Vitamin D Supplementation and the Effects on Glucose Metabolism During Pregnancy: A Randomized Controlled Trial

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OBJECTIVE

Vitamin D deficiency in pregnancy is associated with an increased risk of gestational diabetes mellitus (GDM) and neonatal vitamin D deficiency. We conducted a double-blind, randomized controlled trial of low-dose (LD) versus high-dose (HD) vitamin D supplementation to investigate the effects of vitamin D supplementation on glucose metabolism during pregnancy.

RESEARCH DESIGN AND METHODS

Women with plasma 25-hydroxyvitamin D (25OHD) levels <32 ng/mL before 20 weeks' gestation were randomized to oral vitamin D3 at 5,000 IU daily (HD) (n = 89) or the recommended pregnancy dose of 400 IU daily (LD) (n = 90) until delivery. The primary end point was maternal glucose levels on oral glucose tolerance test (OGTT) at 26–28 weeks' gestation. Secondary end points included neonatal 25OHD, obstetric and other neonatal outcomes, and maternal homeostasis model assessment of insulin resistance. Analysis was by intention to treat.

RESULTS

There was no difference in maternal glucose levels on OGTT. Twelve LD women (13%) developed GDM versus seven (8%) HD women (P = 0.25). Neonatal cord 25OHD was higher in HD offspring (46 ± 11 vs. 29 ± 12 ng/mL, P < 0.001), and deficiency was more common in LD offspring (24 vs. 10%, P = 0.06). Post hoc analysis in LD women showed an inverse relationship between pretreatment 25OHD and both fasting and 2-h blood glucose level on OGTT (both P < 0.001). Baseline 25OHD remained an independent predictor after multiple regression analysis.

CONCLUSIONS

HD vitamin D supplementation commencing at a mean of 14 weeks' gestation does not improve glucose levels in pregnancy. However, in women with baseline levels <32 ng/mL, 5,000 IU per day was well tolerated and highly effective at preventing neonatal vitamin D deficiency.

Vitamin D deficiency has become a common problem globally. It has been estimated that one billion people worldwide are vitamin D deficient (1). In the U.S., vitamin D deficiency is prevalent across populations of all age-groups (2). Even in a country with high ultraviolet exposure like Australia, the prevalence of vitamin D deficiency (25-hydroxyvitamin D [25OHD] level <20 ng/mL [<50 nmol/L]) was 31% among

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adults age 25 years or older in a recent study (3). In pregnant women, the prevalence is even greater (4), especially among women with dark skin color or who have other risk factors (5). The skeletal benefits of vitamin D are well established (1,6), but there is evidence to suggest that vitamin D may also play a role in nonskeletal conditions (7,8), including diabetes (9). This may be mediated through effects of vitamin D on pancreatic β-cell function or insulin resistance (10,11). Cross-sectional studies have shown a relationship among plasma 25OHD, hyperglycemia, and the risk of diabetes or gestational diabetes mellitus (GDM) (12,13).

GDM affects up to 11% of pregnant women in Australia (14). Hyperglycemia in pregnancy is associated with increased morbidity to mother and infant (15,16), and glucose control in GDM reduces the risks associated with this condition (17,18). We postulated that vitamin D deficiency may be a factor in the pathogenesis of GDM and that high-dose (HD) vitamin D supplementation during pregnancy would lead to an improvement in maternal glucose metabolism. We chose to study glucose metabolism in pregnancy, as GDM occurs in groups vulnerable to future diabetes, and the stress of pregnancy unmasks this tendency.

RESEARCH DESIGN AND METHODS

We conducted a double-blind randomized controlled trial of low-dose (LD) versus HD vitamin D3 supplementation at a single institution in Sydney between February 2010 and November 2011. An LD control arm was chosen rather than placebo, as it is clearly unethical to leave women with known vitamin D deficiency in pregnancy untreated. Women with singleton pregnancies who were age 18 years or older and at a gestational age of <20 weeks at study entry were eligible to participate. This cutoff was to ensure at least 6 weeks of treatment prior to evaluation of the primary outcome (glucose levels on oral glucose tolerance test [OGTT] at 26–28 weeks).

