

Early pregnancy vitamin D status and risk for adverse maternal and infant outcomes in a bi-ethnic cohort: the Behaviors Affecting Baby and You (B.A.B.Y.) Study

Carrie J. Nobles¹, Glenn Markenson² and Lisa Chasan-Taber^{1*}

¹Department of Biostatistics and Epidemiology, School of Public Health & Health Sciences, Division of Biostatistics & Epidemiology, University of Massachusetts, Amherst, MA 01003-9304, USA

²Baystate Medical Center, Division of Maternal-Fetal Medicine, Springfield, MA 01199, USA

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Abstract

Vitamin D deficiency is common during pregnancy and higher in Hispanic as compared with non-Hispanic white women. However, the association between vitamin D deficiency and adverse pregnancy outcomes remains unclear and may vary across ethnic groups, in part because of genetic variation in the metabolism of vitamin D. Few studies have included Hispanic women. Therefore, we investigated this association among 237 participants in the Behaviors Affecting Baby and You Study, a randomised trial of an exercise intervention among ethnically diverse prenatal care patients in Massachusetts. Baseline serum 25-hydroxyvitamin D (25(OH)D) was measured at 15.2 (SD 4.7) weeks' gestation. Information on adverse pregnancy outcomes was abstracted from medical records. Mean 25(OH)D was 30.4 (SD 12.0) ng/ml; 53.2% of participants had insufficient (<30 ng/ml) and 20.7% had deficient (<20 ng/ml) 25(OH)D levels. After adjusting for month of blood draw, gestational age at blood draw, gestational age at delivery, age, BMI and Hispanic ethnicity, women with insufficient and deficient vitamin D had infants with birth weights 139.74 (SE 69.16) g ($P=0.045$) and 175.52 (SE 89.45) g ($P=0.051$) lower compared with women with sufficient vitamin D levels (≥ 30 ng/ml). Each 1 ng/ml increase in 25(OH)D was associated with an increased risk for gestational diabetes mellitus among Hispanic women only (relative risk 1.07; 95% CI 1.03, 1.11) in multivariable analysis. We did not observe statistically significant associations between maternal vitamin D status and other pregnancy outcomes. Our findings provide further support for an adverse impact of vitamin D deficiency on birth weight in Hispanic women.

Key words: Epidemiology; Latina; Prospective cohort studies; Vitamin D; Gestational diabetes

Low levels of 25-hydroxyvitamin D (25(OH)D) during pregnancy have been proposed as a potential contributor to adverse maternal and infant outcomes, including gestational diabetes mellitus (GDM), low birth weight/small for gestational age (SGA), spontaneous preterm delivery, caesarean delivery and gestational hypertension and preeclampsia^(1,2). Among pregnant women in the USA surveyed in the National Health and Nutrition Examination Survey from 2001 to 2006, 42% were deficient in vitamin D (defined as <20 ng/ml) and 36% were insufficient (defined as 20 to <30 ng/ml)^(3,4).

Prior observational studies of these associations have been conflicting, however, and a recent systematic review concluded that the current evidence base is insufficient to support clinical recommendations regarding vitamin D supplementation in pregnancy⁽⁵⁾. Specifically, in terms of adverse maternal complications, nine observational studies found that increasing 25(OH)D levels were associated with a reduced risk for

GDM^(6–14), ten found no significant association^(15–24), whereas one found that increasing 25(OH)D levels were associated with a borderline-significant increased risk for GDM⁽²⁵⁾. Eight studies found increasing 25(OH)D levels to be associated with a lower risk for preeclampsia and gestational hypertension^(26–33), whereas six found no significant association^(15,17,25,34–36).

In terms of adverse infant outcomes, three studies found a positive association between 25(OH)D level and birth weight^(37–39), whereas four studies found no significant association^(23,25,40,41). Similarly, seven studies have found a lower risk of delivering an SGA infant with increasing 25(OH)D levels^(37,38,42–46), whereas three studies found no significant association^(15,17,35). Four studies found increasing 25(OH)D levels to be associated with decreased risk for preterm or spontaneous preterm delivery^(47–50), one found an increased risk⁽²⁵⁾, and six found no significant association^(15,17,35,51–53). Three studies found a lower risk for caesarean delivery with

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; B.A.B.Y., Behaviors Affecting Baby and You; GDM, gestational diabetes mellitus; LGA, large for gestational age; RR, relative risk; SGA, small for gestational age.

* **Corresponding author:** Dr L. Chasan-Taber, fax +1 413 545 1645, email lct@schoolph.umass.edu

increasing 25(OH)D levels^(49,54,55), whereas three found no significant association^(15,25,56).

The proportion of pregnant women with vitamin D deficiency and insufficiency varies by race/ethnicity⁽⁵⁷⁾, with Hispanic women having higher proportions of vitamin D deficiency (45%) compared with non-Hispanic white women (13%)^(4,58). This wide variation in vitamin D deficiency levels between Hispanic and non-Hispanic white women may be due to a variety of factors including differences in the use of dietary supplements and the amount of vitamin D intake from foods. In turn, differences in the association between vitamin D and pregnancy outcomes between Hispanic and non-Hispanic whites may be due to residual confounding by social status and wealth⁽⁵⁹⁾ or, in part, due to genetic variation in the metabolism of vitamin D⁽⁶⁰⁾. However, the majority of the observational studies have been limited to predominantly non-Hispanic white populations. In spite of this, several studies have found that low vitamin D increased the risk for SGA only among white women as compared with African-American women^(37,45,46); one study found that low vitamin D increased the risk for spontaneous preterm birth only among non-white women (black and Puerto Rican women combined) but not among white women⁽⁴⁷⁾; and one found that deficient vitamin D increased the risk for preterm birth only among white women but not among black women⁽⁵²⁾. However, none assessed the role of Hispanic ethnicity as an effect modifier of the relationship between vitamin D and adverse pregnancy outcomes.

Additional limitations of the prior literature include the fact that few studies have been conducted among populations at high risk for GDM, the most common treatable maternal complication of pregnancy⁽⁶¹⁾. Incidence rates of GDM are increasing in the USA and there is evidence that this increase corresponds to the rising rates of obesity and sedentary lifestyle among women of reproductive age⁽⁶²⁾. Therefore, evaluating the potential impact of a modifiable risk factor such as vitamin D in this group has high public health significance. Finally, to our knowledge, no studies have evaluated the association between 25(OH)D level and gestational weight gain as an outcome.

