Randomized Controlled Trial (RCT) of Vitamin D Supplementation in Pregnancy in a Population With Endemic Vitamin D Deficiency

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Background: Vitamin D (vD) deficiency in pregnancy is a global health problem and the amount of vD supplementation to prevent vD deficiency is controversial.

Objective: The objective of the study was to determine effectiveness and safety of prenatal 2000 IU and 4000 IU/d compared with 400 IU/d vD3 supplementation in a randomized controlled trial in population in which vD deficiency is endemic.

Design/Methods: Arab women were randomized at 12–16 weeks of gestation to 400, 2000, and 4000 IU/d vD3, which were continued to delivery. Serum 25-hydroxyvitamin D [25(OH)D] concentrations were measured during pregnancy and at delivery. The primary outcome was the maternal and cord blood 25(OH)D, and the secondary outcomes were the achievement of sufficient serum 25(OH)D of 32 ng/mL or greater (≥80 nmol/L) at delivery.

Setting: The locations were primary care and tertiary perinatal care centers.

Results: Of 192 enrolled, 162 (84%) continued to delivery. Mean serum 25(OH)D of 8.2 ng/mL (20.5 nmol/L) at enrollment was low. Mean serum 25(OH)D concentrations at delivery and in cord blood were significantly higher in the 2000 and 4000 IU than the 400 IU/d group ($P < .001$) and was highest in the 4000 IU/d group. The percent who achieved 25(OH)D greater than 32 ng/mL and greater than 20 ng/mL concentrations in mothers and infants was highest in 4000 IU/d group. Safety measurements were similar by group and no adverse event related to vD supplementation.

Conclusions: Vitamin D supplementation of 2000 and 4000 IU/d appeared safe in pregnancy, and 4000 IU/d was most effective in optimizing serum 25(OH)D concentrations in mothers and their infants. These findings could apply to other populations in which vD deficiency is endemic. **(*J Clin Endocrinol Metab* 98: 2337–2346, 2013)**

Evidence is accumulating of adverse effects of low maternal vitamin D status in pregnancy on the mother, fetus, and infant. Inadequate maternal vitamin D status in pregnancy is associated with poor fetal growth (1, 2) and impaired bone development (3, 4). Maternal vitamin D deficiency is associated with rickets (5) and severe hypocalcemia (6) in infants after birth in populations with a high prevalence of vitamin D deficiency. Low cord blood 25-hydroxyvitamin D [25(OH)D] is associated with increased risk of lower respiratory tract infections and infantile eczema in the first year of life (7, 8). Furthermore, higher rates of preeclampsia (9), gestational diabetes (10), and bacterial vaginosis (11) are associated with low maternal vitamin D status during pregnancy, and vitamin D

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deficiency is associated with hypertension in observational and clinical studies (12).

Vitamin D deficiency serum 25(OH)D less than 20 ng/mL (<50 nmol/L) (13, 14) is common globally during pregnancy, particularly among Arab and Asian women (15). Studies from India (16), Kuwait (17), and United Arab Emirates (UAE) (18) found vitamin D deficiency in 74%, 83%, and 98% of pregnant women, respectively. Despite the reported high prevalence and risks of vitamin D deficiency during pregnancy, there is a lack of randomized controlled trials (RCTs) of vitamin D supplementation to optimize the vitamin D status during pregnancy in such high-risk populations.

Studies from North America in men and nonpregnant women indicated that vitamin D intake exceeding 2000 IU/d would maintain serum 25(OH)D 32 ng/mL or greater (≥80 nmol/L) considered by some researchers as vitamin D sufficiency (19, 20). Pharmacokinetic data in adults indicated that 100 IU vitamin D supplementation would increase serum 25(OH)D by 0.4–0.8 ng/mL (1–2 nmol/L) (13, 20, 21). Based on these data and the high prevalence of vitamin D deficiency in the UAE population (22), we estimated that 4000 IU/d vitamin D_3 supplementation would achieve vitamin D sufficiency. This study is an addition to the protocol of an ongoing National Institutes of Health-funded study (NCT02292591) of 2000 and 4000 IU/d of vitamin D_3 supplementation in pregnancy, which was not associated with adverse events. We conducted a RCT of vitamin D supplementation in the UAE to optimize vitamin D status in pregnant Arab women and hence their infants.

We tested the hypotheses that vitamin D doses of 2000 or 4000 IU/d but not 400 IU/d (existing standard recommendation) would substantially improve maternal vitamin D status during pregnancy and that 4000 IU would be superior to 2000 IU to achieve vitamin D sufficiency and thus improve the vitamin D status of her infant at birth without vitamin D toxicity. A priori cutoff point of serum 25(OH)D of 32 ng/mL or greater as vitamin D sufficiency was based on reports from the literature (19).

