JBMR

Vitamin D Supplementation During Pregnancy: Double-Blind, Randomized Clinical Trial of Safety and Effectiveness

Bruce W Hollis,[1] Donna Johnson,[3] Thomas C Hulsey,[2] Myla Ebeling,[2] and Carol L Wagner[1]

[1]Division of Neonatology and Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, USA
[2]Division of Epidemiology, Department of Pediatrics, and Medical University of South Carolina, Charleston, SC, USA
[3]Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, USA

ABSTRACT

The need, safety, and effectiveness of vitamin D supplementation during pregnancy remain controversial. In this randomized, controlled trial, women with a singleton pregnancy at 12 to 16 weeks' gestation received 400, 2000, or 4000 IU of vitamin D_3 per day until delivery. The primary outcome was maternal/neonatal circulating 25-hydroxyvitamin D [25(OH)D] concentration at delivery, with secondary outcomes of a 25(OH)D concentration of 80 nmol/L or greater achieved and the 25(OH)D concentration required to achieve maximal 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] production. Of the 494 women enrolled, 350 women continued until delivery: Mean 25(OH)D concentrations by group at delivery and 1 month before delivery were significantly different ($p < 0.0001$), and the percent who achieved sufficiency was significantly different by group, greatest in 4000-IU group ($p < 0.0001$). The relative risk (RR) for achieving a concentration of 80 nmol/L or greater within 1 month of delivery was significantly different between the 2000- and the 400-IU groups (RR = 1.52, 95% CI 1.24–1.86), the 4000- and the 400-IU groups (RR = 1.60, 95% CI 1.32–1.95) but not between the 4000- and. 2000-IU groups (RR = 1.06, 95% CI 0.93–1.19). Circulating 25(OH)D had a direct influence on circulating 1,25$(OH)_2D_3$ concentrations throughout pregnancy ($p < 0.0001$), with maximal production of 1,25$(OH)_2D_3$ in *all* strata in the 4000-IU group. There were no differences between groups on any safety measure. Not a single adverse event was attributed to vitamin D supplementation or circulating 25(OH)D levels. It is concluded that vitamin D supplementation of 4000 IU/d for pregnant women is safe and most effective in achieving sufficiency in all women and their neonates regardless of race, whereas the current estimated average requirement is comparatively ineffective at achieving adequate circulating 25(OH)D concentrations, especially in African Americans. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: VITAMIN D; CHOLECALCIFEROL; PREGNANCY; NEONATE

Introduction

The function of vitamin D during pregnancy for both mother and fetus remains largely undefined. Vitamin D is known to be involved in skeletal homeostasis during pregnancy, as evidenced by a recent publication dealing with craniotabes in the newborn, and severe vitamin D deficiency may lead to neonatal seizures in neonates with profound hypocalcemia.[1–5] The function of vitamin D during this sensitive period, however, also may have potential effects on other systems, including immune,[6–10] pancreatic,[11–13] musculoskeletal,[14–17] and cardiovascular function,[18–20] as well as neural development.[21–24] Recent publications suggest relationships between maternal vitamin D status and adverse pregnancy outcomes such as preeclampsia and cesarean section.[25–28]

A Cochrane Review published in 2000 highlighted the relative dearth of data dealing with vitamin D supplementation during human pregnancy.[29] This review listed seven studies on the topic,[30–36] of which four reported clinical outcomes.[30–32,36] From these limited data, the Cochrane Review concluded that there was insufficient evidence to evaluate the effects of vitamin D supplementation during pregnancy.[29] Since that time, few studies have addressed this issue.[37–39]

In 2004, we initiated a National Institute of Child Health and Human Development (NICHD)–sponsored 6-year randomized, double-blind, placebo-controlld trial of vitamin D supplementa-

Received in original form March 29, 2011; revised form June 7, 2011; accepted June 10, 2011. Published online June 27, 2011.
Address correspondence to: Bruce W Hollis, PhD, Medical University of South Carolina, 173 Ashley Avenue, MSC 513, Charleston, SC 29425, USA.
E-mail: hollisb@musc.edu
Presented in part at the 14th Vitamin D Workshop, Brugge, Belgium, October 2009, and the Pediatric Academic Societies Meeting, Vancouver, British Columbia, Canada, May 2010.
For a Commentary on this article, please see Abrams (J Bone Miner Res. 2011;26:2338-2340. DOI: 10.1002/jbmr.498).

Journal of Bone and Mineral Research, Vol. 26, No. 10, October 2011, pp 2341–2357
DOI: 10.1002/jbmr.463

tion during pregnancy to assess safety and pregnancy outcomes with an approved Investigational Drug Application from the US Food and Drug Administration (FDA; No. 66,346). We hypothesized that 4000 IU/d of vitamin D_3 would be more efficacious and effective than the standard dosing regimen of 400 IU/d and the 2000 IU/d (the former upper limit for vitamin D) dosing regimen in achieving a total circulating 25-hydroxyvitamin D [25(OH)D] level of at least 80 nmoL/L (32 ng/mL) in pregnant women regardless of race throughout pregnancy and at the time of delivery without causing any safety concerns. This minimal value of 80 nmol/L was based on years of research with regard to circulating 25(OH)D levels suppressing secondary hyperthyroidism and having optimal intestinal calcium absorption and bone mineral density.[41] These results are presented here.

Methods

Study design

This study was a single-center, randomized, controlled, double-blind study of vitamin D supplementation stratified by race (FDA IND No. 66,346; ClinicalTrials.gov No. NCT00292591). Women at fewer than 16 weeks' gestation with a singleton pregnancy were eligible for participation in the study.

Study participants and setting

This study was approved by Medical University of South Carolina's (MUSC's) Institutional Review Board for Human Research (HR No. 10725) and was conducted from January 4, 2004, through July 31, 2009, at MUSC (Charleston, SC, USA). The inclusion criteria for the subjects included the following: (1) maternal age of 16 years or greater at the time of consent, (2) confirmed singleton pregnancy of fewer than 16 completed weeks of gestation at the time of consent, (3) planned to receive ongoing prenatal care in the Charleston, SC, area, and (4) the ability to provide written informed consent at the first visit. If a woman received her obstetrical care at a facility separate from MUSC, then she came to MUSC's Clinical and Translational Research Center (CTRC) outpatient research facility for each of the study visits. Women were consented at their first prenatal visit, at which time baseline 25(OH)D levels were measured. Irrespective of gestational age at enrollment, subjects began vitamin D supplementation between the start of the twelfth and the start of the sixteenth weeks of gestation (12 0/7th and 16 0/7th weeks), as defined by their last menstrual period.

Exclusion criteria

Women with a pregnancy at greater than 16 weeks of gestation as calculated by their last menstrual period were not eligible to participate. Pregnant women with preexisting calcium or parathyroid conditions or who required chronic diuretic or cardiac medication therapy, including calcium channel blockers, or who suffered chronic hypertension were not eligible for enrollment in the study. Pregnant women with active thyroid disease (eg, Graves disease, Hashimoto disease, or thyroiditis) also were excluded, but mothers on thyroid supplement with normal serologic parameters could participate in the study if they were without any other endocrine dysfunction.

Study protocol

Gestational age at enrollment

Subjects could be consented and enrolled into the study before the initiation of vitamin D supplementation at 12 to 16 weeks of gestation. Gestational age was based on last menstrual period. If a woman was unsure of her gestational age, the obstetrical estimate at the time of the visit was used. If, at the 20-week fetal ultrasound it was determined by the obstetrician that the gestational age was incorrect, the revised gestational age was used and the discrepancy noted.

Initial study visit

Baseline blood and urine samples were obtained following subject consent at the initial visit, but the earliest time of randomization following measurement of baseline total circulating 25(OH)D level was 12 weeks' gestation, with the target upper limit of gestation of 16 weeks. Irrespective of enrollment gestational age, vitamin D supplementation did not begin before the twelfth week of gestation (12 and 0/7th weeks).

Subsequent study visits

Subjects were followed with monthly study visits, which continued until delivery. The visits coincided with routine obstetrical visits or were performed in conjunction with those visits if the obstetrical care was provided outside MUSC. The subjects also were seen at the GCRC/CTRC for a study visit at 16 weeks of gestation and with their infant at 2 weeks' postpartum.

Completion of questionnaires

Following their written informed consent, mothers completed questionnaires regarding sociodemographic information, baseline health status, and medical history at the first visit. At the second visit, the Block Food Frequency Questionnaire (FFQ) was completed to ascertain generalized eating pattern, with specific calculation of calcium and vitamin D intake (Block, Berkeley, CA, USA).[42–47] Each completed FFQ form was sent to the processing center (Berkeley, CA, USA), and these data were reviewed later for accuracy by a registered dietician who was blinded to subject treatment group assignment. Total caloric, vitamin D, and calcium intakes were recorded for each subject.