Therefore, we conducted a prospective analysis utilising data from the Behaviors Affecting Baby and You (B.A.B.Y.) Study. The B.A.B.Y. Study was a randomised trial of an exercise intervention during pregnancy among ethnically diverse prenatal care patients in Western Massachusetts. We had two specific goals: (1) to investigate the association between vitamin D status in early pregnancy and the risk for subsequent adverse maternal and infant outcomes; and (2) to investigate whether these associations differed according to Hispanic ethnicity.

Methods

Study design and population

The B.A.B.Y. Study was a randomised trial assessing the effectiveness of an exercise intervention during pregnancy on reducing the risk for GDM. The B.A.B.Y. Study was conducted between 2007 and 2012 at Baystate Medical Center, a large tertiary care facility primarily serving the ethnically and socio-economically diverse communities of Springfield and

Holyoke, MA. Details regarding the B.A.B.Y. Study have been published elsewhere⁽⁶³⁾.

The primary inclusion criterion for the B.A.B.Y. Study was being at high risk for GDM, identified as meeting one or both of the following criteria: (1) having experienced GDM in a prior pregnancy, and/or (2) having a family history of type 2 diabetes mellitus and a BMI ≥ 25 kg/m². Participants were excluded if they met or exceeded the American Congress of Obstetricians and Gynecologists guidelines for physical activity (30 min of moderate or vigorous activity on ≥ 3 d/week), were younger than 18 or older than 40 years of age, were at more than 20 weeks' gestation, were unable to read English at a sixth-grade level, did not plan to deliver at Baystate Medical Center, had a non-singleton pregnancy, had contraindications to engaging in moderate exercise, were currently taking medications that adversely influenced glucose tolerance, or had a personal history of diabetes outside of pregnancy, hypertension, heart disease or chronic renal disease.

A total of 252 women met the eligibility criteria for the B.A.B.Y. Study and completed a baseline assessment before randomisation. For the purposes of the current analysis, we also excluded participants who did not deliver at Baystate Medical Center (*n* 13) or who experienced a miscarriage or terminated pregnancy after randomisation (*n* 2), for a final sample size of 237. Women were included in the current analysis whether or not they were subsequently randomised. Reasons for not being randomised (*n* 50) included unanticipated events that occurred at the time between enrolment and randomisation, including the following: (1) contraindications to exercise noted at a subsequent prenatal medical exam (*n* 14; 28%); (2) gestational age at or near the end of the second trimester at the time of randomisation (*n* 24; 48%); or (3) lack of interest in continuing the study (*n* 12; 24%).

After a baseline assessment at which time blood was collected and covariates, including pre-pregnancy BMI, were self-reported, participants were randomised to either a 12-week exercise intervention or a comparison health and wellness intervention. Both the exercise and health and wellness interventions consisted of one face-to face meeting followed by weekly and bi-weekly mailed material and telephone booster calls.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the University of Massachusetts and Baystate Medical Center Institutional Review Boards. Written informed consent was obtained from all subjects/patients. This B.A.B.Y. Study is registered at ClinicalTrials.gov under 'An Exercise Intervention to Prevent Gestational Diabetes', under registration number NCT00728377 (<https://clinicaltrials.gov/ct2/show/NCT00728377?term=chasan-taber&rank=1>).

Assessment of 25-hydroxyvitamin D

After enrolment, participants provided a fasting blood draw as part of their baseline assessment (mean 15.2 (SD 4.7) weeks' gestation). Blood draws were centrifuged, aliquoted and stored at -80°C . Assessment of total vitamin D (25(OH)D₂ and D₃) in single aliquots was conducted by Heartland Assays, in

Ames, IA, using DiaSorin Liaison (DiaSorin). DiaSorin Liaison has a concordance correlation coefficient of 0.95 as compared with liquid chromatography-tandem MS, which is considered to be the gold standard for assessment of total 25(OH)D⁽⁶⁴⁾. For values >8 ng/ml, DiaSorin Liaison has CV for total and repeatability variation of <10%⁽⁶⁵⁾.

Although no consensus exists for defining cutoff points for vitamin D deficiency and insufficiency most strongly associated with risk for adverse pregnancy outcomes, for the purpose of this study 25(OH)D level was categorised as deficient for levels below 20 ng/ml, insufficient for levels between 20 and <30 ng/ml, and sufficient for levels at or above 30 ng/ml, based on summary recommendations from Holick⁽³⁾.

Assessment of adverse maternal and infant outcomes

After delivery, data on GDM, hypertension in pregnancy and preeclampsia, infant birth weight, birth length, gestational age at birth, spontaneous preterm delivery, caesarean delivery and mother's weight at delivery were abstracted from medical records. Diagnoses were confirmed by the study obstetrician. GDM diagnosis was based upon American Diabetes Association criteria⁽⁶⁶⁾. Women were screened with a non-fasting 1-h, 50 g oral glucose challenge test at 24–28 weeks' gestation. Women with glucose concentrations >135 mg/dl (7.5 mmol/l) were referred for a fasting 3-h, 100 g oral glucose tolerance test. If women exceeded two or more of the following cutoff points – 95 mg/dl (5.3 mmol/l) at fasting, 180 mg/dl (10.0 mmol/l) at 1 h, 155 mg/dl (8.6 mmol/l) at 2 h and 140 mg/dl (7.8 mmol/l) at 3 h – they were diagnosed with GDM. Gestational hypertension was defined on the basis of criteria from the American College of Obstetricians and Gynecologists task force on hypertension in pregnancy, defined as two blood pressure measurements >140/90 mmHg after 20 weeks' gestation in a previously normotensive woman⁽⁶⁷⁾. Preeclampsia was defined as the new onset of hypertension (blood pressure \geq 140/90 mmHg) after 20 weeks' gestation in association with proteinuria, either \geq 1+ by dipstick or \geq 300 mg/24 h in the absence of urinary infection⁽⁶⁷⁾.

Gestational weight gain was calculated as the difference between maternal weight at delivery and pre-pregnancy weight. Total gestational weight gain was further categorised as 'inadequate', 'adequate' or 'excessive' based on pre-pregnancy BMI, following the Institute of Medicine criteria⁽⁶⁸⁾. SGA was calculated as an infant birth weight less than the lower 10th percentile based on gestational age at birth, and large for gestational age (LGA) was calculated as an infant birth weight at or above the 90th percentile, based on estimates in the general US population by Alexander *et al.*⁽⁶⁹⁾, which were established in a diverse, nationally representative cohort. Estimates were applied independent of infant race/ethnicity and sex as these data were unavailable. Birth weight was adjusted for gestational age, and investigated independently, and also standardised for birth length by taking the birth weight:birth length ratio.

