

Maternal Serum 25-Hydroxyvitamin D Concentrations Are Associated with Small-for-Gestational Age Births in White Women^{1–3}

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Abstract

Maternal vitamin D deficiency has been associated with numerous adverse health outcomes, but its association with fetal growth restriction remains uncertain. We sought to elucidate the association between maternal serum 25-hydroxyvitamin D [25(OH)D] concentrations in early pregnancy and the risk of small-for-gestational age birth (SGA) and explore the association between maternal single nucleotide polymorphisms (SNP) in the vitamin D receptor (VDR) gene and the risk of SGA. We conducted a nested case-control study of nulliparous pregnant women with singleton pregnancies who delivered SGA infants ($n = 77$ white and $n = 34$ black) or non-SGA infants ($n = 196$ white and $n = 105$ black). Women were followed from <16 wk gestation to delivery. Women's banked sera at <22 wk were newly measured for 25(OH)D and DNA extracted for VDR genotyping. SGA was defined as live-born infants that were <10th percentile of birth weight according to nomograms based on gender and gestational age. After confounder adjustment, there was a U-shaped relation between serum 25(OH)D and risk of SGA among white mothers, with the lowest risk from 60 to 80 nmol/L. Compared with serum 25(OH)D 37.5–75 nmol/L, SGA odds ratios (95% CI) for levels <37.5 and >75 nmol/L were 7.5 (1.8, 31.9) and 2.1 (1.2, 3.8), respectively. There was no relation between 25(OH)D and SGA risk among black mothers. One SNP in the VDR gene among white women and 3 SNP in black women were significantly associated with SGA. Our results suggest that vitamin D has a complex relation with fetal growth that may vary by race. *J. Nutr.* 140: 999–1006, 2010.

Introduction

Maternal vitamin D deficiency is a major public health problem. Approximately 2 in 3 pregnant women in the United States have suboptimal vitamin D status, with even higher prevalences reported among black and Mexican-American women (1). Vitamin D deficiency is prevalent even in settings of widespread prenatal vitamin use (2), because prenatal vitamins contain doses of vitamin D that are too low to meaningfully raise serum 25-hydroxyvitamin D [25(OH)D]¹¹ concentrations (3) and

dietary intakes of vitamin D among American women are inadequate (4). More than one-half of U.S. women report always or most of the time practicing sun protective behaviors, including using sunscreen or staying in the shade (1), which limit cutaneous production of vitamin D.

Poor vitamin D status during pregnancy has been associated with preeclampsia (5), gestational diabetes (6), and bacterial vaginosis (7), as well as offspring rickets (8), reduced bone density (9), asthma (10), and schizophrenia (11). The link between maternal vitamin D status and fetal growth, as measured most frequently by infant birth weight and birth length, has been explored by a number of investigators with mixed results (12–20). The inconsistency of past findings may be related to the study of continuous birth size rather than a pathologic condition such as growth restriction, or the varied populations studied and methods used. Furthermore, the majority of past studies examined the effect of vitamin D in the 3rd trimester of pregnancy, when fetal growth velocity is greatest (21). Yet, evidence indicates that fetal growth trajectory in late gestation is determined much earlier, most likely in early

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³ Supplemental Table 1 is available with the online posting of this paper at jn.nutrition.org.

¹¹ Abbreviations used: 25(OH)D, 25-hydroxyvitamin D; HWE, Hardy-Weinberg Equilibrium; OR, odds ratio; SES, socioeconomic score; SGA, small-for-gestational age; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

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pregnancy (22). Moreover, the effects of vitamin D status on birth outcome may depend in part on race/ethnicity and maternal or fetal genotype. Some evidence suggests that associations between vitamin D status and adverse health outcomes that are present in whites do not exist among blacks (23–25). Additionally, polymorphisms in vitamin D-metabolizing genes have been related to a number of common, complex conditions for which low 25(OH)D concentration is a risk factor, such as osteoporosis and prostate cancer (26), and a recent study observed that infant vitamin D receptor (VDR) polymorphisms modified the effect of maternal 25(OH)D on offspring size (27). Because fetal growth restriction is associated with perinatal morbidity and mortality (28,29) and later-life chronic disease (30), it is imperative to find modifiable risk factors for this adverse birth outcome.

Our objective was to elucidate the association between maternal serum 25(OH)D concentrations in early pregnancy and the risk of small-for-gestational age birth (SGA), a measure of fetal growth restriction that is defined based on birth weights <10th percentile for a given standard. We also sought to determine the association between maternal single nucleotide polymorphisms (SNP) in the VDR gene and SGA risk.