An interim maternal health history questionnaire also was completed at each visit with the assistance of the study coordinator to ascertain adverse events, discussing types and frequencies of acute illnesses such as respiratory, gastrointestinal, and other viral and/or bacterial illnesses. A review of medications and doctor's visits was obtained at that time.

After delivery, the newborn record of each infant was reviewed for mode of delivery and level of neonatal care required (normal newborn nursery or level 2 or level 3 intensive care). Birth weight (g) and gestational age also were recorded.

Blood and urine samples

Maternal blood and urine samples were collected at each visit. Cord blood was obtained at delivery. If the cord blood sample

could not be obtained, a neonatal blood sample was drawn within 2 weeks of delivery.

Intervention

Multivitamin and vitamin D supplementation

Pregnant women who presented for prenatal care at 16 or fewer weeks of gestation were randomized into one of three treatment regimens of vitamin D_3 after establishing their baseline serum 25(OH)D level. All patients received a total of two pills daily: a standard prenatal multivitamin vitamin containing 400 IU of vitamin D and an additional vitamin D_3 supplement of 0 IU (placebo), 1600 IU, or 3600 IU of vitamin D_3 for a total of 400 IU, 2000 IU, and 4000 IU of vitamin D supplementation, respectively.

In order to obtain Institutional Review Board approval for the study, the following safety measure was put into place: Baseline total circulating 25(OH)D levels were measured, and women with levels of 100 nmol/L (40 ng/mL) or less were eligible for randomization into one of the three arms (400, 2000, or 4000 IU/d of vitamin D_3) with further substratification by race within each treatment group. Women with baseline 25(OH)D levels greater than100 to 150 nmol/L (>40 to 60 ng/mL, levels considered to be in the normal range at the time of study implementation) were randomized into one of two treatment groups (400 or 2000 IU/d of vitamin D_3), whereas women with a baseline 25(OH)D level greater than 150 nmol/L (>60 ng/mL) were given 400 IU/d of vitamin D_3. The doses of vitamin D used in our study were selected based on current recommendations (400 IU/d), the upper safe intake level established in 1997 (2000 IU/d),[40] and the amount we calculated to be required to achieve nutritional vitamin D sufficiency (4000 IU/d).[48].

Adherence to medication regimen

Adherence to the prescribed vitamin D supplementation regimen of one prenatal vitamin and the vitamin D supplement was measured by maternal self-report and pill counts at each follow-up visit.[49] The number of vitamin D pills returned was divided by the expected number of pills that would have been taken between study visits to generate a percentage that served as a measure of adherence of medication regimen between study visits. The adherence measures were used to generate an average adherence for each subject.[49] If a woman missed one prenatal visit, her next month supply of vitamins was either mailed to her or dropped off at her residence. In such cases, medication adherence was based on the pill count from the date of the last visit to the current prenatal visit over the expected number of pills taken. If a woman had more than two missed visits or if she failed to take at least 50% of the prescribed vitamin D pills, she was exited from the study.

Randomization

Our study used stratified blocked randomization to balance by ethnicity and also to balance by enrollment (as a cautionary measure against a potential temporal or seasonal bias). A randomization scheme was developed separately for each of the three ethnic groups (ie, the strata). Within each stratum, the treatments were assigned within blocks. Because there were three treatment groups, the block size had to be divisible by 3; the data team selected a block size of six, which was unknown to the investigators or the pharmacists. In this way, at the end of each block (ie, enrollment of six subjects), each ethnic group was balanced in the number randomly assigned to the 400-, 2000-, and 4000-IU treatment groups.

Materials

Source of vitamin D

Vitamin D tablets were manufactured by Tishcon Corporation (Westbury, NY, USA), a Good-Manufacturing-Practice (GMP) facility. The cholecalciferol contained in the vitamin D tablet was supplied to Tishcon Corporation by Hoffman-La Roche, Ltd. (Basel, Switzerland). The tablet vitamin D concentration was verified by the company every 6 months and by an independent laboratory chosen by the investigators (Heartland Assays, Ames, IO, USA) using high-performance liquid chromatography with UV detector (HPLC-UV) to ensure that the tablets met label claims throughout the study; these results were reported to the Investigational Drugs Department at MUSC. Tablets were maintained in MUSC's Research Pharmacy until the time that they were dispensed to each enrolled subject.

Source of prenatal vitamins

Prenatal vitamins prescribed at the time of each subject's enrollment were manufactured by Myadec Multivitamin-Multimineral Supplement (distributed by Pfizer Consumer Healthcare, Morris Plains, NJ, USA) with 400 IU of vitamin D_3 per tablet. Mothers who were unable to swallow a prenatal vitamin were given Flintstones Complete chewable vitamin (Bayer Healthcare, Morristown, NJ, USA), which provided 400 IU of vitamin D_3 per tablet.

Measures

Maternal sociodemographic measures included maternal age at time of enrollment, her self-defined race, insurance status, educational status, and occupation and employment outside of the home.

Pregnancy health status and labor and delivery characteristics and complications

Characteristics of each mother's health status and complications during pregnancy, labor, and delivery were recorded and reviewed by an obstetrician (DDJ, blinded to treatment). If the mother required hospitalization, a copy of the hospital record was obtained after the mother had signed a release of medical information form. Any acute illnesses, hospitalizations, or development of pregnancy-related conditions that were not preexisting also were recorded. The Data Monitoring and Safety Committee (DSMC) was notified of all such events.

Anthropomorphic measurements

Prepregnancy height and weight of each mother were recorded at the first outpatient visit to determine BMI (weight [kg]/height^2

[m^2]). During subsequent visits, only the subject's weight was recorded. Birth weight (g) was recorded for each infant.

Laboratory measurements

Maternal and cord blood/neonatal vitamin D and metabolite assays

Circulating vitamin D_2 and D_3 were measured in serum using direct ultraviolet detection preceded by organic extraction and high-performance liquid chromatography, as described previously.[50] This assay has a coefficient of variation of 10% or less and a 5 nmol/L vitamin D detection limit. There is no normal established circulating range of vitamins D_2 or D_3 in human subjects.

A rapid, direct RIA developed in the Hollis laboratory and manufactured by Diasorin Corporation (Stillwater, MN, USA) was used to measure total circulating 25(OH)D concentration in serum samples.[51] This RIA is an FDA-cleared device and, in fact, is the FDA predicate device for the measurement of circulating 25(OH)D in humans.

Based on clinical laboratory classifications,[52,53] a priori, deficiency was defined as a total circulating 25(OH)D level of less than 50 nmol/L (20 ng/mL), insufficiency as 50 nmol/L or greater to less than 80 nmol/L ($\geq$20 to $<$32 ng/mL), and sufficiency as 80 nmol/L or greater ($\geq$32 ng/mL).[41,53–55] The inter- and intraassay coefficients of variation are 10% or less.

An RIA manufactured by Diasorin Corporation and developed in the Hollis laboratory was used to measure total circulating 1,25-dihydroxyvitamine D_3 [1,25$(OH)_2D_3$] concentration.[56] This assay uses an ^{125}I-labeled tracer, and samples are processed using acetonitrile followed by solid-phase extraction and quantitation. This RIA is an FDA-cleared device. The normal circulating level of 1,25$(OH)_2D_3$ is 48 to 120 pmol/L (20 to 50 pg/mL). The inter- and intraassay coefficients of variation are 15% or less.

Maternal and cord blood/neonatal circulating intact parathyroid hormone (PTH) concentrations

Intact PTH (iPTH) was measured by immunoradiometric assay (IRMA) that uses two different polyclonal antibodies (Diasorin). The first antibody, specific for PTH(39–84), is bound to a solid-phase bead. The second antibody is specific for PTH(1–34) and is labeled with ^{125}I. The adult normal range for iPTH in our laboratory is 1.3 to 5.4 pmol/L. Higher vitamin D levels are associated with lower iPTH levels because iPTH declines as vitamin D status improves.[57]

Maternal baseline and follow-up serum calcium, creatinine, and phosphorus studies

Maternal serum total calcium, creatinine, and inorganic phosphorus levels were measured by MUSC's Clinical Chemistry Laboratory using standard methodology and laboratory normative data. Results were reported to the clinical principal investigator (PI; CLW) and downloaded to the research database from the clinical chemistry registry. All results were reviewed by the clinical principal investigator of the study on a weekly basis for any abnormal values and reported to the DSMC.

Circulating vitamin D–binding protein (VDBP)

VDBP was measured using a commercial ELISA purchased from R&D Systems (Minneapolis, MN, USA). Circulating VDBP levels in normal individuals using this ELISA are stated by the manufacturer to be 3.93 ± 1.62 μmol/L.