Women were excluded if they had a history of diabetes (type 1 or type 2 diabetes or glucose intolerance already diagnosed in this pregnancy), calcium or vitamin D metabolism disorders, hypercalcemia (serum corrected calcium >10.4 mg/dL [>2.6 mmol/L]), or significant renal impairment (serum creatinine >1.7 mg/dL [>150 μmol/L]) or were taking vitamin D supplements of ≥1,000 IU daily. Women who had a fasting blood glucose level (BGL) >126 mg/dL (7.0 mmol/L) or HbA_{1c} >6.5% (48 mmol/mol) at baseline received an early OGTT to exclude undiagnosed diabetes.

All participants provided informed consent. The study was approved by an independent regional ethics committee.

Treatment allocation was made after measurement of baseline plasma 25OHD at recruitment. Subjects with plasma 25OHD of <32 ng/mL (80 nmol/L) were randomly assigned to receive either 5,000 IU vitamin D3 daily (HD) or 400 IU daily (LD). The recommended intake in pregnancy according to guidelines is 400 IU daily (19,20). Randomization was in a 1:1 ratio, with a permuted block size of six and sequential assignment. Women with baseline 25OHD of ≥32 ng/mL were all allocated to a nonrandomized LD group to maintain blinding. Baseline and progress plasma 25OHD levels were reviewed by a safety officer whose only role in the conduct of the study was to examine calcium and 25OHD levels. All other study investigators and participants were blinded to the intervention allocated. Both doses of vitamin D3 capsules were provided by Blackmores Pty Ltd and appeared identical. Participants were instructed to take one capsule daily until delivery of their baby. They were permitted to also take routine pregnancy vitamin supplements in the recommended dosage, which could contribute an additional 200–500 IU per day.

Baseline blood tests, including plasma 25OHD, random BGL, HbA_{1c}, parathyroid hormone (PTH), liver function tests, and serum calcium, were performed at recruitment. A medical history and examination were performed. Participants completed a questionnaire about sunlight exposure and pregnancy multivitamin intake. Ethnicity was self-reported by the participants. Classification is shown in Table 1. Vitamin D deficiency is defined by 25OHD ≤20 ng/mL (≤50 nmol/L) with subclassifications as follows: severe (25OHD <5 ng/mL [<12.5 nmol/L]), moderate (5–10 ng/mL [12–25 nmol/L]), and mild (10–20 ng/mL [25–50 nmol/L]) (19).

The primary end point was a 75-g OGTT with fasting plasma insulin performed between 26 and 28 weeks' gestation. GDM was diagnosed according to the Australasian Diabetes in Pregnancy Society criteria: fasting glucose ≥99 mg/dL (≥5.5 mmol/L) or 2-h glucose ≥144 mg/dL (≥8.0 mmol/L) (21). Women diagnosed with GDM were treated according to standard practice. Additional study visits were scheduled at 34–36 weeks' gestation and within 2 days after delivery. At these times, maternal blood was obtained for measurement of plasma calcium, 25OHD, random BGL, and HbA_{1c}. Umbilical cord blood was collected for measurement of fetal serum calcium, glucose, insulin, C-peptide, plasma 25OHD, PTH, and alkaline phosphatase. Parents were informed if the plasma 25OHD in infant cord blood was low (<10 ng/mL), and they were advised to commence infant vitamin D supplementation as per Australian consensus guidelines (19).

Secondary end points included maternal obstetric outcomes (maternal hypertension, mode of delivery, prematurity, and homeostasis model assessment of insulin resistance [HOMA-IR]) and neonatal biochemistry and anthropometric measures (birth weight, crown-heel length, and occipitofrontal head circumference), which were recorded by examination of the newborn within the first 2 days of life. Obstetric outcome data were collected from the Hospital ObstetriX database after delivery.