Materials and Methods

Trial design and participants

This was a randomized, controlled, double-blind study of daily vitamin D supplementation of 400, 2000, or 4000 IU during pregnancy in Arab women (clinicaltrials.gov, number NCT00610688, protocol addition to IND 66346). The study was conducted at primary health care clinics affiliated with Tawam Hospital (UAE University Teaching Hospital) in Al Ain from May 2008 to December 2011. Participants were Arab expectant mothers receiving prenatal care at the clinics and later delivered at Tawam hospital. Eligible patients who consented were enrolled in the study. The study was approved by the Faculty of Medicine and Health Sciences, UAE University, Institutional Review Board (number 06-82) and Cincinnati Children's Hospital Medical Center Institutional Review Board (number 06-04-30). Subjects were eligible for participation if they met the following criteria: 1) were at 12–16 weeks of gestation after their last menstrual period or by ultrasound assessment; 2) had a singleton pregnancy; and 3) planned to receive prenatal and delivery care in Al Ain. Exclusion criteria were preexisting calcium and parathyroid conditions, active thyroid disease, liver or kidney disease, or type 1diabetes, which are likely to affect vitamin D and calcium status.

Initial visit

Sociodemographic, health status, and pregnancy information were completed by questionnaires. Each mother completed a standardized food frequency questionnaire appropriate for Middle Eastern culture (22) to calculate vitamin D and calcium intake. The date of blood draw defined as the hot season (April through September) and the cool season (October through March). Maternal weight and height were recorded to determine the body mass index (BMI) (weight [kilograms]/height [square meters]). Baseline maternal blood was drawn by venipuncture and urine samples were collected.

Interventions

Vitamin D supplementation

The study vitamin D tablets, 1600 IU and 3600 IU vitamin D_3, and placebo with a similar color and taste were manufactured and supplied by Tischon Corp (Salisbury, Maryland). The concentrations of the vitamin D_3 in the study tablets were verified by the company at the end of the study. Each subject received a 40-day supply of vitamin D_3 study tablets at 1 of 3 dosing regimens determined by randomization and a 90-day supply of prenatal vitamins containing 400 IU vitamin D_3 per tablet. Therefore, each subject received a total of 400 IU vitamin D_3 (existing recommended intake), 2000 IU vitamin D_3 [existing upper safe intake

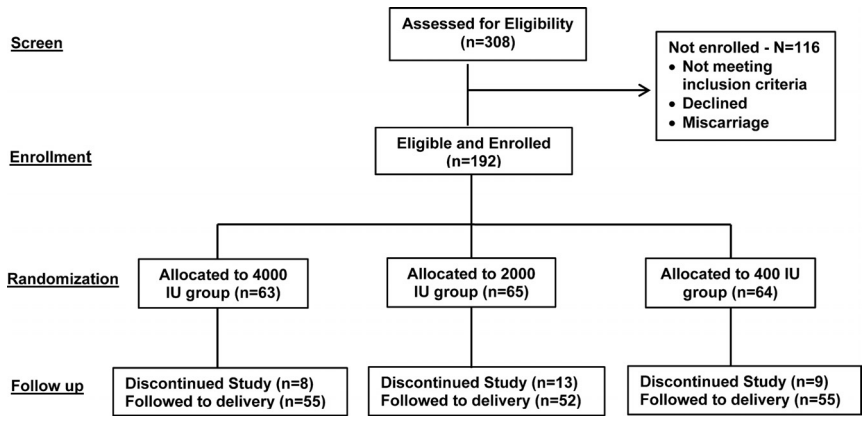

Figure 1. Flow chart of the subjects throughout the study.

Table 1. Baseline Demography and Vitamin D Status by Supplementation Group[a]

Variables	4000 IU (n = 63)	2000 IU (n = 65)	400 IU (n = 64)	*P* Value
Ethnicity/race, %				.903
Gulf Arab	92	94	92	
Non-Gulf Arab	8	6	8	
Maternal age, y (mean ± SD)	25.6 (5.5)	27.3 (4.9)	27.5 (5.5)	.128
BMI (mean ± SD)	26.3 (5.4)	26.3 (6.4)	25.8 (6.3)	.677
Gestation, wk, at enrollment (mean ± SD)	12.6 (1.1)	12.5 (1.1)	12.2 (0.9)	.139
Education, %				.373
None	0	3.1	1. 6	
Elementary	14.3	15.4	12.5	
High school	54.0	36.9	35.9	
College	7.9	16.9	17.2	
University	23.8	22.7	32.8	
Subjective health score (mean ± SD)	7.7 (1.5)	7.7 (1.5)	7.6 (1.4)	.423
Season at entry (hot), %	52.4	49.2	50.0	.933
Vitamin D intake, IU/d (mean ± SD)	108 (79)	132 (125)	93 (62)	.201
Ca intake, mg/d (mean ± SD)	458 (267)	501 (344)	364 (201)	.02
Serum 25(OH)D, nmol/L (mean ± SD)	19.6 (7.7)	20.5 (11.9)	21.5 (13.0)	.622

[a] Number of pregnancies and deliveries were similar.

(23)], or 4000 IU vitamin D_3 [estimated intake to achieve mean 25(OH)D concentration of ≥ 32 ng/mL considered vitamin D sufficiency at the time of the study (19)].