Maternal urinary calcium/creatinine ratio

A nonfasting urine sample was obtained from the mother at each obstetrical visit and was sent to the Clinical Chemistry Laboratory at MUSC for urinary calcium (Ca) and creatinine (Cr) measurements and derivation of the urinary Ca:Cr (mg/dL) ratio (converted to mmol/L/mmol/L). (To convert mg/dL of calcium to mmol/L, multiply the value by 0.25. To convert mg/dL of creatinine to mmol/L, multiply by 0.088.)

Safety measures throughout the study

All study subjects were monitored for hypervitaminosis D. The circulating 25(OH)D level of 225 nmol/L (90 ng/mL) was used to define hypervitaminosis D, as required by the FDA and our IRB. This conservative maternal level was arbitrarily chosen to ensure the safety of all study patients, particularly those assigned to the 4000 IU of vitamin D_3 per day regimen.[58] Subsequent vitamin D supplementation trials have demonstrated that circulating levels of 25(OH)D exceeding 300 nmol/L (120 ng/mL) do not cause hypercalciuria, the first indicator of hypervitaminosis D.[48] Even in women who are vitamin D deficient, urinary calcium excretion increases during pregnancy secondary to increased glomerular filtration rate.[59] Given this, urinary calcium/creatinine ratio was used and is the most sensitive early indicator of hypervitaminosis D. Operationally, we defined a priori *caution* limits for hypervitaminosis D as a nonfasting urinary calcium/creatinine ratio of 0.8 mg/mg or 2.27 mmol/mmol or greater.

Whenever any patient exceeded the caution limit or had an abnormal clinical chemistry value, a specific case study by the Data Safety and Monitoring Committee (DSMC) was to be initiated to examine the contribution of confounding factors (eg, diet, sunlight exposure, etc.). Operationally, vitamin D_3 supplementation stopped if the urinary calcium/creatinine ratio exceeded 1.0 (mg/dL/mg/dL) *or* if the circulating 25(OH)D level exceeded 225 nmol/L (90 ng/mL).

Statistical methods

Sample size and power considerations

To detect a statistically significant increase in 25(OH)D by 10 ng/mL between any two groups, it was calculated to require a minimum of 32 patients per group at 90% power, $\alpha = 0.05$, two-tailed test for the primary analysis. This calculation assumed that the standard deviation of 25(OH)D measurements at a single time point was approximately 10, that there would be a low correlation ($r = 0.25$) between the baseline and final measurements, and that a substantial proportion (up to 50%) of participants may be lost to follow-up owing to moving, termination of care, or discontinuation of participation. Because the primary outcome—maternal and neonatal vitamin D status at or around the time of delivery—a prerequisite for inclusion in the final analysis was that the mother had to have had a live birth

and had to have subject participated until the day of delivery. Lastly, since one of the secondary goals of this study was to explore vitamin D differences by ethnicity, the three supplemented groups (400, 2000, and 4,000 IU/d) were balanced by ethnicity (equal numbers of whites, blacks, and Hispanic).

Statistical analysis

The main variables of interest were: (1) differences in mean maternal and infant total circulating 25(OH)D levels at the time of delivery between supplement groups (ANOVA), and (2) differences between supplement groups in the proportion of women achieving a 25(OH)D level of 80 nmol/L or greater within 1 month and at the time of delivery (chi-square). Secondary analyses employed chi-square for categorical variables; ANOVA or Student's *t* test, as appropriate, for normally distributed variables (with the Bonferonni option for pairwise analysis in ANOVA); and paired Student's *t* test for within-group changes from baseline to delivery. Multiple regression was used to assess the association between final vitamin D concentrations and baseline values, ethnicity, dose group, and the dose × race interaction. Stratified analysis was used to more fully explore any evidence of interaction. Variables that were not normally distributed were analyzed with Wilcoxon-Mann-Whitney test. The association between vitamin D [25(OH)D and $1,25(OH)_2D_3$] and urinary calcium/creatinine ratio was explored with a combination of exponential and linear models. Data were analyzed with SAS 9.22[60] (SAS Institute, Cary, NC, USA) and SigmaPlot software (Systat Software, Inc., San Jose, CA, USA).

The analysis was conducted as an intention-to-treat (ITT) study.[61] The ITT approach (effectiveness) compares the outcomes between supplement groups, as assigned, and makes no assumption regarding whether or not subjects were adherent to the dosing regimen. (Data on adherent subjects will be made available to any investigator on request following publication.) The ITT design was used as a measure of the effectiveness of increasing vitamin D levels via oral dosing. This approach presents a conservative finding of potential benefits that could be shown from a population- or public health–based intervention. (Adherence efficacy and outcome data with detailed pharmacokinetics will be presented in a separate article.)

Results

Study population

Figure 1 shows the enrollment, allocation, and follow-up of the women who participated in the trial. A total of 516 women were interviewed, and 502 consented to participate in this study and were randomly assigned to a treatment group: 166 were assigned to group 1 (400-IU group), 167 were assigned to group 2 (2000-IU group), and 169 were assigned to group 3 (4000-IU group). Of the 502 women consented, there were 23 women with a valid initial 25(OH)D greater than 100 nmol/L (40 ng/mL) who were not eligible for enrollment in the 4000-IU group: 2 black, 6 Hispanic, and 15 white women. Of those, 12 were enrolled into the 400-IU group, 10 were enrolled into the 2000-IU group, and 1 was enrolled in the 4000-IU group [the latter being a protocol deviation early in the study where one woman with a baseline 25(OH)D level of 41 ng/mL was randomized to the 4000-IU group]. Seventeen continued until delivery: 1 black, 6 Hispanic, and 10 white women; 10 were in the 400-IU group,

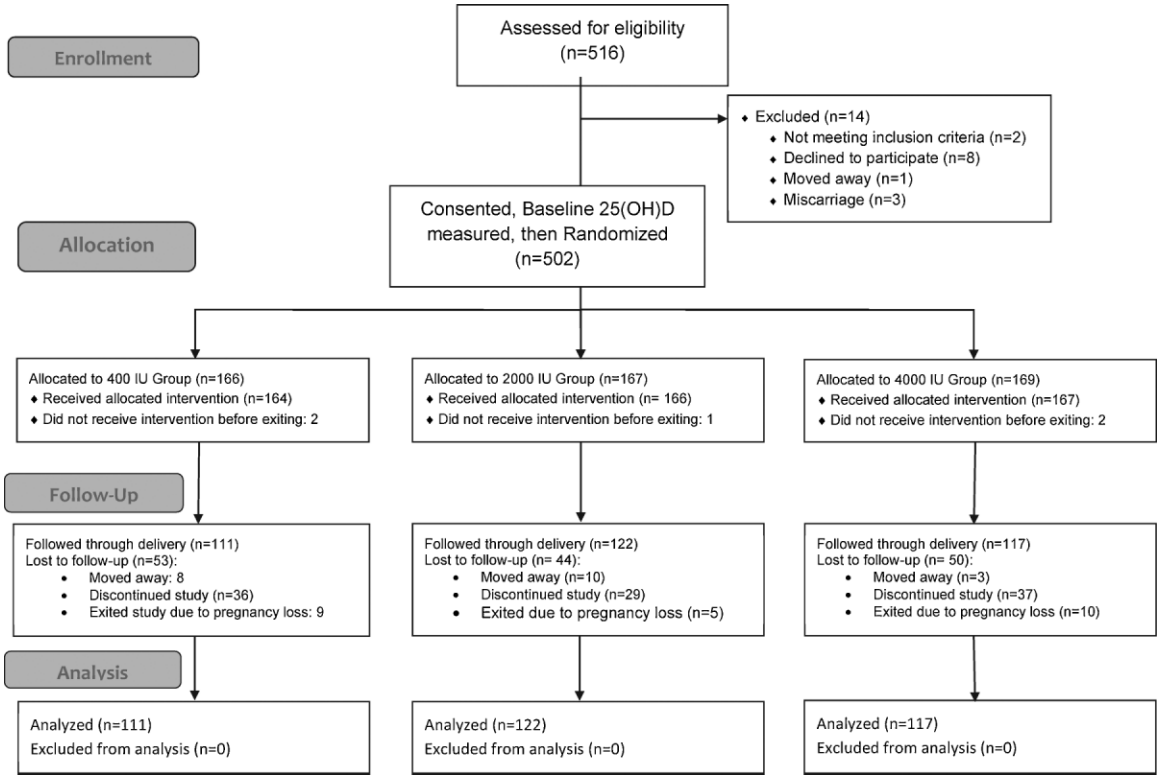


Fig. 1. Flow diagram of pregnancy study. IU = international units.

6 were in the 2000-IU group, and 1 was in the 4000-IU group. Finally, there was one white woman whose baseline 25(OH)D level was 172.5 nmoL/L (69 ng/mL) who was placed into the 400-IU group. After allocation into treatment groups, there were no statistically significant differences among the groups with regard to lost to follow-up, dropouts, or pregnancy losses.