Safety thresholds for the study included persisting severe or moderate maternal vitamin D deficiency (plasma 25OHD <10 ng/mL) because of increased risk of neonatal rickets and osteomalacia (19), hypervitaminosis D (25OHD >100 ng/mL), or hypercalcemia (corrected serum calcium >10.4 mg/dL). After completion of their 26- to 28-week OGTT, women with persisting vitamin D deficiency while receiving LD were switched to 5,000 IU vitamin D3 daily and unblinded. The protocol allowed for subjects with hypervitaminosis D or hypercalcemia to stop supplementation. All women who were unblinded remained in the study and received scheduled follow-up visits.

25OHD was measured in plasma using the DiaSorin LIAISON chemiluminescent immunoassay, which has a concordance correlation coefficient of 0.95 compared with the gold standard of liquid chromatography–tandem

Table 1—Baseline characteristics of randomized groups and nonrandomized group

Variables	400 IU vitamin D	5,000 IU vitamin D	*P*	Nonrandomized group
n	90	89		24
Age, mean (SD), years	28.8 (4.9)	29.5 (4.7)	0.34	28.7 (5.4)
BMI, mean (SD), kg/m^2	26.1 (5.8)	25.4 (5.2)	0.39	23.9 (4.3)
Gestational age at baseline, median (IQR), weeks	15.6 (4.4)	15.1 (3.6)	0.68	14.1 (5)
Sunlight exposure, *n* (%)				
Summer (>30 min/week)	66 (73)	52 (58)	0.04	22 (92)
Winter (>120 min/week)	34 (38)	27 (30)	0.29	10 (42)
Biochemistry				
25OHD, mean (SD); *n*, ng/mL	18 (7)	20 (7)	0.22	37 (6)
Multivitamin use	20 (6); 60	21 (7); 70	0.23	38 (6); 21
No multivitamin use	15 (7); 28	13 (7); 19	0.42	36 (6); 4
Calcium, mean (SD), mg/dL	9.2 (0.3)	9.1 (0.3)	0.57	9 (0.3)
PTH, median (IQR), pg/mL	12 (14)	13 (14)	0.23	11 (13)
Random BGL, mean (SD), mg/dL	76 (13)	74 (11)	0.32	71 (9)
HbA_{1c}, mean (SD), %	5.2 (0.4)	5.2 (0.4)	0.49	5.1 (0.4)
HbA_{1c}, mean (SD), mmol/mol	34 (4)	33 (4)	0.49	32 (4)
Risk factors, *n* (%)				
Previous history of GDM	3 (3)	2 (2)	0.66	1 (4)
Family history of diabetes (first degree)	29 (32)	21 (24)	0.20	7 (29)
Parity			0.28	
0	40 (44)	45 (51)		9 (38)
1	26 (29)	29 (33)		10 (42)
≥2	24 (27)	15 (17)		5 (21)
Ethnicity			0.30	
Caucasian	23 (26)	29 (33)		14 (58)
Indian/subcontinental	27 (30)	26 (30)		0 (0)
Middle Eastern	13 (14)	9 (10)		1 (4)
Asian	11 (12)	13 (15)		4 (17)
African	7 (8)	1 (1)		4 (17)
Pacific Islander	5 (6)	2 (2)		0 (0)
Other	4 (4)	7 (8)		1 (4)

IQR, interquartile range.

mass spectrometry and good intra- and interassay precision, with CV <10% for 25OHD >8 ng/mL (22).

Applying an SD in fasting BGL of 9 mg/dL based on a cross-sectional pilot study, we estimated that a sample size of 200 women would provide 90% power (α = 0.05) to detect a 5 mg/dL difference between groups in fasting BGL at the OGTT. We allowed for a 20% dropout and 10% allocation to the nonrandomized group (baseline 25OHD ≥32 ng/dL). We chose this magnitude of change in fasting BGL because the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study suggests that a change in fasting BGL of 6.9 mg/dL (corresponding to 1 SD) is associated with significant increased odds of adverse pregnancy outcomes. We were also adequately powered to detect a 23.5 mg/dL (1-SD increase in 2-h BGL in HAPO), which is also associated with a clinically significant effect (16). All statistical analyses were performed using SPSS for Macintosh version 20.0 or SPSS for PC version 22.0. The data were analyzed by intention to treat. For continuous data, *t* test or Pearson correlation was used for normally distributed data and a nonparametric test (Mann-Whitney *U* or Spearman correlation) for nonnormally distributed data. The χ^2 test was used for categorical variables. Multiple regression was used to assess the association between 25OHD levels and blood glucose levels, adjusting for known GDM risk factors.