Randomization

The random assignment was a stratified block design so that each month an approximately equal number of subjects were randomly assigned to 400 IU/d, 2000 IU/d, and 4000 IU/d to achieve a seasonally balanced study population. The randomization list was computer generated by the statistician. A secretary not involved in the project allocated and kept a list of the randomization code of the enrolled patients. The research nurses assisted by the coinvestigators (G.B. and H.F.S.) enrolled the participants and the research nurse provided the allocated study vitamin D and prenatal vitamin D. The investigators, patients, health care providers, and the laboratory staff performing the biochemical tests were blinded to the treatment.

Follow-up visits

Subjects were seen monthly from enrollment until delivery by the research nurse. The monthly visits coincided with the routine prenatal visits of each participant, and the research nurse completed a questionnaire on interval maternal health and medication history as well as any hospital admissions and the medical diagnosis. The mode of delivery, complications during delivery, infant's health, weight (grams), head circumference (centimeters), and crown-heel length (centimeters) were recorded at delivery.

Medication compliance

The number of pills taken during the interval between the visits divided by the number that should have been taken was used to calculate compliance that served as her adherence to medication between study visits. The research coordinator made phone calls a day or two prior to a scheduled visit to remind the subject of her upcoming visit. If the subject then missed her appointment, a follow-up call was made and every effort was made to reschedule the subject.

Outcome variables

Vitamin D metabolites and PTH

Serum 25(OH)D and intact PTH were measured at enrollment and 16, 28, and 40 weeks or the time of delivery and in the

Table 2. Subjects Who Delivered Compared With Those Who Withdrew Before Delivery[a]

	Delivered (n = 162)	Exited (n = 30)	*P* Value
Treatment group			.657
4000 IU	55	8	
2000 IU	52	13	
400 IU	55	9	
Ethnicity/race, %			.657
Gulf Arab	92.0	96.5	
Non-Gulf Arab	6.8	3.0	
Maternal age, y (mean ± SD)	26.8 (5.3)	26.7 (6.1)	.935
BMI (mean ± SD)	26.1 (6.0)	25.7 (6.2)	.747
Gestation at enrollment, wk (mean ± SD)	12.5 (1.1)	12.4 (0.9)	.698
Education, %			
None	1.8	0.0	.882
Elementary	14.1	13.8	
High school	42.9	37.9	
College	14.1	13.8	
University	27.0	34.5	
Subjective health score (mean ± SD)	7.7 (1.5)	7.6 (1.6)	.460
Season at entry (hot), %	49.1	51.7	.793
Vitamin D intake, IU/d (mean ± SD)	117 (104)	85 (108)	.001
Ca intake, mg/d (mean ± SD)	451 (279)	387 (298)	.10
Baseline 25(OH)D, nmol/L (mean ± SD)	20.7 (11.6)	19.7 (7.8)	.66

[a] Number of pregnancies and deliveries were similar.

cord blood at Cincinnati Children's Hospital. Serum 25(OH)D was measured using a RIA (DiaSorin, Stillwater, Minnesota) as previously described (24). The intra- and interassay coefficients of variation were 4% and 11%, respectively. The a priori cutoff point of serum 25(OH)D 32 ng/mL or greater was used as the definition of vitamin D sufficiency (19). The PTH concentrations were measured by immunoradiometric assay (DiaSorin). The normal range for adults using this assay is 13–54 pg/mL.

Serum calcium and urine calcium and creatinine

Maternal serum and cord blood calcium concentrations were measured in Tawam Hospital to assess calcium homeostasis and safety. A nonfasting midmorning urine sample at monthly visits was used to measure urine calcium and creatinine for calculating urine calcium (Ca) to creatinine (Cr) ratio as early indicators of hypervitaminosis D.

Safety assessment

Based on the IND protocol, the upper limit of serum 25(OH)D was defined as 90 ng/mL or greater (≥225 nmol/L). Conservatively, the urine Ca to Cr ratio greater than 0.8 mmol/mmol was used to define caution limits, above which a case study was conducted to examine the contribution of confounding variables such as dietary and sunlight exposure. Confirmed urinary Ca to Cr ratio greater than 1 mmol/mmol (21) and serum calcium greater than 2.75 mmol/L were criteria to stop vitamin D supplementation. However, evidence for hypervitaminosis D was not found in studies of vitamin D_3 supplementation of 1000 to 10 000 IU/d among adult men and nonpregnant women and recently in pregnant women (20, 21, 25).

We monitored for persistent vitamin D deficiency using serum 25(OH)D less than 10 ng/mL (<25 nmol/L) which is in the osteomalacic range (13, 23). For ethical reasons, patients with serum 25(OH)D concentrations less than 10 ng/mL after 3–4 months of vitamin D supplementation were referred to the Data Safety and Monitoring Committee (DSMC) consisting of an endocrinologist, biostatistician, clinical pathologist, and internist with obstetrics/gynecology adjunct appointment who reviewed the supplementation allocation to prevent continued vitamin D deficiency if compliance had been judged to be adequate. Based on data among UAE women (26) who reportedly took 400 IU/d vitamin D in the last trimester of pregnancy, we anticipated that less than 5% would have serum 25(OH)D less than 10 ng/mL after 3 months of supplementation.