Methods

We used existing data and banked serum and DNA samples collected as part of a large prospective pregnancy cohort study. Women carrying singleton fetuses were enrolled at <16 wk gestation from outpatient clinics at Magee-Womens Hospital in Pittsburgh, PA (latitude 40° N) and affiliated private practices from 1997 to 2001. The response rate was 72%. After providing informed, written consent, all women completed an interviewer-administered questionnaire at enrollment to collect data on sociodemographic factors, medical history, and health behaviors. Blood samples from nonfasting participants were collected at times of usual blood draws for clinical indications (initial visit, 16–18 wk, 26–29 wk, and predelivery). Maternal serum was processed and DNA was extracted. Both specimens were stored at –80°C. Medical records were abstracted to ascertain antepartum and delivery events and neonatal outcomes. The study was approved by the University of Pittsburgh and Magee-Womens Hospital Institutional Review Boards.

We conducted a nested case-control study among the 1198 women in the cohort who self-identified their race as black or white, were nulliparous, had no preexisting medical conditions, and delivered a live-born infant. In this cohort, there were 124 SGA births (10.4% incidence). Of these, 112 had a maternal blood sample at <22 wk banked and were therefore eligible for the current analysis. We randomly selected 303 non-SGA pregnancies in the cohort to serve as controls. We sought to select 1 sample at <22 wk from each woman, because early pregnancy is likely the time when exposures have the greatest impact on fetal growth trajectory (21). If a woman had more than 1 sample collected at <22 wk gestation, we randomly selected 1 sample using a random number generator. One white control and 2 white cases were excluded because their serum 25(OH)D concentrations were outside the detectable range (discussed below). The final sample size for the primary analyses was 111 SGA cases ($n = 77$ white and $n = 34$ black) and 301 controls ($n = 196$ white and $n = 105$ black).

Definition of SGA. SGA births were live-born infants that were <10th percentile of birth weight according to nomograms based on gender and gestational age from a reference population of over 50,000 infants delivered at Magee-Womens Hospital in Pittsburgh, PA. Gestational age was based on a reliable, self-reported estimate of last menstrual period or an ultrasound early in pregnancy. If gestational age estimates were available from both the last menstrual period and the ultrasound, the ultrasound was used for dating.

Quantitation of serum 25(OH)D. Maternal serum samples were stored from 1997 to 2001 in aliquots at –80°C until assay in 2006. Quantitation

of serum 25(OH)D [25(OH)ergocalciferol + 25(OH)cholecalciferol] was performed using a commercial ELISA from Immunodiagnostic Systems Limited and validated against a HPLC method in the laboratory of Dr. James Roberts at the Magee-Womens Research Institute. The ELISA could detect 25(OH)D in the range of 5–300 nmol/L. Three samples were >300 nmol/L and were excluded from this analysis due to probable sample deterioration. No samples in our analysis fell below the detectable range. The inter-assay CV for the ELISA was 10.3%. The ELISA recognized 100% of serum 25(OH)cholecalciferol and 75% of 25(OH)ergocalciferol but did not distinguish between these 2 forms. In our initial HPLC validation, we observed that only 3 of 32 samples (<10%) had any measurable 25(OH)ergocalciferol and within these samples, 25(OH)ergocalciferol accounted for only 10% of the total measurable 25(OH)D.

Details describing our method for validating the results of the ELISA against HPLC were described previously (2). The inter-assay CV for serum 25(OH)cholecalciferol using the HPLC method was 5.8%. The sensitivity of the HPLC method was <10 nmol/L and had a linear range to 1000 nmol/L. The relationship between serum 25(OH)D concentrations obtained from the ELISA and those from HPLC was as follows: slope = 1.14, intercept = 22, $r = 0.88$. Because studies have shown that the ELISA we used for this analysis overestimates serum 25(OH)D, particularly at concentrations >100 nmol/L (31), we also conducted all analyses limiting the sample to women with values ≤ 100 nmol/L.

There is no universally acceptable definition of vitamin D deficiency, so we used cut-points suggested recently: <37.5 nmol/L (deficiency), 37.5–75.0 nmol/L (insufficiency), and >75.0 nmol/L (sufficiency) (32,33).

SNP selection. We used the International Haplotype Map Project Database (34) to identify SNP within a 83.49-kbp region from positions 46,511,588 to 46,595,080 on chromosome 12 (NCBI Build 35 coordinates) that encompassed ~10 kbp upstream and 10 kbp downstream of the VDR gene. A reference SNP set was initially generated using race-specific genotypes from the HapMap database. We required that a widely studied C/T polymorphism in exon 2 (rs10735810) that introduces a translation start site and protein product that differs in length by 3 amino acids be included in the final SNP set (35). Tagging SNP that capture common variation within the VDR gene region were then selected on the basis of linkage disequilibrium by a pairwise correlation method (36). We forced the Caucasian tagging SNP in the analysis of the HapMap Yoruban genotypes. We required that tagging SNP have a minor allele frequency ≥ 0.05 and predicted the remaining SNP with a minimum $r^2 = 0.80$. A total of 75 tagging SNP were identified for genotyping.