RESULTS

Two hundred and nine women were recruited to the study (Fig. 1). Twenty-four (11.5%) had baseline plasma 25OHD ≥32 ng/mL and were allocated to the nonrandomized LD group. Six women discontinued the study prior to randomization, and the remaining 179 subjects were randomized: 90 to LD and 89 to HD. Baseline characteristics were well balanced between the LD and HD groups (Table 1). Characteristics of the nonrandomized group are shown in Table 1.

At 26–28 weeks, plasma 25OHD levels were higher than baseline in both groups. The HD group achieved significantly higher plasma 25OHD than the LD group (36 ± 11 vs. 24 ± 8 ng/mL, $P < 0.001$). Median duration of supplementation was similar (Table 2). Despite supplements, 34% of LD women and 10% of HD women ($P < 0.001$) remained vitamin D deficient (plasma 25OHD ≤20 ng/mL). Eighty women receiving LD and 78 receiving HD underwent the planned 75-g OGTT at 26–28 weeks' gestation. Mean fasting and 2-h blood glucose levels were not different (Table 2). Similarly, HOMA-IR did not differ between groups. Overall, 19 women (10.5%) in the entire cohort developed GDM: 12 women (13%) in the LD group compared with 7 (8%) in the HD group (Table 2), $P = 0.25$. These seven women in the HD group all had sufficient 25OHD levels (between 24 and 42 ng/mL) at the time of OGTT, suggesting adequate compliance with vitamin D3 supplements in these individuals.