The sociodemographic characteristics of the active cohort are found in Table 1. Baseline characteristics were similar between the groups on the basis of race/ethnicity, maternal age, gestational age at enrollment, educational and employment status, health rating, planned pregnancy, BMI, and season at study entry. There was a trend toward differences between the groups on the basis of maternal gravidity and parity and insurance status. A total of 62 women (12.4%) were taking a prenatal vitamin at the time of randomization. Of the 502 women who were randomized to treatment, 350 women continued in the study until delivery and had outcome data available for analysis: 98 black, 137 Hispanic, and 115 white women evenly distributed into the three treatment groups with 111 controls, 122 in 2000-IU and 117 in 4000-IU groups. There were no differences in baseline vitamin D status among treatment groups.

A comparison of women who completed the study and those who electively discontinued the study is found in Table 2. Women who had a pregnancy loss or who moved away were excluded from the analysis. Individuals who exited the study did not differ by treatment group. Women who electively exited the study were more likely to be black than Hispanic or white.

Table 1. Maternal Sociodemographic and Clinical Characteristics at Study Enrollment by Vitamin D Supplementation Group

Maternal characteristic	400-IU group ($n = 111$)	2000-IU group ($n = 122$)	4000-IU group ($n = 117$)	p Value
Race/ethnicity,[a] *n* (%)				0.9
Black	28 (25.2)	37 (30.3)	33 (28.2)	
Hispanic	45 (40.5)	48 (39.3)	44 (37.6)	
White	38 (34.2)	37 (30.3)	40 (34.2)	
Maternal age (years), mean ± SD	27.0 ± 5.6	27.4 ± 5.7	26.6 ± 5.4	0.6
Range	18–41	17–41	17–44	
Gestation at enrollment (weeks), mean ± SD	12.5 ± 1.9	12.6 ± 1.6	12.4 ± 2.0	0.8
Range	7.1–18.4	8.4–17.6	6.4–21.4	
Maternal gravidity, median	2	2	2	0.08
Range	1–8	1–7	1–9	
Maternal parity, median	2	2	1	0.052
Range	0–5	0–7	0–9	
Education, *n* (%)				0.4
<HS education	18 (17.3)	23 (19.7)	13 (11.6)	
HS graduate	17 (16.4)	24 (20.5)	22 (19.6)	
College or more	69 (66.4)	70 (59.8)	77 (68.8)	
Employed at study entrance, *n* (%)	61 (55.0)	67 (54.9)	65 (55.6)	0.9
Insurance, *n* (%)				0.07
Medicaid/none	62 (55.9)	85 (69.7)	69 (59.0)	
Commercial	49 (44.1)	37 (30.3)	48 (41.0)	
Subjective health rating scale, Median[b]	9	10	10	0.4
Range	5–10	5–10	1–10	
Planned pregnancy, *n* (%)	59 (54.6)	61 (50.4)	59 (50.4)	0.8
BMI, *n* (%)[c]				0.6
≤30	78 (70.3)	87 (71.3)	89 (76.1)	
>30	33 (29.7)	35 (28.7)	28 (23.9)	
Season at study entry, *n* (%)				0.9
April–September	54 (48.7)	60 (49.2)	56 (47.9)	
October–March	57 (51.4)	62 (50.8)	61 (52.1)	
Vitamin D intake in IU,[d] mean ± SD	181.6 ± 108.4	195.8 ± 135.0	204.2 ± 148.2	0.6
Range	21.4–470.6	8.2–693.8	5.3–737.3	
Calcium intake, mg/d, Mean ± SD	1063.6 ± 539.6	993.9 ± 514.0	1073.6 ± 491.9	0.6
Range	252.9–2888.1	285.4–2754.1	275.6–2925.9	
kcal Intake, mean ± SD	2148.3 ± 778.6	2059.4 ± 803	2212.9 ± 920.8	0.5
Range	977.3–4668.2	993.4–4793.4	929.3–5516	

[a]Race/ethnicity as defined by mother.
[b]Self-reported maternal health status rating from 1 (poor) to 10 (excellent).
[c]BMI = prepregnancy body mass index.
[d]International units (IU): dietary intake calculated from the Block 1998 Food Frequency Questionnaire;[42,43] amount did not include prenatal vitamin intake.

Table 2. Subjects Who Completed the Study Compared With Subjects Exited Before Delivery[a]

Maternal characteristic	Delivered ($n = 350$)	Exited ($n = 129$)	p Value
Treatment group, *n* (%)			0.5
400 IU	111 (74.0)	39 (26.0)	
2000 IU	122 (79.7)	31 (20.3)	
4000 IU	117 (75.5)	38 (24.5)	
Ethnicity,[b] *n* (%)			0.003
Black	98 (66.7)	49 (33.3)	
Hispanic	137 (81.1)	32 (18.9)	
White	115 (81.0)	27 (19.0)	
Maternal age (years), mean ± SD	27.0 ± 5.6	25.5 ± 5.1	0.01
Range	17–44	18–42	
Gestational age at enrollment (weeks), mean ± SD	12.5 ± 1.8	12.1 ± 2.1	0.053
Range[c]	6.3–21.4	6.1–17.7	
Maternal gravidity, median (range)	2.0 (0–9)	3.0 (1–10)	<0.0001
Education, *n* (%)			0.01
<HS education	54 (73.0)	20 (27.0)	
HS graduate	63 (71.6)	25 (28.4)	
College or more	216 (84.4)	40 (15.6)	
Employed at entrance into study, *n* (%)			0.04
Yes	193 (81.1)	45 (18.9)	
No	157 (44.9)	48 (51.6)	
Insurance, *n* (%)			0.4
Medicaid/none	216 (75.3)	71 (24.7)	
Commercial	134 (78.4)	37 (34.3)	
Subjective health rating scale,[d] median (range)	9.0 (1–10)	9.0 (5–10)	0.6
Planned pregnancy, *n* (%)			0.01
Yes	179 (51.7)	34 (37.4)	
No	167 (74.6)	57 (25.5)	
BMI,[e] *n* (%)			0.02
≤30	254 (73.8)	90 (26.2)	
>30	96 (84.2)	18 (15.8)	
Season at study entry, *n* (%)			0.6
April–September	170 (75.9)	54 (24.1)	
October–March	180 (77.9)	51 (22.1)	
Baseline 25(OH)D, nmol/L, mean ± SD (range)			
Total	59.5 ± 23.8 (6.0–172.5)	50.5 ± 25.1 (6.5–120.5)	0.001
Black	39.4 ± 18.6 (6.0–108.8)	37.4 ± 17.6 (6.5–87.8)	0.6
Hispanic	59.3 ± 20.0 (17.3–103.8)	54.7 ± 20.6 (23.0–95.3)	0.3
White	74.6 ± 20.2 (29.5–172.5)	68.8 ± 28.8 (23.3–120.5)	0.2

[a]Exited included patients who chose not to continue. It does not include those with pregnancy losses, those who became medically ineligible, or those who moved from the geographic area.
[b]Race/ethnicity as defined by the mother.
[c]Gestational age at enrollment based on last menstrual period; change in gestational age occurred in 11 cases at the time of the 20-week fetal ultrasound.
[d]Self-reported maternal health status rating from 1 (poor) to 10 (excellent).
[e]BMI = prepregnancy body mass index.

Compared with women who continued in the study until delivery, women who exited the study were more likely to be younger ($p = 0.01$), black ($p = 0.003$), of higher gravidity ($p < 0.0001$), less educated ($p = 0.01$), employed at entrance into the study ($p = 0.04$), with an unplanned pregnancy ($p = 0.01$), and with a BMI of less than 30 ($p = 0.02$). Baseline vitamin D status by ethnicity of those who completed versus those who exited also did not differ (see Table 2).

With regard to pregnancy losses, there were 8 women in the 400-IU group (baseline mean ± SD 16.5 ± 7.6 weeks, median 15.5 weeks, range 10.0 to 34.0 weeks), 5 in the 2000-IU group (baseline mean ± SD 17.2 ± 4.6 weeks, median 15.0 weeks, range 12.0 to 23.0 weeks), and 10 in the 4000-IU group (baseline mean ± SD 16.4 ± 6.3 weeks, median 16.0 weeks, range 9 to 32 weeks) who experienced a loss after enrollment into the study. The 25(OH)D level around or at the time of the loss did not differ by treatment group ($p = 0.8$) There were no statistically significant differences in mean gestational age at loss among the treatment groups ($p = 0.9$) or in the percent losses per treatment group ($p = 0.4$). When looking at baseline 25(OH)D

levels of women who delivered a live-born infant versus those who experienced a pregnancy loss, the mean levels were 57.8 ± 24.4 nmol/L versus 50.5 ± 23.3 nmol/L, but this did not reach statistical significance.