Genotyping. Genotyping was completed using genomic DNA prepared from whole blood. Genotyping was completed using the fluorogenic 5'-nuclease TaqMan allelic discrimination assay system (Applied Biosystems) under standard conditions on a 7900HT real-time PCR instrument with probes and reagents purchased from Applied Biosystems. Of the 75 SNP identified for genotyping, 70 were successfully genotyped. Eighty-nine of the SGA cases and 265 of the non-SGA controls had DNA available in sufficient quantity and quality for genotyping.

Covariates. Race/ethnicity was self-reported (white; black). Prepregnancy BMI [weight (kg)/height (m)²] was based on maternal self-report of prepregnancy weight and height at the initial visit. Women self-reported their smoking status in the year before the index pregnancy and in the past 3 mo (nonsmoker, 1–10, ≥ 10 cigarettes/d). The season of sample collection was defined as winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November). Self-reported data were available for marital status, private insurance, use of periconceptional multivitamins at least once per week, any prepregnancy physical activity, and intensity of physical activity.

We created a socioeconomic index variable by averaging the component scores of 3 self-reported variables: household income, maternal education, and maternal occupation. For each variable, we

computed the cumulative percentage distribution, and the midpoint of the percentage interval for each level of the variable constituted the score for that level, as done previously (37). For example, women who reported college as their highest level of education were distributed between the 75th and the 80th percentile, with a midpoint of 77.8. This score was therefore assigned to the education variable for all women in this level. The summary socioeconomic score (SES) was obtained by averaging these 3 scores and dividing by 10. For women with only 2 of 3 reported variables (16%), the mean of the 2 variables was used. This cumulative score was highly correlated with each individual component ($r = 0.81-0.87$).

Analysis. Data are presented as mean (95% CI) or odds ratio (OR) (95% CI). Serum 25(OH)D concentrations were skewed, so they were log-transformed before statistical tests were performed. Multivariable logistic regression was used to assess the association between maternal serum 25(OH)D and the risk of SGA birth after confounder adjustment. Serum 25(OH)D was categorized using the deficiency cut-points as well as quartiles defined by the distribution among the non-SGA controls. Because serum 25(OH)D concentrations were curvilinear in the logit of SGA, we tested numerous splines and fractional polynomials to assess the dose-response relation between serum 25(OH)D levels and SGA risk (19). The curvilinear association between serum 25(OH)D and SGA risk in whites was best described using a quadratic spline with knot at 70 nmol/L.

We fit parsimonious regression models by specifying full models with potential confounding variables: race/ethnicity, prepregnancy BMI, smoking, SES, season, maternal age, gestational age at blood sampling, marital status, insurance status, periconceptional multivitamin use, and preconception physical activity. Potential confounding factors were considered to not be influential and were removed from the model if their inclusion did not satisfy our a priori change-in-estimate criterion ($>10\%$ change in OR). Effect modification by race/ethnicity was tested using a likelihood ratio test ($\alpha = 0.10$) and comparing stratum-specific effect estimates for meaningful differences. Prepregnancy BMI, smoking during pregnancy, and SES met our definition of confounding and were included in the race-stratified models.

Finally, as a sensitivity analysis, all analyses were rerun after the exclusion of women with serum 25(OH)D >100 nmol/L because of the concern surrounding the potential error with the assay at high values. Notably, we reassessed the dose-response relation and fit the best new model to the restricted dataset. In the restricted dataset, the best fit of the data among white women was a quadratic term for vitamin D.

For the genotyping analysis, Haploview 4.1 (38) was used to calculate Hardy-Weinberg Equilibrium (HWE) for each SNP (threshold $P > 0.001$) and to evaluate linkage disequilibrium. HWE was tested in each race group separately. SNP were excluded from subsequent analyses if their minor allele frequency was $<1\%$ or they were out of HWE in the sample of controls. Allelic and genotypic distributions were compared among race groups and between cases and controls using the chi-square test. The association between genotype and SGA was analyzed using logistic regression, where SNP were included as linear (additive) terms. Genotype models were analyzed with and without serum 25(OH)D in the model.

Results

Among both white and black women, those who delivered SGA infants tended to be younger, unmarried, of low socioeconomic status, users of cigarettes, and leaner compared with women delivering non-SGA infants (Table 1). Because most women were recruited at their initial visit in the first trimester, the majority of blood samples were from 0 to 13 wk of gestation.