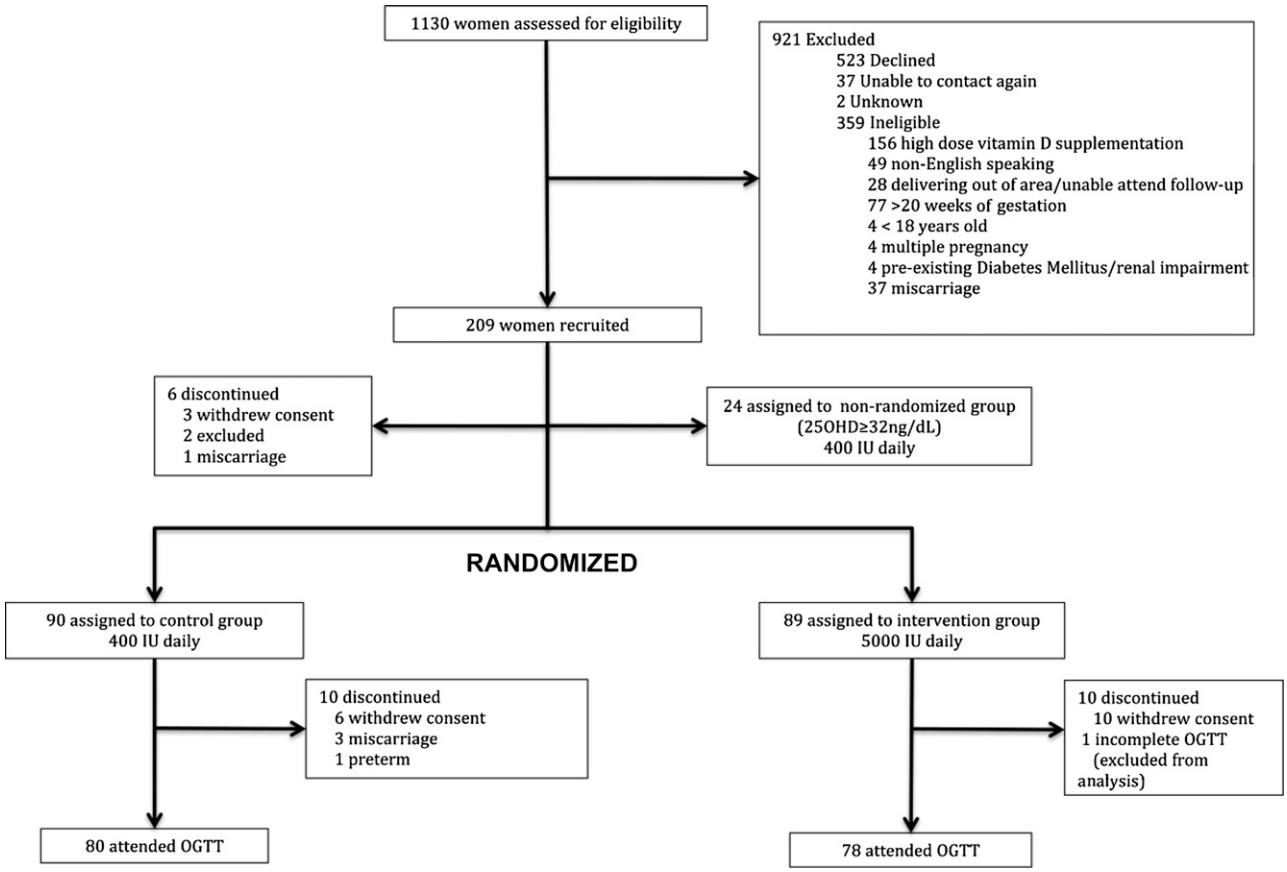

Figure 1—Trial profile.

The study was not powered to detect a difference in risk of GDM. A sample size of 760 would be needed for adequate power to detect a true 40% decrease in risk of GDM.

At postpartum testing, 27% of LD women and 11% of HD women (P = 0.02) were vitamin D deficient. No difference was noted between the two groups in terms of obstetric outcomes of gestational hypertension, preeclampsia, preterm delivery, and mode of delivery (Table 2). There were two stillbirths in each group. All cases had identifiable causes (infection or maternal hypertension); there was no association with 25OHD levels or treatment group.

Mean neonatal cord 25OHD was higher in offspring of the HD group (46 ± 11 vs. 29 ± 12 ng/mL, $P <$ 0.001). Other biochemistry and anthropometric measures were similar between infants (Table 2). Eleven infants (24%) in the LD group and 5 (10%) infants (P = 0.06) in the HD group had cord plasma 25OHD ≤20 ng/mL. The lowest cord plasma 25OHD in the nonrandomized group was 23 ng/mL.

Post hoc analysis was performed in all women (randomized and nonrandomized) taking LD. An inverse relationship was noted between baseline 25OHD and both fasting and 2-h blood glucose levels on the75-g OGTT (r = −0.368 and −0.349, respectively, $P <$ 0.001). Multiple regression analysis was performed and included documented risk factors for GDM, including age, BMI, parity, ethnicity (with Caucasian as reference group), previous history of GDM, and family history of diabetes in first-degree relatives. Baseline 25OHD levels, age, BMI, and Indian subcontinental ethnicity were independent predictors of fasting BGL on the 75-g OGTT. Baseline 25OHD, parity, and Indian subcontinental and Asian ethnicity were independent predictors of 2-h blood glucose levels on the 75-g OGTT (Table 3).

An additional post hoc subgroup analysis examined whether women with deficiency at baseline benefitted from HD supplements. Women with plasma 25OHD of ≤20 ng/mL at recruitment were examined. There was no benefit in this group either in the fasting OGTT result (74 mg/dL in LD vs. 76 mg/dL in HD, $P >$ 0.5) or in the 2-h result at OGTT (112 vs. 108 mg/dL, $P >$ 0.4).