Among the 350 women who continued in the study until delivery, the median ratio of the number of study capsules taken to the number that should have been taken between the time of randomization and delivery was similar between the groups. Adherence to protocol was not statistically different between treatment groups: 69% (400-IU group), 68% (2000-IU group), and 69% (4000-IU group, $p = 0.9$).

Study outcomes

As shown in Table 3, the primary outcome—mean circulating 25(OH)D level one month prior to delivery and at delivery—was statistically different between treatment groups, with the highest mean level achieved in the 4000-IU group. Overall, the mean 25(OH)D level by dose group one month before delivery and at delivery and as chronic levels measured as the average from 20 to 36 weeks of gestation were significantly different between control and 2000 IU, control and 4000 IU, and 2000 and 4000 IU ($p < 0.0001$).

The secondary outcome measure of attaining a total circulating 25(OH)D level of at least 80 nmol/L at the time of delivery was met by 43 of 86 (50%) women in the 400-IU group, 63 of 80 (70.8%) in the 2000-IU group, and 68 of 83 (82%) in the 4000-IU group (Table 3), but there were 92 women with missing levels at delivery ($p < 0.0001$). Because of the high correlation ($r = 0.72$, $p < 0.0001$) between 1 month prior to delivery and delivery 25(OH)D values, 1 month prior to delivery values were used as a surrogate for delivery values for the women with missing delivery room values. When combined, 57 of 109 (52.3%) women in the 400-IU group, 93 of 117 (79.5%) in the 2000-IU group, and 94 of 112 (83.9%) in the 4000-IU group achieved a minimal circulating 25(OH)D level of at least 80 nmol/L around the time of delivery ($p < 0.0001$). Expressed as relative risk ratios, as shown in Table 4, there were significant differences between the 2000- versus 400-IU groups (relative risk [RR] = 1.52, 95% CI 1.24–1.86) and between the 4000- versus 400-IU groups (RR = 1.60, 95% CI 1.32–1.95), but there was not a significant difference between the 2000- and 4000-IU groups in this regard.

Vitamin D supplementation at various treatment doses given to our pregnant population had a variable effect on circulating levels of vitamin D_3 and its metabolites (Fig. 2*A*). Supplementation of vitamin D_3 at double the prior 1997 institute of Medicine (IOM) recommendation of 200 IU/d[40] and the current IOM estimated average requirement (EAR)[62] provided essentially no increase in circulating vitamin D_3 levels and only a minimal 5 ng/mL rise in circulating 25(OH)D levels over the duration of the study (Fig. 2*A*, *B*). Conversely, supplementing 2000 or 4000 IU/d vitamin D_3 had a profound effect on increasing both circulating levels of vitamin D_3 and 25(OH)D levels (Fig 2*A*, *B* and Table 5*A*, *B*). Figure 3*A* describes the substrate-product relationship in all patients between vitamin D_3 and 25(OH)D. The relationship is biphasic with respect to 25(OH)D production, requiring at least 10 ng/mL of circulating vitamin D_3 to saturate the vitamin D-25-hydroxylase.

Table 5*A*, *B* provides additional information with respect to circulating 25(OH)D concentrations analyzed by race, vitamin D dose, and stage of gestation. Clearly, race and duration of supplementation have profound effects on the circulating level of 25(OH)D attained. Black women lag at every time point and dose in relation to circulating 25(OH)D level. This is especially noticeable in the 400-IU group. In contrast, a greater proportion of black women achieved a 25(OH)D level of 80 nmol/L or greater by the second trimester in the 4000-IU group when compared with both the 400- and 2000-IU groups (Table 5*B*).

One of the most interesting biochemical findings in our study was the association between circulating $1,25(OH)_2D_3$ levels and of circulating 25(OH)D levels (Figs. 2*C* and 3*B*). In exploring the association between 25(OH)D and $1,25(OH)_2D_3$ levels, 25(OH)D level was found to have a direct influence on $1,25(OH)_2D$ levels throughout pregnancy ($p < 0.0001$). While the baseline $1,25(OH)_2D_3$ level in all groups at 12 weeks' gestation were not significantly different (Fig. 2*C*), within a few weeks, however, the circulating $1,25(OH)_2D_3$ levels became significantly

Table 3. Total Circulating 25(OH)D Concentrations (nmol/L) During Pregnancy

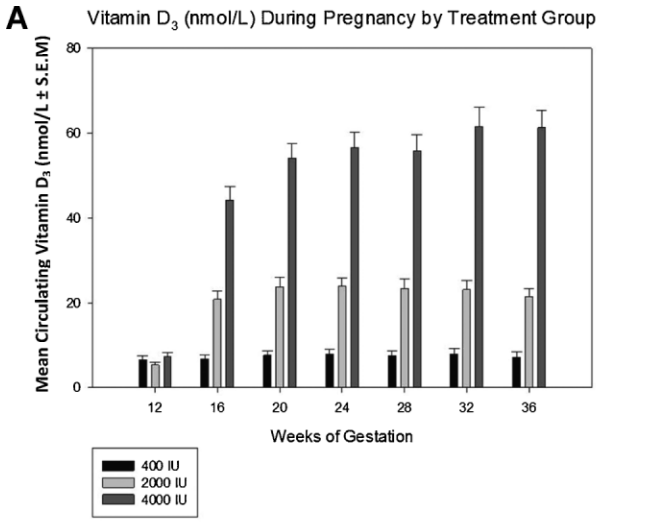
Measure	400-IU group	2000-IU group	4000-IU group	*p* Value
25(OH)D at baseline, mean ± SD	61.6 ± 27.1	58.3 ± 22.3	58.2 ± 21.8	0.5
Range	(6.0–172.5)	(14.0–115.3)	(11.8–109.3)	
25(OH)D 1 month before delivery, mean ± SD	79.4 ± 34.3	105.4 ± 35.7	118.5 ± 34.9	<0.0001
Range	(16.0–193.0)	(17.3–176)	(26.3–243.5)	
25(OH)D at delivery, mean ± SD	78.9 ± 36.5	98.3 ± 34.2	111.0 ± 40.4	<0.0001
Range	(12.5–159.5)	18.0–177.0	25.0–251.0	
25(OH)D, 20 to 36 weeks,[a] mean ± SD	79.1 ± 29.5	94.4 ± 26.1	110.8 ± 28.3	<0.0001
Range	(17.1–162.3)	(16.7–149.1)	(26.5–212.3)	
Achieved 25(OH)D level ≥ 80 nmoL at 1 month prior to delivery, *n* (%)	51 (50.0)	82 (73.9)	91 (82.0)	<0.0001
Achieved 25(OH)D level ≥ 80 nmoL at delivery, *n* (%)	43 (50.0)	63 (70.8)	68 (82.0)	<0.0001
Achieved 25(OH)D level ≥ 80 nmoL at 1 month prior to delivery or at delivery, *n* (%)	57 (52.3)	93 (79.5)	94 (83.9)	<0.0001
Infant birth 25(OH)D, mean ± SD	18.2 ± 10.1	22.8 ± 9.8	26.5 ± 10.3	<0.0001
Range	(2.4–48.4)	(3.6–47.9)	(2.4–52.0)	

[a]Mean value was the average 25(OH)D steady-state value obtained at visits between 20 and 36 weeks of gestation.

Table 4. Secondary Outcome: Achieving Total Circulating 25(OH)D ≥ 80 nmol/L Around Time of Delivery by Treatment Group

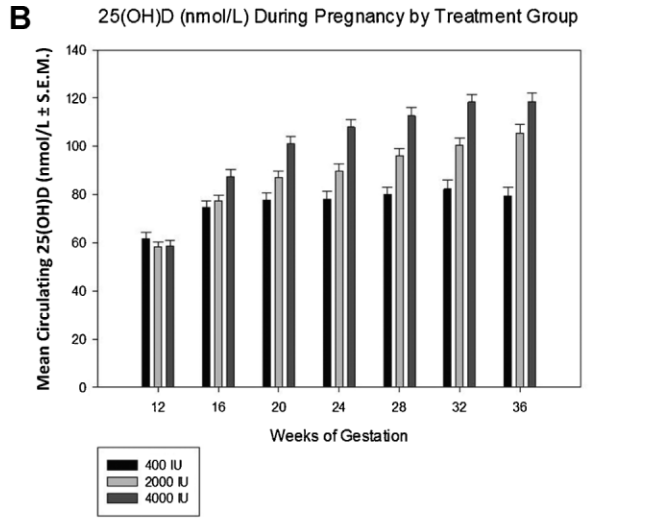
25(OH)D, nmol/L	2000 IU, *n* (%)	400 IU, *n* (%)	Risk ratio (95% CI)	Risk difference (95% CI)
≥80 nmol/L	93 (79.5)	57 (52.3)	1.5200 (1.2426–1.8594)	0.2719 (0.1530–0.3909)
	4000 IU, *n* (%)	400 IU, *n* (%)	Risk ratio (95% CI)	Risk difference (95% CI)
≥80 nmol/L	94 (83.9)	57 (52.3)	1.6049 (1.3183–1.9540)	0.3163 (0.2005–0.4322)
≥80 nmol/L	4000 IU, *n* (%)	2000 IU, *n* (%)	Risk ratio (95% CI)	Risk difference (95% CI)
	94 (83.9)	93 (79.5)	1.0559 (0.9340–1.1936)	0.0444 (–0.0555–0.1443)

Note: Vitamin D sufficiency was defined a priori as a total circulating 25(OH)D concentration of 80 nmoL (32 ng/mL) or greater. The following comparisons were made: 2000-IU group versus the 400-IU group, the 4000-IU group versus the 400-IU group, and lastly, the 4000-IU group versus the 2000-IU group. Risk ratios and risk differences were reported for each comparison with 95% CIs.