Covariate assessment

Data on socio-demographics, medical history factors and health behaviours were obtained through self-report at entry into the

study using standardised questionnaires. Socio-demographic characteristics included age, socio-economic status (household income: \geq \$15 000 *v.* <\$15 000/year), educational attainment (>high school *v.* \leq high school), family structure (marital status: married *v.* unmarried) and number of children in a household (any children *v.* no children in household). Hispanic ethnicity (Hispanic *v.* non-Hispanic) was assessed through self-report in the manner of the US Census, the Behavioral Risk Factor Surveillance Survey, and the National Health Interview Survey. Specifically, participants were asked 'Are you Latina or of Spanish or Hispanic origin or descent? no/yes'. Medical history factors included pre-pregnancy BMI (<25, 25 to <30, \geq 30 kg/m²), parity (parous *v.* nulliparous) and personal history of GDM (yes *v.* no). Information on pre-pregnancy weight and height was abstracted from medical records.

Health behaviours include smoking (\geq 1 cigarette *v.* <1 cigarette/d), sports/exercise activity (\geq 7.5 *v.* <7.5 MET-h/week) and sedentary behaviour (\geq 2 *v.* <2 h/d). The Pregnancy Physical Activity Questionnaire⁽⁷⁰⁾ was used to assess sports/exercise activity and sedentary behaviour, and asked participants to report the relative frequency with which they engaged in a variety of sports/exercise activities of light, moderate and vigorous intensity and sedentary activities. The time spent in sports/exercise activities was multiplied by the metabolic equivalent of task (MET) for each activity to derive MET-h/week^(71,72). Smoking during early pregnancy was assessed using a modified version of the Pregnancy Risk Assessment Monitoring System questionnaire⁽⁷³⁾.

Additional covariates were obtained from study records and included gestational age at blood draw, month of blood draw and assigned intervention arm (exercise arm, health and wellness arm, or not randomised). Season of blood draw was calculated by dividing month of blood draw into 'winter' and 'summer'. To account for the estimated 4–8-week half-life of 25(OH)D in serum after exposure to UV radiation⁽⁷⁴⁾, date cutoff points for 'summer' and 'winter' were derived by modelling the sinusoidal relationship between 25(OH)D level and month of blood draw ('1 May–31 October' for summer, winter otherwise)⁽⁷⁵⁾.

Statistical analysis

All socio-demographic, health behaviour and medical history factors, 25(OH)D level and outcome variables were summarised as numbers and percentages for categorical variables and as means and standard deviations for continuous variables. Associations between socio-demographic, health behaviour and medical history factors with vitamin D insufficiency were estimated using unadjusted logistic regression.

Associations between 25(OH)D levels and dichotomous pregnancy outcomes (i.e. GDM, gestational hypertension, spontaneous preterm birth, SGA, LGA and caesarean delivery) were calculated using modified Poisson's regression. Associations between 25(OH)D and the multilevel pregnancy outcome of gestational weight gain were calculated using multinomial logistic regression. Associations between 25(OH)D levels and continuous pregnancy outcomes (i.e. weight gain to delivery, gestational age at delivery and birth weight, birth length, and birth weight:length ratio) were calculated using multivariable linear regression.



Multivariable models were adjusted for potential confounding factors determined *a priori* by reviewing past studies and based on physiological considerations, and included month of blood draw, maternal age, pre-pregnancy BMI and Hispanic ethnicity^(20,46,47,54). Gestational age at blood draw was also included to account for variability in 25(OH)D level due to the increase in 25(OH)D that occurs with increasing gestation⁽⁷⁶⁾. Because of the particularly strong association between personal history of GDM and subsequent GDM, personal history of GDM was evaluated as a potential confounder, and included in any model in which its addition resulted in a change in effect estimate >10%⁽⁷⁷⁾. We additionally assessed the significance of measures of socio-economic position, including household income, educational attainment and marital status. As the adjustment of these factors did not result in a change in effect estimate >10%, none were included in the final models. Models with SGA, LGA and gestational weight gain as the outcomes of interest were additionally adjusted for gestational age at delivery (weeks) to account for variability in birth weight and gestational weight gain due to length of gestation, and birth weight was adjusted for the square of gestational age. Month of blood draw was modelled as a sinusoidal function⁽⁷⁵⁾.

We evaluated Hispanic ethnicity, trimester of blood draw and BMI as potential effect modifiers of the association between 25(OH)D and pregnancy outcomes by including an interaction term in the models. Because the evaluation of effect modification by Hispanic ethnicity was one of our primary aims, we present overall and ethnicity-stratified estimates for all adverse pregnancy outcomes. Study group assignment was added to all models to assess its importance as a confounder, and, as no differences in effect estimates were observed, this covariate was not retained in final multivariable models. Results of analyses were determined to be significant at $P < 0.05$. All analyses were conducted in SAS version 9.4 (SAS Institute Inc.).

Finally, we conducted several sensitivity analyses. First, we evaluated differences in baseline characteristics between participants excluded *v.* those included in the final analysis using χ^2 or Fisher's exact tests. Second, we evaluated the impact of missing data in the GDM analysis by assigning all participants with missing outcome data as GDM cases. Finally, we evaluated the effect of controlling for mid-pregnancy BMI (approximately 20 weeks' gestation) *v.* pre-pregnancy BMI.

Power

Because the original goal of the B.A.B.Y. Study was to evaluate the effectiveness of an exercise intervention during pregnancy on reducing the risk for GDM, power for the current analysis was based on *post hoc* calculations. We had excellent power to detect mean differences in continuous outcomes, with >80% power to detect differences in birth weight as small as 31.4 g (based on an SE of 89.2 g), in total gestational weight gain of 1.23 pounds (based on an SE of 3.6 pounds) and in gestational age at delivery of 1.27 weeks (based on an SE of 0.36 weeks). In terms of dichotomous outcomes, we had >80% power to detect OR of 0.48 or stronger magnitude for excessive gestational weight gain (based on an expected incidence rate of 0.63), of 0.23 or stronger magnitude for GDM (based on an expected incidence

rate of 0.14), and of 0.50 or stronger magnitude for caesarean delivery (based on an expected incidence rate of 0.37).