The association between serum 25(OH)D and risk of SGA varied by race/ethnicity ($P = 0.01$). Among white mothers, mean maternal serum 25(OH)D at <22 wk did not differ between SGA cases [geometric mean (95% CI): 73.2 (69.7, 76.8) nmol/L] and controls [71.5 (64.0, 79.9) nmol/L]. However, white

mothers with SGA infants were more likely than white controls to have serum 25(OH)D <37.5 nmol/L (deficiency) and >75 nmol/L (sufficiency) ($P < 0.0001$) (Table 2). Case mothers were also more likely to be in the lowest and highest quartile of serum 25(OH)D concentrations ($P < 0.05$) (Table 2). These results did not change after limiting the analysis to women with serum 25(OH)D ≤ 100 nmol/L ($n = 217$; data not shown).

Among white mothers, vitamin D deficiency and sufficiency were both associated with increased risk of SGA, independent of pregravid BMI, smoking, and SES (Table 2). Excluding women with serum 25(OH)D >100 nmol/L did not meaningfully change the results for the vitamin D-sufficient group [serum 25(OH)D 75.1–100 nmol/L; adjusted OR 2.4 (1.2, 4.7)]. In quartile analysis, women in the bottom quartile and upper quartile were ~ 2.7 and 3.9 times as likely, respectively, as women in the second quartile to have an SGA infant after confounder adjustment. These results also remained significant even after limiting the analysis to serum 25(OH)D ≤ 100 nmol/L [upper quartile adjusted OR 9.3 (95% CI: 2.8, 30.6)].

The U-shaped nature of the relation between serum 25(OH)D at <22 wk and risk of SGA was confirmed with spline regression (Fig. 1A; $P < 0.01$ for spline terms). Indeed, this model suggested a steep downward trend in the probability of SGA from 20 to 70 nmol/L and then a significant gradual increase in risk at concentrations >70 nmol/L. The U-shape did not change after the exclusion of observations >100 nmol/L ($n = 217$; Fig. 1B) or additional adjustment for other measured covariates.

Vitamin D deficiency and insufficiency were pervasive among black women, and their geometric mean concentration was almost one-half that of white women (39.8 vs. 72.7 nmol/L). Among black women, the mean maternal serum 25(OH)D concentration at <22 wk did not differ [SGA cases: 39.8 (36.7, 43.2) vs. non-SGA controls: 39.8 (33.6, 47.0) nmol/L] and the distribution of vitamin D status did not vary by case status (Table 2). Likewise, there was no association between maternal serum 25(OH)D and the risk of SGA after confounder adjustment (Table 2), regardless of how the 25(OH)D was specified in the model (e.g. linear, categorical, spline). These results did not change when we excluded serum 25(OH)D >100 nmol/L.

The results for white and black women did not meaningfully differ when pregnancies with preeclampsia, preterm birth, or infants with a birth weight >90 th percentile were excluded.

VDR gene sequence variation was significantly associated with risk of SGA and differed by race (Table 3; Supplemental Table 1). Among white women, a single SNP (rs11168292) demonstrated a significant association with SGA, whereas among black women, 3 SNP in this gene (rs11168287, rs3782905, and rs2239179) were significant. Inclusion of serum 25(OH)D concentration in these models revealed that, for both black and white women, VDR genotype and vitamin D concentration have independent contributions to risk of SGA.

Discussion

We observed that maternal vitamin D status at <22 wk gestation had a U-shaped relation with SGA risk in white women. Among whites, the risk of SGA declined precipitously as serum 25(OH)D increased to ~ 70 nmol/L, after which the rise in risk was less steep, but still significant, even after excluding women with serum 25(OH)D >100 nmol/L. Thus, the lowest risk of SGA among whites was at serum 25(OH)D concentrations from ~ 60 to 80 nmol/L. No association was found among black women.

To our knowledge, our study is the first to examine the relation between maternal serum 25(OH)D in early pregnancy