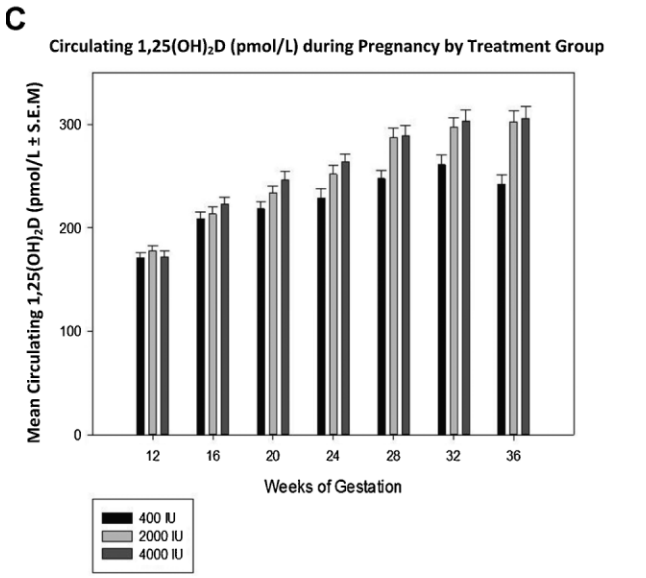
Statistical difference (p-value) in mean Vitamin D_3 between treatment groups at each week of gestation

Treatment Group	12	16	20	24	28	32	36
400 IU vs. 2000 IU	0.3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
400 IU vs. 4000 IU	0.6	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
2000 IU v 4000 IU	0.1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001



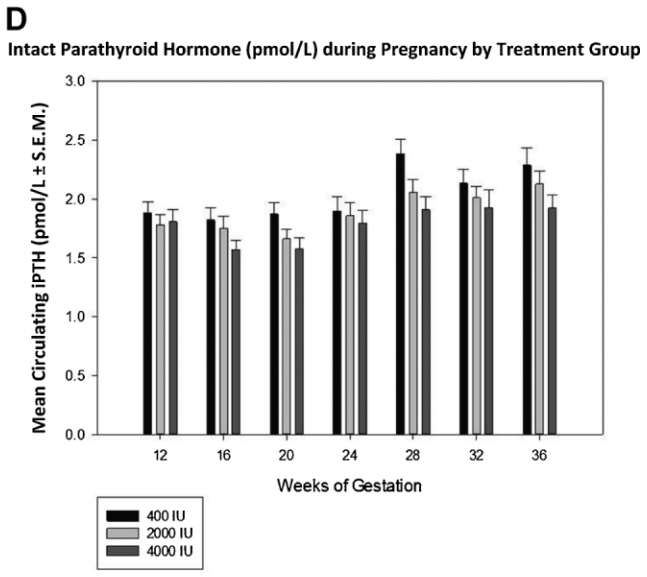
Statistical difference (p-value) in mean 25(OH)D between treatment groups at each week

Treatment Group	12	16	20	24	28	32	36
400 IU vs. 2000 IU	0.3	0.4	0.02	0.007	0.0001	0.0002	<0.0001
400 IU vs. 4000 IU	0.4	0.002	<.0001	<0.0001	<0.0001	<0.0001	<0.0001
2000 IU v 4000 IU	0.9	0.008	0.009	<0.0001	0.0003	<0.0001	0.009



Statistical difference (p-value) in mean circulating $1,25(OH)_2D$ between treatment groups at each week

Treatment Group	12	16	20	24	28	32	36
400 IU vs. 2000 IU	0.3	0.6	0.1	0.06	0.001	0.007	<0.0001
400 IU vs. 4000 IU	0.9	0.1	0.01	0.003	0.001	0.003	<0.0001
2000 IU v 4000 IU	0.4	0.3	0.3	0.3	0.9	0.7	0.8



Statistical difference (p-value) in mean intact PTH between treatment groups at each week

Treatment Group	12	16	20	24	28	32	36
400 IU vs. 2000 IU	0.4	0.6	0.1	0.8	0.049	0.4	0.4
400 IU vs. 4000 IU	0.6	0.06	0.03	0.5	0.006	0.3	0.049
2000 IU v 4000 IU	0.9	0.2	0.5	0.7	0.3	0.6	0.2

Fig. 2. Circulating vitamin D, its metabolites, and intact PTH as a function of vitamin D_3 dose and time during pregnancy. (*A–D*) The mean (± SEM) circulating concentrations of vitamin D, 25(OH)D, $1,25(OH)_2D_3$, and intact PTH at defined time points during pregnancy.

Table 5. Circulating 25(OH)D and PTH Changes During Pregnancy by Treatment Group and Race/Ethnicity

A. Circulating 25(OH)D (nmol/L) by Trimester Stratified by Treatment Group

Treatment group	Baseline 25(OH)D, mean ± SD	Second trimester,[a] mean ± SD	One month prior delivery, mean ± SD
400 IU	61.2 ± 27.1	76.1 ± 27.5	81.2 ± 35.9
2000 IU	57.5 ± 22.4	84.2 ± 23.0	102.6 ± 36.4
4000 IU	59.8 ± 25.4	98.6 ± 27.3	114.2 ± 35.5
p Value	0.5	<0.0001	<0.0001

B. Circulating 25(OH)D (nmol/L) By Trimester Stratified by Treatment Group and Race/Ethnicity

	400 IU				2000 IU				4000 IU			
Characteristic	Baseline 25(OH)D, mean ± SD	Second trimester, mean ± SD	One month prior to delivery, mean ± SD	Δ Baseline to 1 month prior[b] (*p* value)	Baseline 25(OH)D, mean ± SD	Second trimester, mean ± SD	One month prior to delivery, mean ± SD	Δ Baseline to 1 month prior (*p* value)	Baseline 25(OH)D, mean ± SD	Second trimester, mean ± SD	One month prior to delivery, mean ± SD	Δ Baseline to 1 month prior (*p* value)
Black	37.3 ± 17.1	48.8 ± 21.1	49.4 ± 28.4	12.7(0.009)	41.0 ± 19.1	72.2± 28.4	91.2 ± 45.1	49.4 (<0.0001)	40.7 ± 20.1	81.0 ± 26.4	97.8 ± 42.4	57.4 (<0.0001)
Hispanic	59.1 ± 21.6	76.9 ± 21.7	79.5 ±30.3	20.3 (<0.0001)	59.2 ± 18.9	85.2 ± 16.8	102.1 ± 28.7	42.1 (<0.0001)	63.3 ±27.6	101.4 ± 28.2	121.1 ± 30.9	60.1 (<0.0001)
White	81.3 ± 23.8	95.2 ± 20.6	106.9 ± 26.4	25.0 (<0.0001)	71.9 ± 19.0	94.9 ± 18.3	115.7 ± 31.8	44.4 (<0.0001)	71.3 ± 17.3	109.8 ± 19.2	120.4 ± 29.7	50.4 (<0.0001)
p Value	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	0.02		<0.0001	<0.0001	0.008	

C. Intact PTH (pmol/L) by Trimester Stratified by Treatment Group

Treatment group	Baseline PTH, mean ± SD	Second trimester,[c] mean ± SD	One month prior to delivery, mean ± SD
Control	1.9 ± 1.0	1.9 ± 1.0	2.2 ± 1.3
2000 IU	1.8 ± 0.9	1.7 ± 0.9	2.1 ± 1.1
4000 IU	1.8 ± 1.1	1.6 ± 0.8	1.9 ± 1.1
p Value	0.5	0.1	0.1