Results

A total of 237 participants enrolled in the B.A.B.Y. Study were included in the analysis. This included ninety-seven participants randomised to the exercise arm (40.9%), ninety-four participants randomised to the health and wellness arm (39.7%) and forty-six participants who were not randomised (19.4%). Mean gestational age at blood draw was 15.2 (SD 4.7) weeks (range 5.7, 36.7 weeks). A total of seventy-three participants had their 25(OH)D level assessed in the first trimester (<13 weeks' gestation), 160 in the second trimester (13 to <26 weeks' gestation) and four in the third trimester (≥ 26 weeks' gestation). Mean level of 25(OH)D in the study population was 30.4 (SD 12.0) ng/ml (range 6.6, 64.4 ng/ml). In all, forty-nine (20.7%) participants had deficient levels of vitamin D (<20 ng/ml), seventy-seven (32.5%) had insufficient levels (20 to <30 ng/ml) and 111 (46.8%) had sufficient levels (≥ 30 ng/ml).

The study population was predominantly young (68.8%, <30 years), Hispanic (57.0%) and of low income (51.7%, household incomes <\$15 000/year) (Table 1). In terms of race, 75.6% of the participants were white and 13.5% were black. The majority of participants were overweight or obese before pregnancy (94.9% with BMI ≥ 25 kg/m²), parous (70.7%) and reported >2 h of sedentary activity/d (52.9%). Mean 25(OH)D level was higher among participants who were older, non-Hispanic, had post-secondary education, were married and had their blood drawn at a more advanced gestational age (Table 1).

We evaluated predictors of vitamin D insufficiency and deficiency combined (25(OH)D <30 ng/ml) *v.* vitamin D sufficiency (≥ 30 ng/ml) (Table 2), and found that each higher year of maternal age, being married, and greater than a high school education were associated with a lower odds of vitamin D insufficiency (OR 0.95; 95% CI 0.91, 0.995, OR 0.38; 95% CI 0.21, 0.70 and OR 0.42; 95% CI 0.24, 0.73, respectively). Hispanic ethnicity was associated with a higher odds of vitamin D insufficiency (OR 2.53; 95% CI 1.49, 4.29). Similarly, increasing kg/m² of BMI was associated with a 5% greater odds of vitamin D insufficiency (OR 1.05; 95% CI 1.01, 1.10). Winter season of blood draw was associated with a 2-fold higher odds of insufficiency (OR 2.04; 95% CI 1.21, 3.44), and increasing week of gestational age at blood draw was associated with decreasing odds of insufficiency (OR 0.90; 95% CI 0.84, 0.95).

Adverse maternal outcomes

We then evaluated the association between vitamin D status and adverse maternal outcomes (Table 3). In all, thirty-one participants (13.7%) were diagnosed with GDM. After adjusting for age, BMI, Hispanic ethnicity, month of blood draw and gestational age at blood draw, each 1 ng/ml increase in 25(OH)D was associated with a borderline-significant greater risk for GDM (relative risk (RR) 1.03; 95% CI 1.00, 1.06) (Table 3).

Hispanic ethnicity was a significant effect modifier of this relationship ($P_{\text{interaction}} = 0.02$). Among Hispanic participants,



Table 1. Characteristics of study participants; the Behaviors Affecting Baby and You Study, 2007–2012 (Numbers and percentages; mean values and standard deviations; *n* 237)*

	Total		Vitamin D level (ng/ml)		Vitamin D insufficiency (<30 ng/ml; <i>n</i> 126)		Vitamin D sufficiency (≥30 ng/ml; <i>n</i> 111)	
	<i>n</i>	%	Mean	SD	<i>n</i>	%	<i>n</i>	%
Socio-demographic factors								
Age (years)								
18–19	28	11.8	28.6	8.9	17	13.5	11	9.9
20–24	84	35.4	29.8	12.3	46	36.5	38	34.2
25–29	51	21.5	26.4	11.0	34	27.0	17	15.3
30–40	74	31.2	34.5	12.5	29	23.0	45	40.5
Ethnicity								
Hispanic	135	57.0	28.1	10.8	85	67.5	50	45.1
Non-Hispanic	102	43.0	33.4	13.0	41	32.5	61	55.0
Household income								
<\$15 000	90	51.7	29.5	11.5	50	58.1	40	45.5
≥\$15 000	84	48.3	32.9	12.7	36	41.9	48	54.6
Education								
≤High school	117	55.2	28.3	11.5	72	65.5	45	44.1
>High school	95	44.8	33.8	12.2	38	34.6	57	55.9
Marital status								
Single/separated/divorced/widowed	170	72.7	28.5	11.4	101	81.5	69	62.7
Married	64	27.4	35.7	12.6	23	18.6	41	37.3
Children in household								
0	53	23.5	29.9	10.3	30	24.8	23	21.9
≥1	173	76.6	30.5	12.7	91	75.2	82	78.1
Medical history factors								
Pre-pregnancy BMI (kg/m ²)								
<25	12	5.1	32.0	11.6	5	4.0	7	6.3
25 to <30	81	34.2	32.6	11.8	41	32.5	40	36.0
≥30	144	60.8	29.0	12.1	80	63.5	64	57.7
Parity								
Nulliparous	68	29.3	30.2	9.5	39	31.5	29	26.9
Parous	164	70.7	30.4	13.0	85	68.6	79	73.2
Personal history of GDM								
Yes	26	11.2	32.0	11.4	11	8.9	15	13.6
No	207	88.8	30.1	12.1	112	91.1	95	86.4
Behavioural factors								
Smoking during pregnancy								
Yes	30	14.7	28.9	12.7	16	15.1	14	14.3
No	174	85.3	31.0	12.2	90	84.9	84	85.7
Sports/exercise activity – baseline (MET-h/week)								
<7.5	135	64.9	31.1	11.9	66	61.7	69	68.3
≥7.5	73	35.1	30.6	12.4	41	38.3	32	31.7
Sedentary behaviour – baseline (h/d)								
<2	97	47.1	32.2	14.0	48	44.4	49	50.0
≥2	109	52.9	29.4	10.2	60	55.6	49	50.0
Factors related to study design								
Gestational age at blood draw (weeks)								
<12	56	23.6	25.7	10.0	40	31.8	16	14.4
12 to <20	142	59.9	30.9	12.0	74	58.7	68	61.3
≥20	39	16.5	35.4	12.8	12	9.5	27	24.3
Season of blood draw								
Summer (1 May–31 October)	98	41.4	32.1	11.7	42	33.3	56	50.5
Winter (1 November–30 April)	139	58.7	29.2	12.2	84	66.7	55	49.6
Study group assignment								
Exercise arm	97	40.9	29.7	11.4	50	39.7	47	42.3
Health and wellness arm	94	39.7	31.0	12.3	48	38.1	46	41.4
Not randomised	46	19.4	30.7	12.9	28	22.2	18	16.2