TABLE 1 Maternal characteristics among SGA age cases and controls¹

	Whites		Blacks	
	Non-SGA controls	SGA cases	Non-SGA controls	SGA cases
Median maternal age, <i>y</i>	23 [15–42]	22 [14–36]	19 [14–35]	20 [15–35]
Maternal age, <i>n</i> (%)				
<20 <i>y</i>	47 (24.0)	18 (23.4)	56 (53.3)	16 (47.1)
20–29 <i>y</i>	105 (53.6)	50 (64.9)	42 (40.0)	17 (50.0)
≥30 <i>y</i>	44 (22.5)	9 (11.7)	7 (6.7)	1 (2.9)
Marital status, <i>n</i> (%)				
Married	77 (39.3)	17 (22.1)	102 (97.1)	32 (94.1)
Unmarried	119 (60.7)	60 (77.9)	3 (2.9)	2 (5.9)
Private insurance, <i>n</i> (%)				
No	110 (62.5)	52 (72.2)	84 (84.9)	27 (84.4)
Yes	66 (37.5)	20 (27.8)	15 (15.2)	5 (15.6)
Median composite of maternal SES	5.3 [0.7–9.4]	4.2 [0.7–9.3]	3.9 [0.7–8.5]	3.3 [0.7–8.5]
Quartiles of maternal SES composite variable, <i>n</i> (%)				
Q1 [median 1.92]	37 (18.9)	21 (27.3)	35 (33.3)	15 (44.1)
Q2 [median 3.82]	42 (21.4)	24 (31.2)	22 (21.0)	10 (29.4)
Q3 [median 5.62]	42 (21.4)	14 (18.2)	40 (38.1)	7 (20.6)
Q4 [median 8.40]	75 (38.3)	18 (23.3)	8 (7.6)	2 (5.9)
Smoking status in the year before the index pregnancy, <i>n</i> (%)				
Nonsmokers	91 (46.4)	27 (35.5)	63 (60.6)	22 (68.8)
1–10 cigarettes/d	38 (19.4)	16 (21.1)	32 (30.8)	9 (28.1)
≥11 cigarettes/d	67 (34.2)	33 (43.4)	9 (8.7)	1 (3.1)
Smoking status in the 3 mo prior to enrollment, <i>n</i> (%)				
Nonsmokers	130 (66.3)	36 (46.8)	86 (81.9)	24 (70.6)
1–10 cigarettes/d	50 (25.5)	33 (42.8)	17 (16.2)	9 (26.5)
≥11 cigarettes/d	16 (8.2)	8 (10.4)	2 (1.9)	1 (2.9)
Prepregnancy BMI, <i>n</i> (%)				
<18.5 kg/m ²	17 (8.7)	10 (13.0)	3 (2.9)	4 (11.7)
18.5–24.9 kg/m ²	109 (55.6)	48 (62.3)	50 (47.5)	16 (47.1)
25.0–29.9 kg/m ²	43 (21.9)	13 (16.9)	26 (24.8)	5 (14.7)
≥30.0 kg/m ²	27 (13.8)	6 (7.8)	26 (24.8)	9 (26.5)
Periconceptional multivitamin use at least once/wk, <i>n</i> (%)				
Yes	88 (44.9)	42 (54.6)	72 (68.6)	23 (67.6)
No	108 (55.1)	35 (45.4)	33 (31.4)	11 (32.4)
Preconception leisure-time physical activity, <i>n</i> (%)				
None	106 (54.1)	51 (66.2)	71 (68.3)	21 (61.8)
Yes, low intensity	13 (6.6)	5 (6.5)	9 (8.7)	3 (8.8)
Yes, medium intensity	42 (21.4)	12 (15.6)	16 (15.4)	6 (17.7)
Yes, high intensity	35 (17.9)	9 (11.7)	8 (7.6)	4 (11.7)
Season of blood sampling, <i>n</i> (%)				
Winter	38 (19.4)	17 (22.1)	25 (23.8)	7 (20.6)
Spring	54 (27.6)	23 (29.9)	38 (36.2)	14 (41.2)
Summer	50 (25.5)	20 (26.0)	18 (17.1)	8 (23.5)
Fall	54 (27.6)	17 (22.0)	24 (22.9)	5 (14.7)
Median birthweight centile	57.1 [11.7–100]	5.4 [0.1–9.9]	53.0 [13.1–98.8]	5.3 [0.1–9.9]
Median gestational age of blood sampling, <i>wk</i>	10.4 [4.7–21.0]	10.1 [5.6–20.4]	10.4 [5.3–20.4]	9.1 [5.4–19.6]
Gestational age of blood sampling, <i>n</i> (%)				
0–13 <i>wk</i>	135 (68.9)	52 (67.5)	66 (62.9)	23 (67.7)
14 to <22 <i>wk</i>	61 (31.1)	25 (32.5)	39 (37.1)	11 (32.3)
Large-for-gestational age, ² <i>n</i> (%)	31 (15.8)	–	12 (11.4)	–
Delivery <37 <i>wk</i> , <i>n</i> (%)	15 (7.7)	6 (7.8)	10 (9.5)	4 (11.8)
Preeclampsia, ³ <i>n</i> (%)	9 (4.6)	4 (5.2)	1 (1.0)	2 (5.9)

¹ Values are *n* (%) or medians [range]. Missing data were as follows: insurance status was missing for 10 white controls, 5 white cases, 6 black controls, and 2 black cases; year-prior smoking status was missing for 1 white SGA case; preconception physical activity was missing for 1 black control.

² Large-for-gestational age was defined as live-born infants that were >90th percentile of birthweight according to nomograms based on gender and gestational age from a reference population of over 50,000 infants delivered at Magee-Womens Hospital in Pittsburgh, PA.

³ Preeclampsia was defined as new-onset hypertension and proteinuria after 20 *wk* of gestation.