Thirteen women had evidence of ongoing severe vitamin D deficiency at the time of OGTT or at the 34–36 weeks' gestation visit (plasma 25OHD <10 ng/mL; 9 in the LD group and 4 in the HD group). They were unblinded and provided 5,000 IU vitamin D3 daily. No maternal hypervitaminosis D (plasma 25OHD >100 ng/mL) was noted in either group. One infant in the HD group had a high cord blood plasma 25OHD of 102 ng/mL. However, this was not associated with hypercalcemia or other clinically adverse effects.

Two cases of mild maternal hypercalcemia (calcium 10.6 and 10.7 mg/dL) were noted postpartum (one in each randomized group) with no associated hypervitaminosis D. Three cases of isolated infant hypercalcemia (calcium 12.0, 12.1, and 12.4 mg/dL [normal range 7.0–11.96]) on cord blood samples were identified. None were clinically significant, and repeat serum calcium levels after birth in two infants

Table 2—Primary and secondary outcomes, 25OHD levels, and OGTT results at 26–28 weeks

	400 IU vitamin D	5,000 IU vitamin D	OR	95% CI	*P*
25OHD at baseline, mean (SD), ng/mL	18 (7)	20 (7)			0.22
25OHD at OGTT visit, mean (SD), ng/mL	24 (9)	36 (11)			<0.001
Treatment duration, median (IQR), weeks	12.1 (4.9)	12.0 (3.8)			0.88
OGTT results at 26–28 weeks					
n	80	78			
Fasting blood glucose, mean (SD), mg/dL	75 (6)	75 (7)			0.72
2-h blood glucose, mean (SD), mg/dL	111 (28)	108 (25)			0.42
HOMA-IR, median (IQR)	0.8 (1.6)	1.0 (1.3)			0.70
GDM, *n* (%)	12 (13)	7 (8)	0.56	0.21–1.50	0.25
Maternal outcomes					
n	80–85	81			
Hypertension, *n* (%)					
Gestational	2 (2)	1 (1)	0.51	0.05–5.76	0.59
Preeclampsia	4 (5)	2 (2)	0.51	0.09–2.84	0.44
Preterm delivery, *n* (%)	6 (7)	4 (5)	0.66	0.18–2.43	0.38
Mode of delivery, *n* (%)					
Normal vaginal delivery	48 (58)	46 (57)	0.96	0.52–1.78	0.89
Instrumental	9 (11)	9 (11)	1.03	0.39–2.74	0.96
Caesarean section	26 (31)	26 (32)	1.04	0.54–2.00	0.92
Stillbirth, *n* (%)	2 (2)	2 (2)	0.66	0.14–7.64	0.96
Neonatal outcomes and cord blood biochemistry					
n	81–83	78–81			
Gestational age, mean (SD), weeks	38.9 (2.2)	39.1 (2.4)			0.60
Birth weight, mean (SD), grams	3,267 (649)	3,337 (671)			0.50
Birth weight, median (IQR), centile	56.6 (48.3)	50.2 (51.7)			0.89
Length, mean (SD), cm	49.9 (2.5)	49.7 (2.4)			0.71
Head circumference, mean (SD), cm	34.3 (1.8)	34.4 (1.6)			0.70
Forearm length, mean (SD), cm	7.5 (0.6)	7.5 (0.5)			0.56
Femur length, mean (SD), cm	10.8 (0.8)	11.0 (0.9)			0.41
Cord blood tests					
n	45–47	47–52			
Vitamin D, mean (SD), ng/mL	29 (12)	46 (19)			<0.001
Calcium, mean (SD), mg/dL	10.7 (0.8)	10.9 (0.7)			0.13
PTH, median (IQR), pg/mL	3 (2)	3 (0)			0.07
Random BGL, mean (SD), mg/dL	77 (20)	79 (20)			0.61
Insulin, median (IQR), μIU/mL	3.0 (4)	3.5 (5)			0.67
C-peptide, median (IQR), ng/mL	0.9 (0.8)	0.8 (0.8)			0.70

IQR, interquartile range.

(days one and five, respectively) were within the normal range, suggesting that hemolysis during cord blood collection and storage may have caused artifactual results in these cases.