GDM, gestational diabetes mellitus, MET, metabolic equivalent of task.
 * Numbers may not total to 237 because of missing data.

unadjusted mean vitamin D levels were 9 ng/ml lower among those who developed GDM (mean 27.1 (SD 10.5) ng/ml) as compared with those who did not develop GDM (mean 36.2 (SD 10.1) ng/ml). In multivariable models, we observed an adverse impact of 25(OH)D on risk for GDM limited to Hispanic

women only (*n* 135) (RR 1.07; 95% CI 1.03, 1.11 ng/ml). Among non-Hispanic women (*n* 102), the RR was 1.00 (95% CI 0.97, 1.04). In addition, Hispanic women with vitamin D insufficiency had a 68% lower risk for GDM as compared with those with vitamin D insufficiency (RR 0.32; 95% CI 0.11, 0.92).

Table 2. The association between covariates and vitamin D insufficiency *v.* sufficiency (<30 *v.* ≥30 ng/ml); the Behaviors Affecting Baby and You Study, 2007–2012 (Odds ratios and 95 % confidence intervals)

	Vitamin D insufficiency (<30 <i>v.</i> ≥30 ng/ml)	
	OR*	95 % CI
Socio-demographic factors		
Age (years)	0.95	0.91, 0.995
Hispanic ethnicity (yes <i>v.</i> no)	2.53	1.49, 4.29
Household income (≥\$15 000 <i>v.</i> <\$15 000/year)	0.60	0.33, 1.09
Education (>high school <i>v.</i> ≤high school)	0.42	0.24, 0.73
Marital status (married <i>v.</i> not married)	0.38	0.21, 0.70
Children in household (yes <i>v.</i> no)	0.85	0.46, 1.58
Behavioural factors		
Smoking during pregnancy (yes <i>v.</i> no)	1.07	0.49, 2.32
Sports/exercise activity (≥7.5 <i>v.</i> <7.5 MET-h/week)	1.34	0.76, 2.37
Sedentary behaviour (≥2 <i>v.</i> <2 h/d)	1.25	0.72, 2.16
Medical history factors		
BMI (kg/m ²)	1.05	1.01, 1.10
Parity (yes <i>v.</i> no)	0.80	0.45, 1.42
Factors related to timing of blood draw		
Gestational age at blood draw (week)	0.90	0.84, 0.95
Season (winter <i>v.</i> summer)	2.04	1.21, 3.44
Study group assignment		
Health and wellness arm <i>v.</i> exercise arm	0.98	0.56, 1.73
Not randomised <i>v.</i> exercise arm	1.46	0.72, 2.99

MET, metabolic equivalent of task.

* Logistic regression was used to independently model the odds of vitamin D insufficiency *v.* sufficiency for each covariate.

Vitamin D insufficiency was not associated with GDM among non-Hispanic women.

In all, fourteen (6.3%) participants were diagnosed with hypertension in pregnancy (inclusive of preeclampsia) and eleven (4.9%) were diagnosed with preeclampsia; thirty-three (16.4%) participants had inadequate total gestational weight gain, and 128 (63.7%) had excessive total gestational weight gain (Table 3). However, there were no observed associations between vitamin D status and overall hypertension in pregnancy, preeclampsia and inadequate or excessive gestational weight gain. Timing of blood draw was a statistically significant effect modifier of the relationship between vitamin D status and gestational weight gain. Among participants who had their blood drawn in the first trimester, a 1 ng/ml increase in 25(OH)D was associated with a 17% greater odds of having excessive gestational weight gain (OR 1.17; 95% CI 1.01, 1.36). In contrast, the OR of excessive gestational weight gain for those who had their blood drawn in the second and third trimesters was 1.01 (95% CI 0.98, 1.05).

Finally, personal history of GDM was not a significant confounder in any model. We also did not observe effect modification of the association between 25(OH)D and other adverse maternal outcomes by Hispanic ethnicity or timing of blood draw. None of the estimates stratified by Hispanic ethnicity reached significance with the exception of those for GDM risk.

Adverse infant outcomes

We then evaluated the association between vitamin D status and adverse infant outcomes (Table 4). A total of twelve women (5.5%) experienced a spontaneous preterm birth, twenty-one women (9.6%) gave birth to an SGA infant, thirty-one (14.2%)

to an LGA infant and eighty-three (36.9%) experienced a caesarean delivery. After adjusting for age, BMI, Hispanic ethnicity, month of blood draw, gestational age at blood draw and gestational age at delivery, women with vitamin D deficiency had a RR of 2.14 (95% CI 0.67, 6.88) for delivering an SGA infant, which was not statistically significant. We did not observe statistically significant effect modification of the relationship between vitamin D status and SGA by ethnicity ($P=0.12$). Similarly, vitamin D levels were not significantly associated with other categorical adverse infant outcomes.

Mean birth weight was 3379.67 (SD 594.48)g and mean gestational age was 39.14 (SD 1.93) weeks (range 26.9, 42.0 weeks). After adjusting for age, BMI, Hispanic ethnicity, month of blood draw, gestational age at blood draw and the square of gestational age at delivery, women who had insufficient vitamin D levels (<30 ng/ml) or deficient vitamin D levels (<20 ng/ml) had infants with birth weights 139.74 (SE 69.16)g ($P=0.045$) and 175.52 (SE 89.45)g ($P=0.051$) lower compared with women with sufficient vitamin D levels (≥30 ng/ml) (Table 4). Lastly, there was no statistically significant effect modification of this relationship by BMI ($P=0.31$).

Levels of 25(OH)D were not statistically significantly associated with gestational age at delivery, birth length or birth weight/length. Finally, no effect modification of any of the associations between 25(OH)D and infant outcomes was observed by Hispanic ethnicity or timing of blood draw. None of the estimates stratified by Hispanic ethnicity reached statistical significance.