Sample size calculation

The primary outcome measure was maternal serum 25(OH)D concentrations at delivery. Based on a previous study (26), we powered the study to detect a difference of 10 ng/mL (25 nmol/L) between the 400 IU/d and the 2000 IU/d or 4000 IU/d of vitamin D supplementation groups and to account for an unexpected attrition rate, which resulted

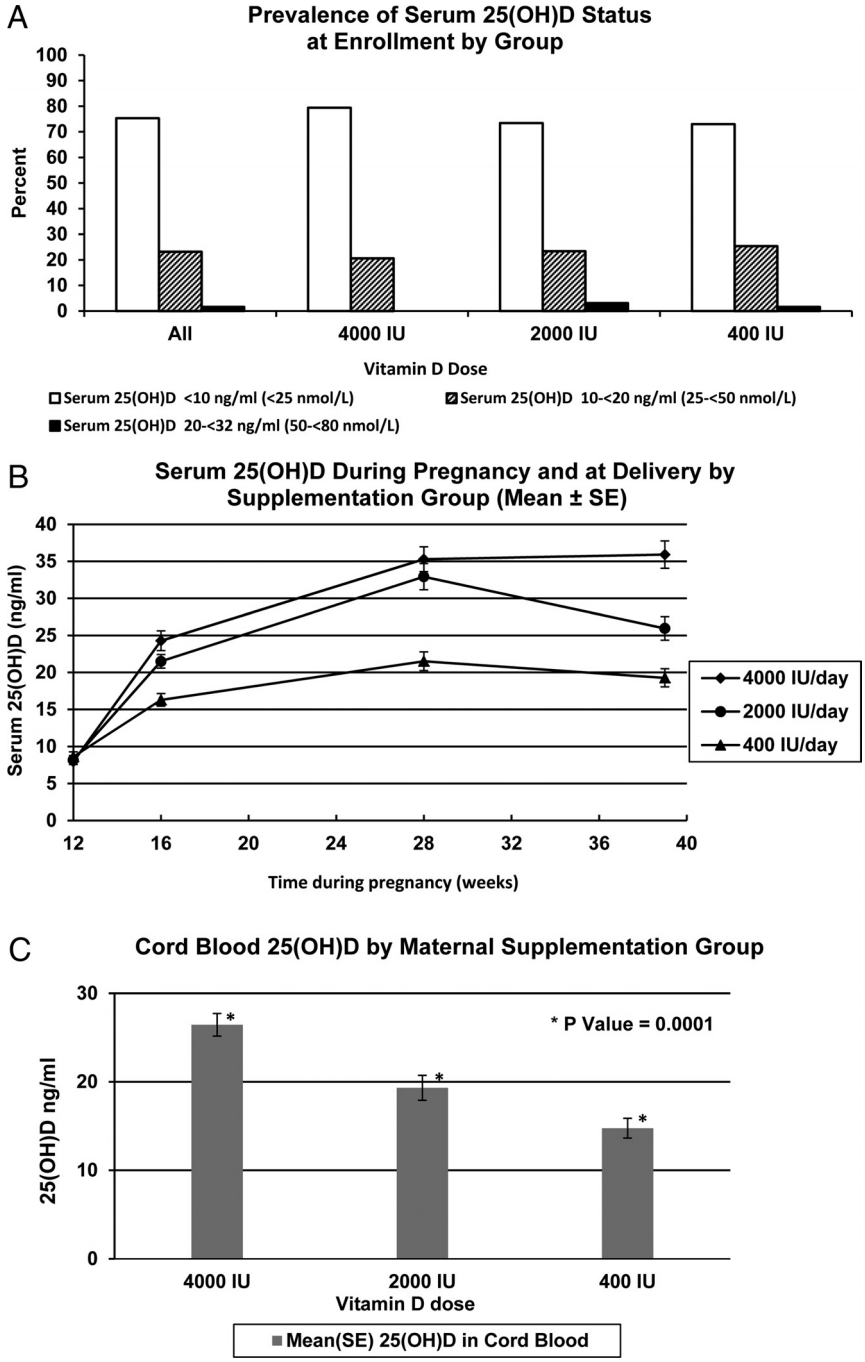

Figure 2. Percentages of mothers with very low (<10 ng/mL), low (<10 to < 20 ng/mL), and insufficient (20 to < 32 ng/mL) serum 25(OH)D concentrations at enrollment were similar among the treatment groups (A). Serum 25(OH)D concentrations at defined time points during pregnancy in response to randomized vitamin D_3 supplementation doses showed significant interactions between the groups and time ($P < .001$) (B). and Cord blood 25(OH)D concentrations as a function of mothers' vitamin D_3 supplementation were significantly different (C).

in a total of 192 subjects with 64 subjects per group for the study.