D. PTH (pmol/L) by Trimester Stratified by Treatment Group and Race/Ethnicity

	400 IU			2000 IU			4000 IU		
Characteristic	Baseline PTH, mean ± SD	Second trimester, mean ± SD	One month prior, mean ± SD	Baseline PTH, mean ± SD	Second trimester, mean ± SD	One month prior, mean ± SD	Baseline PTH, mean ± SD	Second trimester, mean ± SD	One month prior, mean ± SD
Black	2.5 ± 1.2	2.6 ± 1.2	3.1 ± 1.8	2.1 ± 1.1	2.0 ± 0.9	2.3 ± 1.3	2.0 ± 0.9	1.9 ± 0.9	2.3 ± 1.1
Hispanic	1.8 ± 0.9	1.8 ± 0.7	2.0 ± 1.0	1.7 ± 0.9	1.8 ± 1.0	2.2 ± 1.1	1.8 ± 1.1	1.5 ± 0.7	1.7 ± 0.9
White	1.6 ± 0.9	1.4 ± 0.8	1.7 ± 1.0	1.6 ± 0.7	1.5 ± 0.7	1.9 ± 1.0	1.7 ± 1.2	1.6 ± 0.8	1.7 ± 1.3
p Value	0.001	<0.0001	0.0002	0.01	0.055	0.3	0.6	0.06	0.06

[a]Second-trimester mean value was the average 25(OH)D value obtained at visits between 16 and 24 weeks of gestation.
[b]Δ baseline to 1 month prior connotes the change from the baseline 25(OH)D level to the level achieved at 1 month prior to delivery.
[c]Second-trimester mean value was the average PTH value obtained at visits between 16 and 24 weeks of gestation.

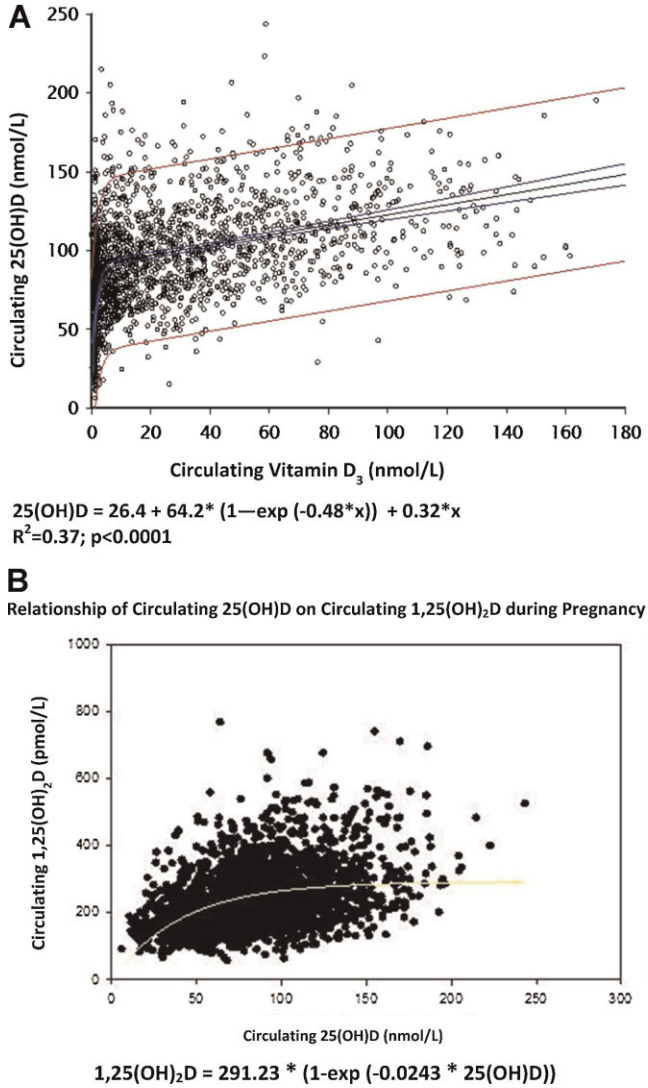


Fig. 3. Substrate-product relationships of vitamin D metabolites during pregnancy. (*A*) The relationship between circulating vitamin D to control the production of 25(OH)D during pregnancy. (*B*) The relationship of circulating 25(OH)D to control the production of $1,25(OH)_2D_3$ during pregnancy. All data points for all subjects in all groups were included in this analysis.

elevated in the 2000- and 4000-IU groups as opposed to the 400-IU group.

The relationship between these vitamin D metabolites is examined more closely in Fig. 3*B*. This figure clearly demonstrates a biphasic relationship between circulating 25(OH)D and $1,25(OH)_2D_3$, with circulating levels of 25(OH)D of at least 100 nmol/L (40 ng/mL) required to support maximum $1,25(OH)_2D_3$ output in the pregnant women. It is also worthy to note that circulating $1,25(OH)_2D_3$ levels at 12 weeks' gestation are approximately triple that of normal, nonpregnant female and normal male subjects, as reported previously[56] (Fig. 2*C*).

Figure 2*D* and Table 5*C*, *D* also display circulating intact PTH levels. The trend of PTH in all subjects was higher as the subjects progressed through pregnancy but was not significantly different by treatment group. Decreases in circulating PTH were observed if the levels attained were analyzed by race. The black group clearly had decreasing circulating PTH as circulating 25(OH)D levels increased (Table 5*C*, *D*).

Circulating levels of VDBP were measured in 80 selected subjects based on their circulating $1,25(OH)_2D_3$ levels, which ranged from 224.9 to 768.0 pmol/L at various stages of gestation. The average level of VDBP detected in these subjects was 5.45 ± 1.26 μmol/L, which represented a 39% increase over normal subjects. Further, using linear regression, no relationship was observed between circulating VDBP and $1,25(OH)_2D_3$ levels.

With respect to the effect of circulating 25(OH)D on either blood calcium or urinary calcium level, no significant effects were observed with one exception—that being the relationship between low circulating 25(OH)D and urinary calcium levels (Figs. 4*A*, *B* and 5). From Fig. 5, it would appear that approximately 75 nmol/L (30 ng/mL) of circulating 25(OH)D was required in the pregnant women to normalize urinary

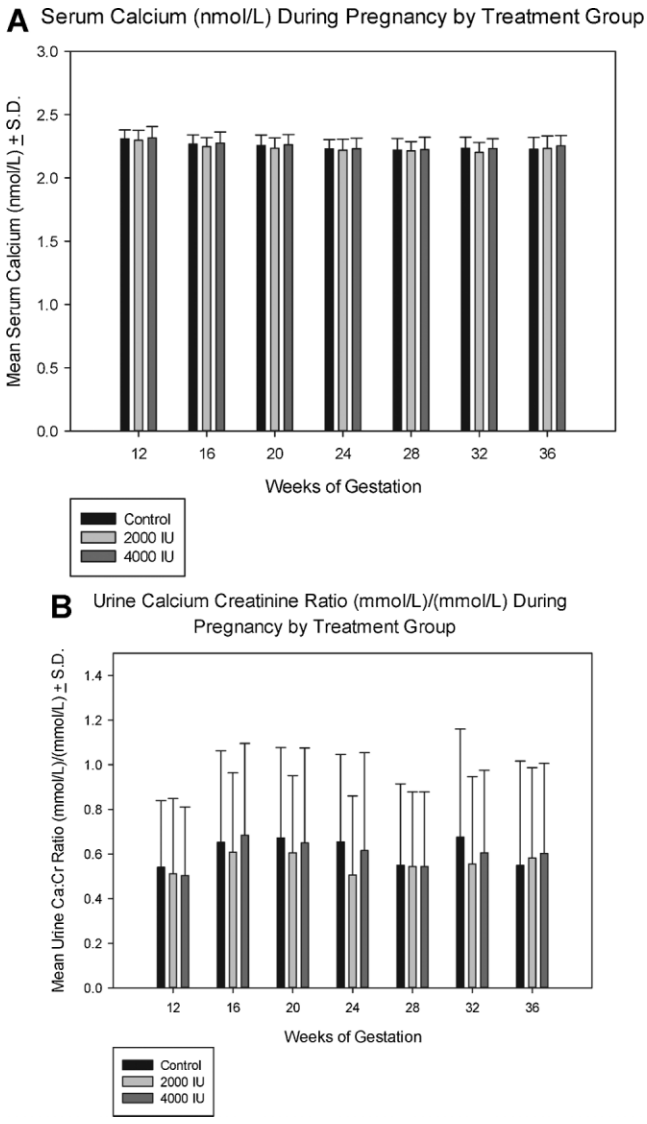


Fig. 4. Serum calcium and urinary calcium/creatinine ratio as a function of vitamin D_3 dose and time during pregnancy. (*A*, *B*) the mean (± SD) serum calcium and urinary calcium/creatinine ratio at defined time points during pregnancy.