TABLE 2 Association between maternal vitamin D status and the risk of SGA by race/ethnicity

	Controls	SGA	Unadjusted OR (95% CI)	Adjusted OR ¹ (95% CI)
<i>n</i> (%)				
White women				
Serum 25(OH)D at <22 wk ²				
<37.5 nmol/L	3 (1.5)	8 (10.4)	10.6 (2.6, 42.5)	7.5 (1.8, 31.9)
37.5–75 nmol/L	107 (54.6)	27 (35.1)	1.0 (ref)	1.0 (ref)
>75 nmol/L	86 (43.9)	42 (54.5)	1.9 (1.1, 3.4)	2.1 (1.2, 3.8)
Quartile 1 [21.0–58.0 nmol/L]	49 (25.0)	25 (32.5)	3.1 (1.3, 7.6)	2.7 (1.1, 6.8)
Quartile 2 [58.1–71.4 nmol/L]	49 (25.0)	8 (10.4)	1.0 (ref)	1.0 (ref)
Quartile 3 [71.5–90.6 nmol/L]	49 (25.0)	15 (19.5)	1.9 (0.7, 4.8)	1.9 (0.7, 5.1)
Quartile 4 [90.7–245.0 nmol/L]	49 (25.0)	29 (37.6)	3.6 (1.5, 8.7)	3.9 (1.6, 9.7)
Black women				
Serum 25(OH)D at <22 wk				
<37.5 nmol/L	48 (45.7)	17 (50.0)	1.4 (0.6, 3.1)	1.5 (0.6, 3.5)
37.5–75 nmol/L	50 (47.6)	13 (38.2)	1.0 (ref)	1.0 (ref)
>75 nmol/L	7 (6.7)	4 (11.8)	2.2 (0.6, 8.7)	2.2 (0.5, 9.0)
Quartile 1 [13.8–30.0 nmol/L]	27 (25.7)	11 (32.4)	1.8 (0.6, 5.5)	1.7 (0.5, 5.5)
Quartile 2 [30.1–38.8 nmol/L]	26 (24.8)	6 (17.7)	1.0 (ref)	1.0 (ref)
Quartile 3 [40.4–49.3 nmol/L]	26 (24.8)	5 (14.7)	0.8 (0.2, 3.1)	0.8 (0.2, 3.2)
Quartile 4 [49.4–137.2 nmol/L]	26 (24.8)	12 (35.3)	2.0 (0.7, 6.1)	1.8 (0.5, 5.8)

¹ Adjusted for prepregnancy BMI, smoking during pregnancy, and SES. Additional adjustment for season, maternal age, gestational age at blood sampling, marital status, insurance status, smoking in the year before pregnancy, periconceptional multivitamin use, or preconception physical activity had no meaningful impact on the results.

² Distribution differs by case status, $P < 0.0001$ (Pearson chi-squared test).

and risk of fetal growth restriction. Brooke et al. (12) conducted a randomized, double-blinded, placebo-controlled trial among 126 Asian mothers living in Britain and found that compared with placebo, supplementation with 1000 IU (25 µg) vitamin D/d starting in the 3rd trimester reduced the risk of SGA (15 vs. 28%; $P < 0.05$) and increased 25(OH)D concentrations (mean 168.0 vs. 16.2 nmol/L; $P < 0.001$). In another trial, supplementation with 600,000 IU (15 mg) twice in gestational mo 7 and 8 reduced the incidence of low birth weight (<2500 g) compared with placebo (4 vs. 19%) among 200 Indian pregnant women (18). Serum 25(OH)D was not assessed.

Other previous studies of vitamin D and fetal growth examined measures of size at birth on a continuum with mixed results. One vitamin D supplementation trial and 4 observational studies of 25(OH)D levels, most of which studied late-pregnancy exposure, found no relation with birth weight or other measures of size at birth (13–16,20,39). In contrast, another 3rd-trimester vitamin D supplementation trial and an observational study of maternal vitamin D intake found positive associations with birth weight (17,19). However, the problem with studying birth weight on a continuum is that it is not an intrinsically pathologic outcome. The relation between vitamin D and fetal growth or size may not have as close a relationship in accounting for variation in normal as it would in accounting for variation in a pathophysiologic condition like growth restriction. The conflicting findings may also be due to the study of vitamin D in late pregnancy, rather than early pregnancy, when factors affecting fetal growth have the greatest impact (40).

Vitamin D has a biologically plausible role in fetal growth. In terms of placental development and function, the vitamin D-activating enzyme CYP27B1 as well as VDR are present in human placenta (41). 1,25-Dihydroxyvitamin D, the active form of vitamin D, acting through the VDR and the cAMP/protein kinase A signaling pathway, regulates human chorionic gonadotropin expression and secretion in human syncytiotrophoblast

(42) and increases placental sex steroid production (43). Vitamin D is also important in glucose/insulin metabolism and homeostasis (44) and so may play a role in glucose availability for transplacental transport and fetal usage. As a regulator of calcium homeostasis and transport, calcitriol also can influence fetal growth directly through influences on skeletal muscle and bone development (45).