Statistical analysis

The primary variables were maternal serum 25(OH)D concentrations across pregnancy. A univariate statistic was generated for each variable to examine the distribution. Log transformation was considered for variables not normally distributed. The primary analysis of comparing the 3 groups across time was conducted using mixed model analysis via SAS PROZ MIXED procedure in which the outcome variable, serum 25(OH)D, was modeled as a function of group, time, and the interaction between group and time while accounting for the repeated measurements across subjects. This was followed by an analysis of difference (comparison of means) between groups at each time point. An intention-to-treat analysis was followed. We also determined as secondary outcome the proportion of mothers who achieved serum 25(OH)D 32 ng/mL or greater (≥80 nmol/L) defined as vitamin D sufficiency (19) at the time of delivery.

Results

One hundred ninety-two eligible mothers consented to participate and were randomly assigned to treatment groups (Figure 1). After allocation, 30 patients (15%) discontinued participation without specific reasons or due to the husband's refusal and 162 were followed up to delivery. All available data were used for the mixed-effect analysis.

Baseline characteristics

The groups were similar in all characteristics including baseline vitamin D status except for lower calcium intake in the 400 IU group (Table 1). The women who exited the study before delivery had similar baseline characteristics as those who were followed up to delivery except for lower vitamin D intake (Table 2). Of 191 subjects with available serum 25(OH)D at enrollment, 143 (75%) had serum 25(OH)D concentration less than 10 ng/mL (<25 nmol/L) and 44 (23.2%) had concentrations of 10 to less than 20 ng/mL (25 to < 50 nmol/L). Only 1 subject had serum 25(OH)D above 32 ng/mL (80 nmol/L). There were no significant differences among the groups in the prevalence of serum 25(OH)D status mentioned above (Figure 2A).

Compliance data

Mean pill counts of 87%, 82%, and 86% in the 400 IU, 2000 IU, and 4000 IU groups, respectively, after randomization were not significantly different.

Primary outcomes findings

The mixed-effect analysis of 25(OH)D concentrations showed significant interactions between group and time ($P < .0001$), indicating that the trajectory of the 3 groups across time is different, depending on the time of the follow-up visit. We compared group differences at each time point to examine this relationship. Figure 2B shows mean (±SE) of the serum 25(OH)D among groups during pregnancy and at the time of delivery. Serum 25(OH)D increased from baseline with supplementation and approached a plateau at 28 weeks in all the groups. The mean serum 25(OH)D in the 2000 IU and 4000 IU groups were higher than in the 400 IU group at 16 weeks ($P < .001$), 28 weeks ($P < .001$), and at delivery ($P < .001$). The mean serum 25(OH)D between the 2000 IU and 4000 IU groups was significantly different at 16 weeks ($P = .034$) and at delivery ($P < .0001$). Two subjects in the 400 IU group and 1 in the 2000 IU group were reallocated to higher doses after 16 weeks of supplementation following the instructions from the DSMC due to serum 25(OH)D less than 10 ng/mL but were treated based on the randomization assignment group to comply with the intent-to-treat analysis.

The mean serum 25(OH)D concentrations in infants at birth were higher in infants of mothers on 4000 IU ($P < .001$) and 2000 IU ($P < .01$) than infants of mothers on 400 IU. The mean serum 25(OH)D concentrations in infants of mothers on 4000 IU was higher than in infants of mothers on 2000 IU ($P < .001$) (Figure 2C). There was a strong correlation ($r = 0.71$, $P < .0001$) between maternal and infant serum 25 (OH)D at birth.

Secondary outcome measures

Serum 25(OH)D concentrations were available in 126 mothers at delivery. The percentage of mothers achieving a priori criteria for vitamin D sufficiency at delivery is different among the groups (Table 3) and is 7-fold higher in mothers who received 4000 IU than those on 400 IU/d ($P < .0001$). If the Institute of Medicine recent recommendation (13) of serum 25(OH)D 20 ng/mL or greater is

Table 3. Categories of Maternal and Infant Vitamin D Status at Delivery by Treatment Group

	4000 IU (n = 43)	2000 IU (n = 41)	400 IU (n = 42)	*P* Value
Mothers achieving 25(OH)D ≥ 32 ng/mL (≥80 nmol/L), n, %	28 (65.1)	10 (24.4)	4 (9.5)	.0001
Mothers achieving 25(OH)D ≥ 20 ng/mL (≥50 nmol/L), n, %	39 (90.7)	31 (75.6)	20 (47.6)	.0001
Infants achieving 25(OH)D ≥ 20 ng/mL (≥50 nmol/L), n, %	34 (79.1)	18 (43.9)	9 (21.4)	.0001

Table 4. Categories of Maternal and Infant Vitamin D Status in This Study and US Study[25]