Sensitivity analyses

There were no statistically significant differences between women who were excluded from the analysis (e.g. not eligible/did not

Table 3. Multivariable* relative risks (RR) for the association between vitamin D and adverse maternal outcomes; the Behaviors Affecting Baby and You Study, 2007–2012 (Numbers and percentages; relative risks, odds ratios and 95% confidence intervals; mean values and standard deviations; regression coefficients with their standard errors)

Maternal outcomes	Cases		Vitamin D continuous (ng/ml)			Vitamin D insufficiency (<30 v. ≥30 ng/ml)			Vitamin D deficiency (<20 v. ≥30 ng/ml)†					
	<i>n</i>	%	RR	95% CI	<i>P</i>	RR	95% CI	<i>P</i>	RR	95% CI	<i>P</i>			
Gestational diabetes mellitus (total group)	31	13.7	1.03	1.00, 1.06	0.08	0.80	0.36, 1.77	0.57	0.37	0.10, 1.43	0.15			
Hispanic‡	14	10.7	1.07	1.03, 1.11	0.001	0.32	0.11, 0.92	0.03						
Non-Hispanic‡	17	17.7	1.00	0.97, 1.04	0.85	1.91	0.77, 4.72	0.16						
Hypertension in pregnancy	14	6.3	0.98	0.93, 1.03	0.48	1.31	0.42, 4.12	0.65	1.41	0.30, 6.64	0.66			
Hispanic	8	6.1	0.97	0.89, 1.06	0.48	1.22	0.26, 5.60	0.80						
Non-Hispanic	6	6.5	0.99	0.95, 1.04	0.77	1.43	0.26, 7.86	0.68						
Preeclampsia	11	4.9	0.97	0.91, 1.03	0.26	2.05	0.55, 7.64	0.29	2.81	0.52, 15.34	0.23			
Hispanic	6	4.6	0.95	0.85, 1.06	0.36	1.75	0.36, 8.60	0.49						
Non-Hispanic	5	5.4	0.98	0.94, 1.03	0.43	2.42	0.38, 15.46	0.35						
	<i>n</i>	%	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>			
Inadequate gestational weight gain§	33	16.4	1.00	0.95, 1.04	0.79	0.96	0.33, 2.82	0.94	0.63	0.16, 2.52	0.51			
Hispanic	21	17.1	1.01	0.95, 1.07	0.83	0.63	0.11, 3.76	0.61	0.31	0.03, 3.93	0.37			
Non-Hispanic	12	15.4	0.98	0.92, 1.05	0.62	1.24	0.31, 4.90	0.76	0.91	0.17, 5.02	0.91			
Excessive gestational weight gain§	128	63.7	1.00	0.96, 1.03	0.93	0.88	0.29, 2.01	0.77	0.62	0.22, 1.71	0.35			
Hispanic	78	63.4	0.98	0.95, 1.04	0.74	1.11	0.30, 4.07	0.88	0.84	0.17, 4.26	0.83			
Non-Hispanic	50	64.1	1.00	0.96, 1.05	0.87	0.40	0.28, 2.15	0.62	0.51	0.15, 1.79	0.29			
	Mean	SD	<i>B</i>	SE	95% CI	<i>P</i>	<i>B</i>	SE	95% CI	<i>P</i>	<i>B</i>	SE	95% CI	<i>P</i>
Weight gain to delivery (kg)	29.0	20.7	0.01	0.13	-0.24, 0.26	0.94	2.57	2.98	-3.30, 8.44	0.39	-2.85	3.80	-10.34, 4.64	0.45

* Multivariable modified Poisson's, multinomial logistic and linear regression models were adjusted for month of blood draw as a sinusoidal function, gestational age at blood draw (weeks), age (years), pre-pregnancy BMI (kg/m²) and Hispanic ethnicity (yes v. no). Inadequate gestational weight gain, excessive gestational weight gain and weight gain to delivery were additionally adjusted for gestational age at delivery (weeks).

† The small number of cases precluded calculation of estimates for vitamin D deficiency v. sufficiency.

‡ *P* value for multiplicative interaction 0.02 for continuous vitamin D and ethnicity and 0.01 for vitamin D insufficiency and ethnicity.

§ Multinomial logistic regression used to model the multilevel outcome of inadequate, adequate and excessive gestational weight gain.

Table 4. Multivariable* relative risks (RR) for the association between vitamin D and adverse infant outcomes; the Behaviors Affecting Baby and You Study, 2007–2012 (Numbers and percentages; relative risks and 95 % confidence intervals; mean values and standard deviations; β coefficients with their standard errors)

Infant outcomes	Cases		Vitamin D continuous (ng/ml)			Vitamin D insufficiency (<30 v. \geq 30 ng/ml)			Vitamin D deficiency (<20 v. \geq 30 ng/ml)					
	<i>n</i>	%	RR	95 % CI	<i>P</i>	RR	95 % CI	<i>P</i>	RR	95 % CI	<i>P</i>			
Spontaneous preterm delivery	12	5.5	1.01	0.96, 1.07	0.59	0.95	0.28, 3.16	0.93	0.87	0.17, 4.57	0.87			
Hispanic	8	6.3	1.00	0.94, 1.07	0.90	0.68	0.19, 2.47	0.56	0.54	0.06, 4.66	0.58			
Non-Hispanic	4	4.4	1.03	0.91, 1.16	0.64	0.83	0.23, 14.80	0.57	1.77	0.13, 23.85	0.67			
Small for gestational age	21	9.6	0.98	0.94, 1.03	0.44	1.85	0.70, 4.90	0.21	2.14	0.67, 6.88	0.20			
Hispanic	12	9.4	0.96	0.90, 1.02	0.17	3.39	0.69, 16.59	0.13	4.70	0.86, 25.85	0.08			
Non-Hispanic	9	10.0	1.01	0.95, 1.07	0.86	1.05	0.29, 3.84	0.94	0.64	0.08, 5.13	0.67			
Large for gestational age	31	14.2	1.01	0.98, 1.04	0.48	0.91	0.46, 1.77	0.77	0.88	0.38, 1.92	0.71			
Hispanic	16	12.5	0.99	0.95, 1.03	0.66	1.11	0.43, 2.89	0.82	1.02	0.32, 3.21	0.98			
Non-Hispanic	15	16.7	1.03	0.98, 1.07	0.23	0.73	0.25, 2.15	0.56	0.74	0.21, 2.59	0.63			
Caesarean delivery	83	36.9	1.00	0.99, 1.02	0.64	1.02	0.71, 1.48	0.91	1.04	0.64, 1.69	0.88			
Hispanic	45	34.4	0.99	0.97, 1.02	0.60	1.12	0.67, 1.88	0.66	1.11	0.56, 2.16	0.76			
Non-Hispanic	38	40.4	1.01	0.99, 1.03	0.28	0.91	0.53, 1.56	0.74	0.98	0.49, 1.97	0.96			
	Mean	SD	β	SE	95 % CI	<i>P</i>	β	SE	95 % CI	<i>P</i>	β	SE	95 % CI	<i>P</i>
Gestational age at delivery (weeks)	39.14	1.93	-0.01	0.01	-0.04, 0.01	0.32	0.07	0.28	-0.48, 0.62	0.80	0.09	0.36	-0.62, 0.80	0.81
Birth weight (g)	3379.67	594.48	4.72	2.95	-1.10, 10.53	0.11	-139.74	69.16	-276.08, -3.41	0.045	-175.52	89.45	-351.86, 0.83	0.051
Birth length (cm)	20.20	1.15	-0.003	0.007	-0.017, 0.011	0.70	-0.21	0.16	-0.54, 0.11	0.20	-0.03	0.21	-0.44, 0.39	0.89
Birth weight/length (g/cm)	170.15	20.08	0.19	0.12	-0.05, 0.43	0.12	-5.35	2.85	-10.97, 0.27	0.06	-5.90	3.67	-13.14, 1.34	0.11

