study, acquisition of data, and critical review of the manuscript; JS, CS, MN: contributed to analysis and interpretation of the data and critical review of the manuscript; and all authors read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data availability

Data described in the manuscript, code book, and analytic code will not be made available because they belong to a third party and are not the property of manuscript authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2024.11.018.

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