	4000 IU		2000 IU		400 IU	
	UAE	US	UAE	US	UAE	US
Mean baseline 25(OH)D, ng/mL	7.8	23.3	8.2	23.3	8.6	24.6
Mean 25(OH)D at delivery, ng/mL	35.9	44.0	25.9	39.3	19.3	31.6
Mothers achieving 25(OH)D ≥ 32 ng/mL, %	65	82	24	71	10	50
Infants achieving 25(OH)D ≥ 20 ng/mL, %	75	79	47	58	22	40

applied as adequate, then 91% of mothers on 4000 IU and less than half of mothers on 400 IU would have achieved adequate vitamin D status at delivery ($P < .0001$). Infants of mothers who received 2000 and 4000 IU/d were 2 times and 4 times, respectively, more likely to achieve serum 25(OH)D 20 ng/mL or greater than infants of mothers on 400 IU/d. The comparison with a similar but larger RCT in South Carolina (25) indicated that the mean baseline 25(OH)D levels were lower and the percentage of mothers and infants who achieved serum 25(OH)D greater than 32 ng/mL and 20 ng/mL, respectively, were lower in the UAE study (Table 4). An interesting observation in this study is the mean increment of 10.7 ng/mL (26.7 nmol/L) in 25(OH)D concentrations from enrollment to delivery in the 400 IU group, which was 3–6 times higher than the expected value of 1.6–3.2 ng/mL (4–8 nmol/L) (13, 20, 21).

Serum PTH at all time points correlated inversely ($r = -0.31$, $P < .0001$) with serum 25(OH)D (Figure 3A). The correlation varied by times of gestation (Figure 3B), with the highest correlation at enrollment ($r = -0.32$, $P = .0001$) and insignificant correlation at 16 weeks of gestation. Mean PTH concentrations fell after enrollment in all the groups, and concentration was significantly higher in the 400 IU group than in mothers on 4000 IU at delivery ($P = .011$) (Figure 4A).

Calcium homeostasis and safety measures

Total serum calcium concentrations were within normal range at enrollment but tended to fall during the pregnancy in all the groups with a slight trend to higher serum concentrations in the 4000 IU group. However, the differences among the groups were not significant (Figure 4B). As expected during pregnancy, the mean urine Ca to Cr ratios increased in all the groups. There were no significant differences among the groups (Figure 4C). Throughout the study period, the DSMC did not find adverse events attributable to vitamin D supplementation, and no safety measure stops were implemented.

Infant growth parameters

There were no significant differences in the mean birth weight, length, head circumference, and gestational age among groups. There were 4 cases of small-for-gestational age infants in the 400 IU group, none in the 2000 IU group, and 5 in the 4000 IU group. These differences were not significant ($P = .08$).

Discussion

In this study, we found a very high baseline prevalence of 98% vitamin D deficiency [serum 25(OH)D < 20 ng/mL]

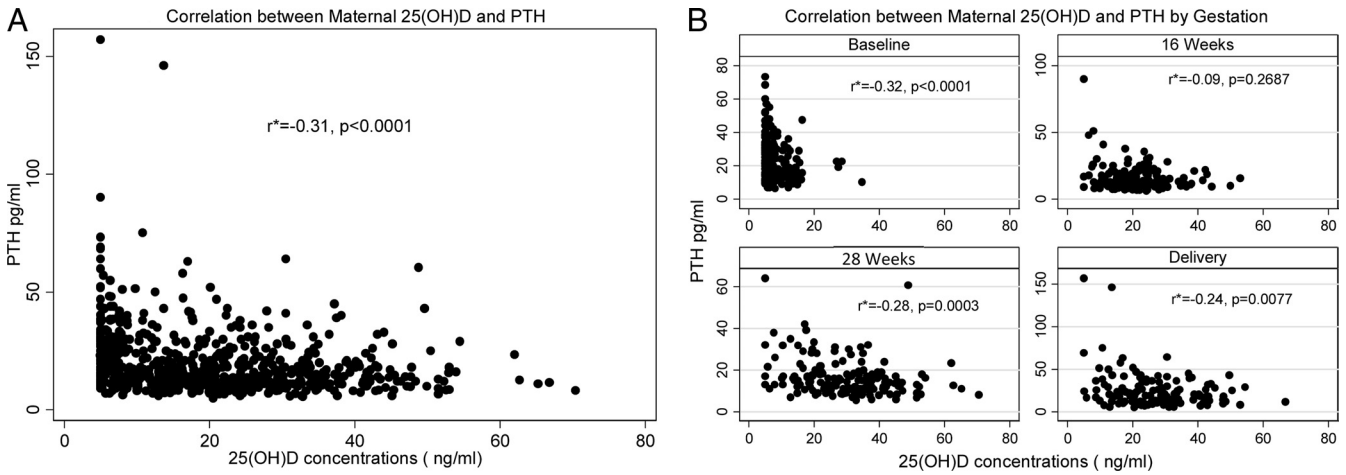

Figure 3. The relationship between serum 25(OH)D and intact PTH concentrations at all time points during pregnancy (A) and at enrollment, 16 weeks, and 28 weeks of gestation and at delivery (B) are shown. Spearman correlation coefficients and *P* values are reported.