LGA, large for gestational age; SGA, small for gestational age.

* Multivariable modified Poisson's and linear regression models were adjusted for month of blood draw as a sinusoidal function, gestational age at blood draw (weeks), age (years), pre-pregnancy BMI (kg/m²) and Hispanic ethnicity (yes v. no). SGA, LGA, birth weight, birth length and birth weight/length were additionally adjusted for gestational age at delivery (weeks).

complete a baseline assessment) as compared with women who were included in terms of socio-demographic, behavioural or medical history factors, with the exception of a personal history of GDM. Women who were excluded were more likely to have a personal history of GDM as compared with those who were included (19.4 *v.* 11.9%, respectively). However, when we compared women with data on GDM with women missing data on GDM, the prevalence of a personal history of GDM did not differ significantly between these groups (12.0 *v.* 13.3%, respectively). When we assigned all participants missing GDM as GDM cases, we found no meaningful differences in effect estimates.

Finally, pre-pregnancy BMI did not appear to substantially modify the observed relationships. Specifically, estimates from a sensitivity analysis adjusting for mid-pregnancy BMI (approximately 20 weeks gestation) in lieu of pre-pregnancy BMI were virtually unchanged.

Discussion

In this prospective study of early-pregnancy maternal vitamin D status and risk of adverse maternal and infant outcomes in an ethnically diverse population at high risk for GDM, we found that women with insufficient or deficient vitamin D had infants with lower birth weights than did women with sufficient levels, after adjustment for age, Hispanic ethnicity, pre-pregnancy BMI, month of blood draw, gestational age at blood draw and gestational age at delivery. Among Hispanic women only, increasing serum vitamin D level in early pregnancy was associated with a small increased risk for GDM. We did not observe statistically significant associations between maternal vitamin D status and risk for gestational hypertension, SGA, LGA, spontaneous preterm birth, caesarean delivery or gestational weight gain overall, nor within strata of ethnicity.

In this ethnically diverse sample (57.0% Hispanic) at high risk for GDM and other pregnancy complications, approximately half of the participants had insufficient levels of vitamin D, with one-fifth having deficient levels of vitamin D. These findings are comparable to prior studies that have included a high proportion of Hispanic participants^(27,54,55). In a study by Scholl *et al.*⁽⁵⁴⁾ in New Jersey, which included 54.1% Hispanic women, the authors found a 34.7% prevalence of vitamin D deficiency (<50 nmol/l). In a study by Merewood *et al.*⁽⁵⁵⁾ in Boston, Massachusetts, which included 52.9% Hispanic women, the authors found that 22.6% of participants had vitamin D levels <37.5 nmol/l. In a study by Baker *et al.*⁽²⁷⁾ in Chapel Hill, North Carolina, which included 26% Hispanic women, the authors found a 33.6% prevalence of vitamin D insufficiency, with 12.4% of participants being vitamin D deficient.

Our study population had a mean BMI of 32.8 (SD 6.3) kg/m² and included a high percentage of participants who were overweight (34.2% BMI ≥25 and <30 kg/m²) or obese (60.8% BMI ≥30 kg/m²) before pregnancy. The majority of prior studies were limited to participants who were of normal weight and/or overweight, with mean BMI ranging from 20⁽²⁵⁾ to 30.5 kg/m²⁽²⁷⁾. However, our findings for the prevalence of

vitamin D deficiency and insufficiency were similar to, or somewhat lower than, those of studies that included higher proportions of obese participants. For example, a study by Baker *et al.*⁽²⁷⁾ in Chapel Hill, North Carolina, in which participants had a mean BMI of 30 (95% CI 28, 35) kg/m² for preeclampsia cases and 31 (95% CI 27, 35) kg/m² for controls, found 46% of participants to be vitamin D deficient or insufficient as compared with 20.7% for vitamin D deficiency and 32.5% for insufficiency in our study. In a study by Gernand *et al.*⁽⁴²⁾ on 792 participants from twelve medical centres throughout the USA, which included 44.0% of participants who were obese and 23.4% who were overweight, the authors found a 36.6% prevalence of vitamin D deficiency, with 31.3% being vitamin D insufficient. Finally, our study population did not exercise for ≥3 d/week, which is comparable to national surveillance data, which found that the prevalence of meeting recommendations of physical activity during pregnancy (at least 150 min/week of aerobic exercise) was only 22.9%⁽⁷⁸⁾.

Consistent with prior studies^(79–81), we found that older age, non-Hispanic ethnicity, higher education, being married and increasing gestational age were associated with lower odds of vitamin D insufficiency, whereas winter season and each 1-unit kg/m² increase in BMI was associated with increased odds of vitamin D insufficiency.