The elevated risk of SGA among vitamin D-sufficient white women that we observed previously remained after excluding serum 25(OH)D levels >100 nmol/L, which might have a greater likelihood of being misclassified because of the assay method we used (31). The increased risk with vitamin D sufficiency has not been observed previously and its potential mechanisms remain uncertain. Notably, others have observed U-shaped associations between 25(OH)D and risk of atopic disorders (46,47) and allergic response (48), lower-extremity function (49), prostate cancer (50), and colorectal cancer (51), which suggests that vitamin D may have dual effects. The U-shaped relationship we observed is further supported by time series analyses of birth weight. Such analyses point to nonlinear risk curves, such as those for vitamin D, in underpinning well-known seasonal variability in infant birth weight (52). However, we cannot rule out residual confounding as a potential explanation for our finding, e.g. by intake of fatty fish, which may raise serum 25(OH)D concentrations (53) while also increasing exposure to growth-restricting organic pollutants (54). We lacked data on other dietary confounders to address this possibility. Research to understand the mechanisms underlying this U-shaped relation is a high priority. Because the definition of vitamin D sufficiency (typically >75 nmol/L) was selected based on parathyroid hormone levels, calcium transport, and bone outcomes (33,55), it is possible that these definitions do not apply to SGA and other pregnancy outcomes. Importantly, researchers should explore whether genotypic differences in the study population may explain this U-shaped relation.

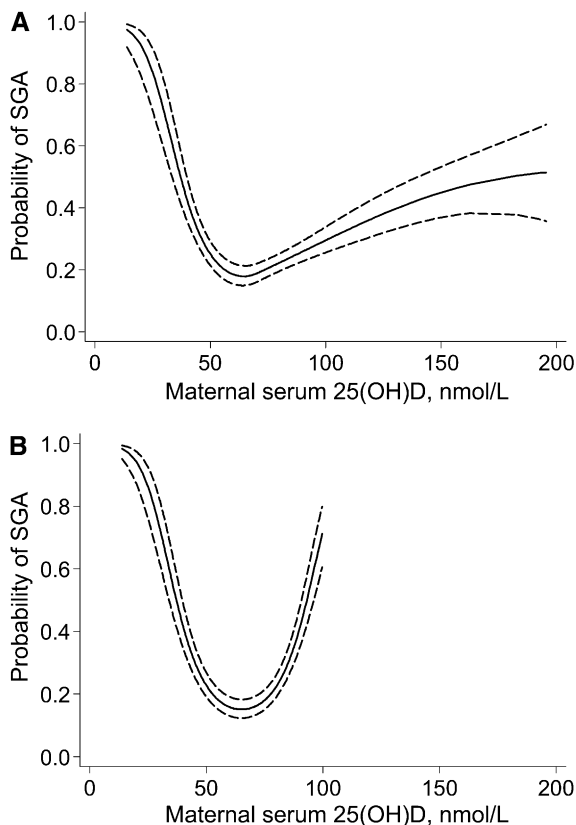


FIGURE 1 Unadjusted association between the probability of SGA births and serum 25(OH)D concentrations among white women (A; $n = 273$) and white women with a 25(OH)D ≤ 100 nmol/L (B; $n = 217$) at <22 wk. The point estimates were derived from logistic regression models with serum 25(OH)D concentrations specified as a quadratic spline with knot at 70 nmol/L ($P = 0.006$; A) or quadratic term ($P < 0.0001$; B). The solid line represents the point estimate and the dotted lines represent the 95% confidence bands.

To our knowledge, ours is the first study to assess serum 25(OH)D in relation to SGA risk among black women. Future studies with large samples of black women are needed to confirm or refute our finding of a lack of an association. The reason for the lack of association remains unclear. It may have been due to a relatively small sample of black cases and/or relatively narrow distribution of serum 25(OH)D among black women. However, others have also observed a differential association between vitamin D and bone health and diabetes by race/ethnicity (23–25). Scientists have postulated that blacks are less sensitive to the negative impacts of vitamin D deficiency. For instance, although secondary hyperparathyroidism results from vitamin D deficiency in blacks, there is a net increase in bone mass and decreased risk of osteoporosis that is observed in blacks compared with whites (25). This race difference indicates that the ability of parathyroid hormones to induce bone turnover is lower among blacks than whites (25). Researchers speculate that blacks may have experienced skeletal and renal adaptations to vitamin D deficiency that explain their lack of association between vitamin D and bone health (23,25). Whether this applies to pregnancy outcomes is unknown. It is possible that exposures other than vitamin D deficiency may contribute to the higher risk of growth restriction in black infants compared with white infants (56).