CONCLUSIONS

An association has been drawn between maternal vitamin D deficiency and impaired maternal glucose metabolism in pregnancy. This has been observed in a number of cross-sectional studies (12,23–26) and provided the rationale for this intervention study. Our findings are that supplementation with 5,000 IU vitamin D3 daily during pregnancy can safely and effectively elevate serum 25OHD concentrations into the desired target range in 90% of women, but it did not lead to an improvement in maternal glucose metabolism, compared with a control group taking a standard pregnancy supplemental dose of 400 IU vitamin D3 daily. More women in the LD group remained vitamin deficient compared with HD at 26–28 weeks and postpartum, indicating that LD supplementation may not be adequate in some women. Compliance may have been a factor in this.

Normal human pregnancy results in a significant additional demand for insulin

Table 3—Multiple regression analysis of baseline 25OHD and OGTT for women on 400 IU vitamin D3 daily

Fasting BGL (OGTT)	B coefficient	SE	*P*
Constant	3.421	0.219	
25OHD	−0.04	0.001	0.002
Age	0.016	0.005	0.004
BMI	0.015	0.005	0.003
Subcontinental ethnicity	0.182	0.063	0.005
2-h BGL (OGTT)			
Constant	6.275	0.219	
25OHD	−0.017	0.005	0.001
Multiparity	0.947	0.327	0.004
Subcontinental ethnicity	0.667	0.254	0.009
Asian ethnicity	0.822	0.304	0.008

secretion, and women with impaired β-cell reserve are at risk for development of GDM. Animal studies have shown that vitamin D deficiency impairs β-cell function (27) and that some mice that lack vitamin D receptors have impaired insulin secretion and abnormal glucose tolerance (28). Vitamin D has also been shown to correlate with β-cell function and insulin resistance in humans (10).

Several studies (12,23–25) have reported that women who developed GDM have lower mean 25OHD levels compared with those with normal glucose tolerance. When results were pooled in a recent meta-analysis, despite a wide range in mean 25OHD levels (6.6–39.7 ng/mL) in women with GDM between studies, it was evident that those who developed GDM had significantly lower vitamin D levels than those who had normal glucose tolerance (29). Similarly, in our subjects, pretreatment measures of serum 25OHD demonstrated a significant inverse association with fasting and 2-h blood glucose levels on a mid-pregnancy OGTT, and this remained significant after adjustment for other risk factors for GDM. We have also previously found an inverse association between vitamin D levels and glycated hemoglobin among women with GDM (13).

Several studies have confirmed this inverse association by reporting an increased risk of GDM in pregnant women who are vitamin D deficient (24–26). Zhang et al. (25) found that women with vitamin D deficiency during early pregnancy had a 3.7-fold increased risk of developing GDM compared with those who were vitamin D replete. However, other studies that have evaluated first-trimester vitamin D status and the subsequent risk of GDM have not found an association (30–32). Among these negative studies, none had information on vitamin supplementation and one was underpowered for the outcome. A meta-analysis of vitamin D status and GDM showed that the odds of developing GDM in those with 25OHD $<$20 ng/mL was higher than in those with 25OHD $\geq$20 ng/mL (odds ratio [OR] 1.61 [95% CI 1.19–2.17]; P = 0.002). After adjustment for maternal age, BMI, and ethnicity in three studies, the association between maternal GDM and vitamin D remained significant (the combined OR was 1.84 [1.07–3.15]; P = 0.03) (29).

Therefore, if vitamin D deficiency is associated with an increased risk of diabetes, especially during pregnancy, does it follow that vitamin D supplementation will improve glucose metabolism? There have been several studies evaluating the effects of vitamin D supplementation on plasma glucose levels and risk of developing diabetes in nonpregnant populations (9). The results have been varied. Some, but not all, studies show a benefit from vitamin D supplementation (33–35), particularly in those with preexisting risk factors for diabetes (36).