Our finding that early-pregnancy vitamin D insufficiency and deficiency was significantly associated with a lower birth weight (of approximately 140–180 g) is consistent with prior studies on this topic^(37–39). For example, in a prospective cohort study of 3730 women in the Netherlands, Leffelaar *et al.*⁽³⁸⁾ found that participants with vitamin D levels in early pregnancy of <12 ng/ml had a mean birth weight that was 114 (95% CI 77.6, 151.2) g lower than those with vitamin D levels ≥20 ng/ml. Vitamin D may help to regulate fetal growth via its role in increasing Ca transport across the placenta, as well as by the action of vitamin D on its receptors present in tissues throughout the body, which play important roles in regulating glucose metabolism⁽⁸⁸⁾.

Although the majority of studies have suggested that increasing levels of vitamin D are either protective against the development of GDM^(6–14) or have no effect on GDM^(15–24), our findings suggest an adverse impact of higher early-pregnancy vitamin D level on risk for GDM among Hispanic women. The only previous study to report an increased risk for GDM with increasing vitamin D level was a prospective cohort among 1953 women conducted by Zhou *et al.*⁽²⁵⁾ in southern China. The authors similarly found that each increasing ng/ml of 25(OH)D was associated with a 2% higher odds of GDM (OR 1.02; 95% CI 1.001, 1.03), after adjustment for maternal age, systolic/diastolic blood pressure, pre-pregnancy BMI and serum Ca level⁽²⁵⁾. A systematic review by Burris & Camargo⁽⁸²⁾ noted that, although an association between vitamin D insufficiency and deficiency and an increased risk for GDM has been found in several studies, many of these studies were limited by lack of control for potential covariates, such as race/ethnicity and BMI.

Differences in the impact of vitamin D on GDM by ethnicity may be due to genetic variation in the metabolism of vitamin D. For example, a recent study by Zheng *et al.*⁽⁸³⁾ suggested that genetic variation in insulin receptor substrate 1 (*IRS1*) may modify the observed protective effect of vitamin D on type 2



diabetes risk. Specifically, the authors found that circulating 25(OH)D modulated the associations of *IRS1* variant *rs2943641* with type 2 diabetes and insulin resistance in Puerto Rican female adults. The authors concluded that this gene–nutrient interaction, which appears to be limited to women, warrants further examination in randomised controlled trials of vitamin D supplementation.

However, our study faced several limitations. Women who were included in the study were less likely to have a personal history of GDM as compared with women who were excluded. Therefore, loss to follow-up, even non-differential, may be one potential explanation for our findings for vitamin D and GDM as those lost were more likely to have a history of GDM (and presumably current GDM). In addition, given the small number of total cases, a lack of data at the lower ranges of vitamin D could be responsible, in part, for the association with continuous vitamin D. However, in a sensitivity analysis assigning all participants with missing data to GDM case status, results remained largely unchanged. In addition, the robustness of our findings was further supported by the observation that unadjusted mean vitamin D was lower among Hispanic women with GDM as compared with Hispanic women without GDM, but comparable among non-Hispanic women. In addition, it is possible that our population has unique social and economic factors related to dietary intake and sun exposure relevant to vitamin D status. Specifically, the Hispanic participants in our study were drawn from predominantly Puerto Rican and Dominican communities, who share a disproportionate burden of adverse pregnancy outcomes^(84–86) and socio-economic risk factors such as lower education, lower income, poor nutrition and lower physical activity levels^(87–89) as compared with other Hispanic groups and non-Hispanic whites. Finally, because differential weight gain during pregnancy could potentially modify our findings for vitamin D and risk for GDM, we conducted a sensitivity analysis adjusting for mid-pregnancy BMI in lieu of pre-pregnancy BMI, but our results were not meaningfully altered.

The small number of cases of several other adverse pregnancy outcomes also constrained our ability to evaluate their association with 25(OH)D. However, although the majority of CI do not provide statistically significant evidence, point estimates were consistent with previous research for outcomes such as gestational hypertension/preeclampsia^(27–33,90) and having an SGA infant^(37,38,42–46). The study was not designed to have sufficient power to investigate effect modification, and findings of lack of effect modification for the majority of study outcomes should not be construed to indicate evidence of a lack of interaction. In addition, the borderline significance of our findings for vitamin D and birth weight must be viewed in the light of multiple comparisons, where the probability of a type I error is greater.

25(OH)D was assessed in early pregnancy using DiaSorin Liaison. Early-pregnancy serum samples were chosen as they help to establish a clear temporal association between 25(OH)D and subsequent adverse pregnancy outcomes, reducing the potential for reverse-causation inherent in cross-sectional designs. In addition, because 25(OH)D levels increase naturally during pregnancy⁽⁷⁶⁾, obtaining samples during early pregnancy

helps to reduce this source of inter-person variability. Comparable to prior studies^(11,33,35), we used a single aliquot, which may lead to a less precise measure than duplicate or triplicate aliquots. However, the strong association of 25(OH)D with expected predictors of 25(OH)D status, such as age, ethnicity, BMI, season and gestational age, suggests that this single measure had reasonably low variability. Indeed, Diasorin Liaison has a low (<10%) total and repeatability CV⁽⁶⁵⁾ and has been utilised in several studies investigating the association between 25(OH)D level in pregnancy and adverse pregnancy outcomes^(11,17,31,35). Any variability in measurement of 25(OH)D is expected to be independent of the outcomes assessed in this study, and therefore would likely lead to an underestimation of the observed effect estimates. In addition, although we utilised cutoff points for 25(OH)D deficiency and insufficiency that are commonly used in research and practice for the general adult population⁽³⁾, cutoff points for vitamin D status during pregnancy have yet to be validated. This potential misclassification of vitamin D status would also tend to bias findings towards the null for the binary categorisation of 25(OH)D, and underestimate the observed effect sizes.

In summary, in this prospective study of women who were ethnically diverse and at high risk for GDM, we found that women with vitamin D deficiency and insufficiency had infants with lower birth weights as compared with women with sufficient levels of vitamin D. A significant increase in risk for GDM with increasing ng/ml of 25(OH)D was found only among Hispanic women. This suggests that further research into the association between vitamin D and GDM among Hispanic women is needed, and highlights the need for large, well-conducted studies in diverse cohorts.

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The author contributions are as follows: L. C.-T. conceived the topic for the manuscript. C. J. N. researched the literature, performed the statistical analysis and drafted the manuscript. L. C.-T., G. M. and C. J. N. participated in the design of the study and interpretation of study findings. G. M. confirmed cases of adverse pregnancy outcomes. All authors read and approved the final version of the manuscript.

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