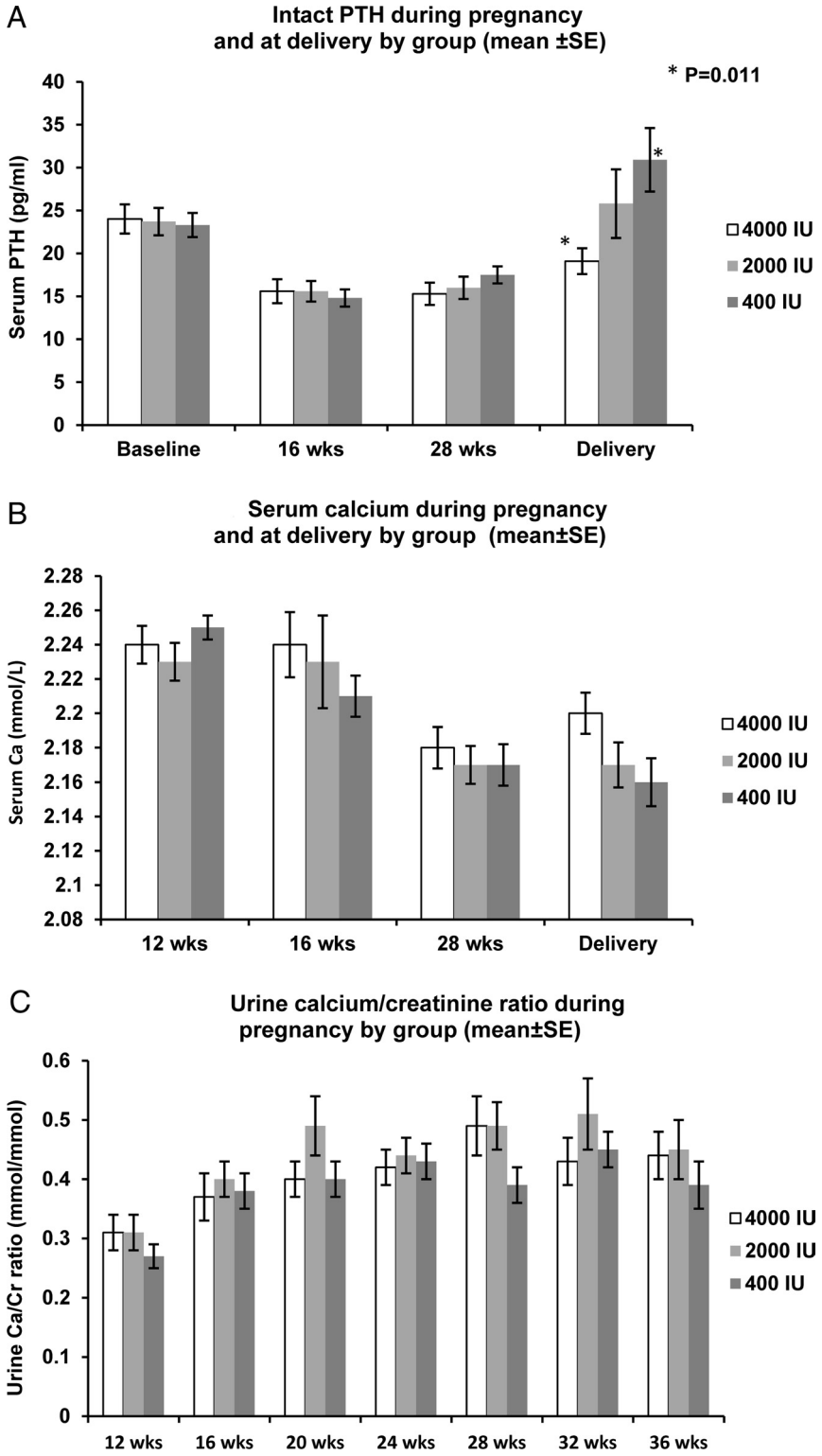

Figure 4. Serum intact PTH (A), serum calcium (B), and urinary Ca to Cr ratios (C) at defined time points during pregnancy by treatment groups are shown. The mean PTH was significantly higher in the 400 IU group than the 4000 IU group at delivery ($P = .011$).

and 99% vitamin D insufficiency [serum 25(OH)D < 32 ng/mL]. Vitamin D_3 supplementation of 4000 IU/d was most effective in achieving vitamin D sufficiency throughout pregnancy when compared with 2000 IU and 400 IU/d. This is consistent with our hypothesis. At the time of delivery, 65% of the mothers on 4000 IU compared with less than 10% of those on 400 IU/d were categorized as vitamin D sufficient. The mean serum 25(OH)D concentrations reached a plateau after 16 weeks of supplementation. There were no reports of documented hypervitaminosis D (serum 25[OH]D > 90 ng/mL) during the period of the study (25). The pattern of response to supplementation is consistent with enzymatic kinetics of first-order to zero-order reactions (27). The reason for the decline in serum 25(OH)D concentrations in the group on 2000 IU/d is unclear. There was no evidence of exposure to the lower dose of vitamin D_3 study drug or a change in compliance. It is, however, noteworthy that compliance as measured by pill count in this study may not be a reliable predictor of compliance as measured by the change in serum 25(OH)D concentrations (28).