Only a handful of intervention studies have evaluated the effects of vitamin D supplementation during pregnancy on glucose metabolism. One small study involved 12 women with GDM who had sequential OGTTs before and 2 h after administration of one intravenous dose of 1,25$(OH)_2$ vitamin D3 (37). Treatment resulted in a transient increase in serum vitamin D levels and fall in fasting glucose. Another study randomized women from early pregnancy to vitamin D supplementation of 200 IU daily, 50,000 IU monthly, or 50,000 IU every 2 weeks until delivery. They found a significant improvement in HOMA-IR at the end of pregnancy compared with early pregnancy in the group receiving 50,000 IU vitamin D every 2 weeks compared with the group receiving 200 IU daily, but no effect on fasting BGL was detected (38). More recently, a double-blinded randomized controlled trial in 54 women diagnosed with GDM reported an improvement in fasting BGL and HOMA-IR after two doses of oral 50,000 IU vitamin D given 21 days apart compared with placebo. However, significant differences in these parameters were noted at baseline, making the results difficult to interpret (39). None of these studies had data on neonatal cord 25OHD levels. In contrast, our randomized controlled trial did not demonstrate a benefit of prolonged oral vitamin D treatment on maternal glucose metabolism in pregnancy. We can be confident that sufficient vitamin D was administered to test the hypothesis, as our treatment protocol achieved a clear separation in vitamin D levels between the HD and LD groups. Furthermore, neonatal vitamin D levels were higher in offspring of women receiving HD supplements, with less incidence of vitamin D deficiency in the HD group compared with the LD group. This is important because while the risk of neonatal rickets is greatest at 25OHD $<$10 ng/mL, cases of rickets have been reported at neonatal 25OHD levels between 10 and 20 ng/mL (40).

There are several possible reasons for the negative result in this study. First, the association between low vitamin D levels and GDM might not be causal. Low serum vitamin D levels may act as a marker of women who are predisposed to GDM through another mechanism, such as ethnicity, diet, or lifestyle. Alternatively, the association might be causal in the other direction, with the prediabetes state causing low vitamin D generation or hydroxylation.

It is also possible that the negative result was due to the study design. First, we did not select women with a high risk for developing GDM, and this may have diluted a potential beneficial effect of supplements. However, the population of women at our institution is high risk, reflected in the 10.5% GDM rate in our entire cohort and 13% GDM rate in the LD group. Second, we did not limit recruitment to women with vitamin D deficiency at baseline. However, there was also no difference in the subgroup of women with low vitamin D status ($\leq$20 ng/mL) at baseline. Third, for ethical reasons, we chose to supplement all women with at least 400 IU vitamin D3 daily rather than leaving women with deficiency untreated. Most (76% of subjects) also took a pregnancy multivitamin (which contain 200–500 IU vitamin D3 depending on type). It is possible that 400–900 IU per day is sufficient for the maximal benefit for maternal glucose metabolism. However, this is unlikely, given the 13% GDM rate in the LD group. Fourth, given the rate of vitamin D deficiency noted in both groups during the study, noncompliance may have been a concern. However, even with a noncompliance rate of 10% (the rate of vitamin D deficiency noted in the HD group), we were well powered for detecting a statistically significant effect.

Additionally, the nonsignificantly lower incidence of GDM that we observed in the HD group in our study was interesting, but our study was not adequately powered to detect a difference in incidence of GDM. Of course, if confirmed, a 40% decrease in GDM incidence would be

important and clinically significant. However, a study of ~800 women would be needed for adequate power.

Finally, because women who develop GDM usually exhibit β-cell dysfunction prior to pregnancy, and changes in β-cell function and proliferation start very early in pregnancy, the commencement of therapy may have been too late for an observable benefit. Our study demonstrated an inverse association between pretreatment 25OHD and fasting BGL and 2-h BGL at OGTT, suggesting that prepregnancy vitamin D status may play an important role in subsequent maternal glucose metabolism, in which case vitamin D supplements may need to be commenced before pregnancy to be effective.

In conclusion, the results of our study do not support routine vitamin D supplementation during pregnancy for the purpose of improving blood glucose levels. However, they do demonstrate safety of HD supplementation at a dose of 5,000 IU daily in women with baseline plasma 25OHD levels <32 ng/mL.

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