Recently, Morley et al. (27) observed that sequence variation in the VDR gene modified the effect of maternal vitamin D

TABLE 3 Association between VDR SNP and the risk of SGA birth by race/ethnicity¹

	OR (95% CI)	P-value	Model r^2	Model adjusted r^2
White women				
Model 1			2.6	3.8
rs11168292	1.8 (1.1, 3.0)	0.02		
Model 2			8.0	12.0
rs11168292	1.7 (1.1, 2.8)	0.04		
Serum 25(OH)D at <22 wk				
<37.5 nmol/L	14.0 (2.7, 73.0)	0.005		
37.5–75 nmol/L	1.0 (ref)			
>75 nmol/L	2.0 (1.1, 3.7)	0.17		
Black women				
Model 1			12.3	18.6
rs11168287	0.4 (0.2, 0.9)	0.03		
rs3782905	5.7 (1.8, 17.7)	0.004		
rs2239179	0.3 (0.1, 0.6)	0.003		
Model 2			14.0	21.0
rs11168287	0.4 (0.2, 0.8)	0.02		
rs3782905	5.3 (1.8, 17.4)	0.003		
rs2239179	0.3 (0.1, 0.7)	0.004		
Serum 25(OH)D at <22 wk				
<37.5 nmol/L	1.4 (0.5, 3.9)	0.61		
37.5–75 nmol/L	1.0 (ref)			
>75 nmol/L	3.4 (0.6, 17.4)	0.18		

¹ Additive models were analyzed for each SNP. OR represent the increase in odds associated with the addition of 1 allele.

deficiency on infant size at birth. These investigators noted that low 25(OH)D concentrations were associated with lower birthweight infants only among infants that were either homozygous for the *FokI* major allele or heterozygotes. Although we did not have the power to study the modifying effect of VDR SNP in our analysis, our data suggest an association of sequence variation in the VDR gene with the risk of SGA. However, we did not find a relation between SGA and 2 commonly studied variants in the VDR gene, *BsmI* (rs1544410) and *FokI* (rs2228570), which previously have been associated with complex phenotypes, including cancer, diabetes, and fracture (26), or SNP in linkage disequilibrium with these variants. These findings should be viewed as preliminary; the number of cases within each race stratum limited our power. Similarly, our sample size limited our exploration of SNP to those with a predicted minor allele frequency of $\geq 5\%$. Thus, we cannot exclude the possible association of less common variants in the VDR gene and risk of SGA. Additionally, we explored only the contribution of maternal genotype to risk of SGA. It is certainly possible that the fetal VDR gene plays a role in regulation of fetal growth and thus also deserves study. Despite these limitations, these findings highlight the importance of future studies with larger sample size, mother-child pairs, and genotyping that includes less common variants in the VDR gene. None of the associated SNP are known to be potentially functional and thus may be in linkage disequilibrium with the causal variant(s). These data also support the performance of more fundamental, mechanistic work exploring the influence of VDR sequence variants on the function of the receptor itself with respect to binding affinity and downstream signal transduction.

Our study was limited by its reliance on the Immunodiagnostic Systems Limited ELISA for assessing serum 25(OH)D, which overestimates serum 25(OH)D >100 nmol/L. We did not

have the resources to reanalyze the samples with an assay that is more accurate at high levels. Nevertheless, the overestimation of serum 25(OH)D would apply equally to cases and controls and would not systematically bias our results. Further, we observed similar U-shaped relations when results were limited to values ≤ 100 nmol/L. Our outcome, SGA, is an imperfect measure of fetal growth restriction, which is defined as the failure of an infant to exercise its genetic growth potential (57). By definition, SGA cases will include some infants that are constitutionally small rather than pathologically small and, conversely, exclude some infants that have failed to achieve their growth potential (57). We used a local population-based birthweight standard to define SGA rather than a national standard, such as those of Alexander et al. (58), but the results were not meaningfully different after applying this national standard (data not shown). Nevertheless, compared with birthweight-based growth standards, customized fetal growth trajectories are able to provide a more precise estimate of pathologic growth (59–61) and should be explored in future studies of maternal vitamin D status.

We also did not have the resources to measure biomarkers of the vitamin D endocrine system, parathyroid hormone concentration, or other functional indicators of maternal vitamin D status. Unmeasured confounders or error in the measurement of existing confounding factors may have biased our results. Future studies of vitamin D and growth restriction would benefit from using serial ultrasound measurements, the gold standard assessment, quantifying other vitamin D-related biomarkers, and considering a wide range of dietary and lifestyle confounding factors.

Our results suggest that vitamin D deficiency is associated with SGA in white women and a more modest increased risk may also exist for higher serum 25(OH)D levels. Additional studies are needed to refute or replicate these findings given the high prevalence of vitamin D deficiency in U.S. pregnant women.

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