Two recent studies of vitamin D supplementation in pregnancy from India, in which the prevalence of vitamin D deficiency is high, used intermittent very large doses of 120 000 IU vitamin D in the fifth and seventh months (29) or second and third trimesters (30). The mean serum 25(OH)D concentrations at delivery were 53.4 nmol/L (29) and 58.7 nmol/L (30) compared with a mean of 89.8 nmol/L in our study. In one of the studies from India (29), only 34% of the subjects in the study compared with 65% of subjects on 4000 IU/d in our study achieved serum 25(OH)D of 32 ng/mL at delivery. A recent RCT from South Carolina showed that vitamin D intake of 4000 IU/d during pregnancy was safe and was more effective than 2000 IU and 400 IU in achieving serum 25(OH)D concentrations of 32 ng/mL or more in mothers and serum 25(OH)D of 20 ng/mL or greater in the infants at birth (25). The results of our study are in agree-

ment with theirs. However, there was a higher mean increment in 25(OH)D concentrations between enrollment and delivery in our study than in the South Carolina study (Table 4). This finding presumably relates to the much lower baseline levels in our Arab population and supports the need for taking into consideration baseline prevalence of vitamin D deficiency when evaluating outcome of vitamin D supplementation (31). Despite the larger increment of 25(OH)D, the percentage of mothers on 4000 IU/d vitamin D_3 who achieved the target level of 32 ng/mL at delivery was still 20% lower than in the US study.

Recent observational studies indicated that serum 25(OH)D concentrations greater than 30 ng/mL (75 nmol/L) is associated with a reduced risk of nonskeletal health disorders, such as preeclampsia (32) and gestational diabetes (33). Furthermore, serum 25(OH)D greater than 30 ng/mL in the cord blood has been associated with an improved newborn innate immune response (34) and a reduced risk of respiratory syncytial virus bronchiolitis and eczema in early childhood (7, 8). Therefore, intervention trials assessing the benefit of prenatal vitamin D supplementation for nonskeletal health conditions should include a treatment arm aimed at achieving the optimal serum 25(OH)D concentrations. Our data and those from another recent study (25) indicate that daily supplementation of 4000 IU vitamin D_3 appears safe and effective to test this hypothesis.

With respect to PTH assessment, the only significant difference among the groups was a lower mean concentration in the 4000 IU group than the 400 IU group at the time of delivery ($P = .011$), supporting a greater suppression of PTH secretion in the 4000 IU group due to higher serum 25(OH)D concentrations than in 400 IU group during the last trimester. An inverse relationship between serum 25(OH)D and PTH has been reported to be weaker in pregnant than nonpregnant women (25). In this study we found a significant negative correlation between 25(OH)D and PTH for all subjects at baseline ($r = -0.31$, $P < .0001$). The relationship was variable during the course of pregnancy, with a significant relationship at 28 weeks of gestation and delivery but not at 16 weeks. A similar degree of an inverse correlation between PTH and 25(OH)D ($r = -0.32$, $P = .002$) was found in healthy Asian and Caucasian pregnant women in the United Kingdom (35). Other authors reported weaker negative correlations ($r = -0.24$, $P = .001$ and $r = -0.18$, $P = .001$, respectively) among US women in South Carolina (36) and Australian women in Victoria (37). A recent study from Saudi Arabia found no significant correlation between serum PTH and 25(OH)D concentrations (38). In our study, there was no sharp inflection point of elevated serum PTH when serum 25(OH)D was low, as has been suggested by some authors (39). Taken together, the results indicate that the inverse relationship between 25(OH)D and PTH seems to vary widely during pregnancy and may not be a very reliable biomarker of vitamin D status as previously suggested (35).

We found no differences in birth weight, length, and head circumference at birth among the 3 supplementation groups. Although several observational studies and 1 RCT have shown no improvement in birth weight in association with maternal vitamin D supplementation or status during pregnancy (25, 40), other studies found improvement in birth weight or other anthropometric measurements (1, 30, 41). Observational studies and a RCT have also found a higher risk of small-for-gestational-age infants in association with maternal vitamin D status in pregnancy (1, 2, 42). The relationship was found to be U shaped in 1 study (42), but this was not proven in other studies (1, 2, 43). The sample size in this study was too small to evaluate the effect of supplementation on growth in a randomized controlled fashion. Our study, however, provided valuable data on vitamin D dosing that appear to be safe and effective in ensuring maternal vitamin D sufficiency and that would be valuable in designing trials to test the effects of vitamin D supplementation on fetal growth and other maternal and infant health outcomes.

Summary

This study provides data from a randomized controlled trial on daily vitamin D supplementation during the second and third trimesters of pregnancy that achieved vitamin D sufficiency (serum 25[OH]D $\geq$ 32 ng/mL) and adequacy (serum 25[OH]D $\geq$ 20 ng/mL) in a population with a high prevalence of severe vitamin D deficiency. Vitamin D supplementation of 4000 IU/d in this population appeared to be safe and most effective in achieving vitamin D sufficiency in mothers and serum 25(OH)D of 20 ng/mL or greater in infants at birth. Large RCTs are needed to test the safety and effectiveness of such supplementation strategy on growth and other nonskeletal health outcomes in the mother and offspring in populations with endemic vitamin D deficiency.

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