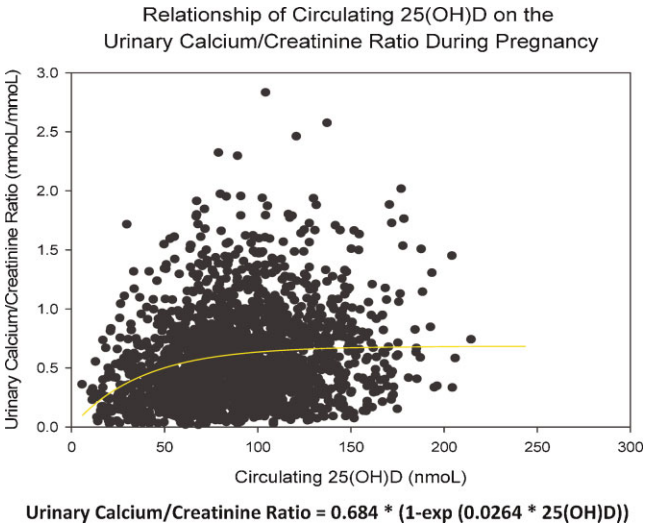


Fig. 5. Relationship of circulating 25(OH)D on the urinary calcium/creatinine ratio during pregnancy. All data points are included for all study patients. Urinary calcium and urinary creatinine were measured in mmol/L. Ratio was calculated from measurement of urinary calcium (mmol/L) divided by measurement of urinary creatinine (mmol/L).

calcium excretion. Above that threshold, 25(OH)D appeared not to influence urinary calcium and subsequent excretion.

Throughout the study, there were no statistically significant differences between groups on any safety measure: serum calcium, creatinine, and phosphorus and urinary calcium/creatinine ratios (p value not significant [pNS] between groups). Review of adverse events by the DSMC showed that not a single adverse event in this trial was attributed to vitamin D supplementation or circulating 25(OH)D levels. There was one safety measure stop implementation: In the 4000-IU group, one woman with a baseline circulating 25(OH)D level of 29.3 nmol/L (13.3 ng/mL) increased to 233.3 nmol/L (93.3 ng/mL) at visit 2. Her follow-up circulating 25(OH)D level at the return visit prior to stopping supplementation had decreased to 66.6 ng/mL. Although her urinary calcium/creatinine ratio and all serum biochemical indices were within normal limits, the woman ceased supplementation per protocol. Two additional women met upper threshold criteria at the time of delivery; both had commenced sunbathing during the weeks prior to delivery; no toxicity by any parameter in either mother or baby was found.

Mode of delivery and neonatal characteristics by maternal treatment group are found in Table 6. There were no differences between the groups in terms of gestational age at delivery or birth weight. In addition, there were no significant differences in level of care (newborn nursery versus higher level of care, ie, level 2 or neonatal intensive care admission) or increased adverse outcomes of pregnancy related to maternal vitamin D intake. There were several differences, however, in terms of neonatal vitamin D status by treatment group. Neonatal 25(OH)D was significantly correlated with maternal 25(OH)D overall, 1 month prior, and at delivery ($r^2 = 0.6$, odds ratio [OR] = 0.50) and was significantly different by treatment group: 45.5 ± 25.3 nmol/L (18.2 ± 10.1 ng/mL, control), 57.0 ± 24.5 nmol/L (22.8 ± 9.8 ng/mL, 2000-IU group), and 66.3 ± 25.8 nmol/L (26.5 ± 10.3 ng/mL, 4000-IU group; $p < 0.0001$). By treatment group, using IOM guidelines for sufficiency [total circulating 25(OH)D $\geq$ 50 nmol/L or 20 ng/mL],[62] 31 of 78 (39.7%) neonates in the 400-IU group, 53 of 91 (58.2%) in the 2000-IU group, and 66 of 84 (78.6%) in the 4000-IU group had a cord blood/neonatal 25(OH)D level in the sufficient range ($p < 0.0001$).

Discussion

In this randomized, controlled trial of vitamin D supplementation during pregnancy involving a diverse group of women living at latitude 32°N, those women randomized to 4000 IU/day compared to those receiving 400- or 2000 IU/day experienced improved vitamin D status throughout pregnancy, 1 month prior to delivery, and improved vitamin D status in their offspring at birth. Irrespective of race and ethnicity, this improvement in vitamin D status was achieved without any evidence of

Table 6. Characteristics at Delivery by Vitamin D Supplementation Group

Characteristic	400-IU group ($n = 111$)	2000-IU group ($n = 122$)	4000-IU group ($n = 117$)	p Value
Maternal age at delivery (years), mean ± SD	27.4 ± 5.7	28.0 ± 5.7	27.1 ± 5.5	0.49
Mode of delivery[a]: n (%)				
Uncomplicated vaginal	69 (62.2%)	81 (66.4%)	81 (69.8%)	
Assisted vaginal	2 (1.8%)	4 (3.3%)	9 (7.8%)	
C/S after labor	23 (20.7%)	19 (15.6%)	19 (16.4%)	
C/S without labor	17 (15.3%)	18 (14.8%)	7 (6.0%)	
Vaginal, any type	71 (74.7%)	85 (79.4%)	90 (85.7%)	0.15
Primary C/S	24 (25.3%)	22 (20.6%)	15 (14.3%)	
Gestational age (weeks) at delivery, mean ± SD	38.6 ± 2.2	38.8 ± 1.8	39.1 ± 1.8	0.17
Birth weight (g) at delivery, mean ± SD	3221.8 ± 674.9	3360.1 ± 585.0	3284.6 ± 597.6	0.23
Admission to level II or III, n (%)	12 (10.8%)	14 (11.5%)	11 (9.4%)	0.9

Delivery Characteristics by Vitamin D Supplementation Group:

[a]Mode of delivery was categorized a priori as either a vaginal delivery (defined as spontaneous vaginal delivery or assisted vaginal delivery [which included use of forceps or vacuum extraction]) or cesarean section (C/S; further subdivided as cesarean following labor, cesarean without labor, and repeat elective cesarean). Primary cesarean section included women who had undergone a cesarean section with or without labor for either a maternal or fetal indication and did not include women who underwent a repeat, elective cesarean section.

hypervitaminosis D or an increase in adverse events during pregnancy and with optimization of 25(OH)D and $1,25(OH)_2D_3$. From the standpoint of enzyme kinetics, this simply means that in the case of vitamin D being converted to 25(OH)D and subsequently to $1,25(OH)_2D_3$, enzyme saturation is occurring; that is, reaction rates are moving from first-order to zero-order enzyme kinetics. In simple terms, this means that an appropriate amount of substrate is being supplied to produce maximum product, that is, 25(OH)D and $1,25(OH)_2D_3$; as such, no substrate "starvation" is occurring.

At no point in human nutrition is it more critical to ensure adequate nutrient intake than during the state of pregnancy. Folate intake during pregnancy and its role in the development of neural tube defect serves as a stark example.[63,64] The limited clinical investigation into meaningful dietary vitamin D supplementation during pregnancy can be traced back to post–World War II Britain. Because of the British experience with idiopathic infantile hypercalcemia attributed to hypervitaminosis D, an inaccurate association occurred that had a profound effect on the potential of vitamin D supplementation not only during infancy but also during pregnancy. In 1963, Black and Bonham-Carter[65] recognized that the elfin facies observed in patients with severe idiopathic infantile hypercalcemia resembled the peculiar facies observed in patients with supravalvular aortic stenosis (SAS) syndrome. By 1966, vitamin D was viewed by the medical community as the cause of SAS syndrome.[66,67] With the advent of molecular genetics, the children with SAS syndrome were discovered to have Williams syndrome, an example of unipaternal disomy, with abnormal vitamin D metabolism.[68–75]

The perception that vitamin D can inflict harm during pregnancy still lives on today because many obstetrical specialists are afraid to undertake vitamin D repletion during this period. Research efforts in this area were further hampered when in 1997 the Institute of Medicine (IOM) issued guidelines that defined the adequate intake (AI) for vitamin D during pregnancy to be 200 IU/d, with intakes greater than 2000 IU/d causing potential harm.[40] Recently, the IOM issued new guidelines with respect to pregnant women that define the estimated average requirement (EAR) and recommended dietary allowance (RDA) to be 400 and 600 IU/d, respectively. The IOM also increased the tolerable upper intake limit (UL) to 4000 IU/d.[62] These new guidelines, with the exception of the UL, are based on old data because limited new data exist. The result of prior and current guidelines is that most prenatal vitamins contain only 400 IU of vitamin D. In our experience, many of today's practicing obstetricians are unaware of the vitamin D content in prenatal vitamins or have a fear of administering additional vitamin D supplements to pregnant women.

Our study was based on two previous vitamin D supplementation studies in nonpregnant adults that appeared to be safe.[48,58] Prior to undertaking the NIH-funded study described here, however, we had to obtain an Investigational Drug Number from the FDA, which entailed writing a complete investigational drug application. This was required by the FDA because we proposed using a vitamin D_3 dose of 4000 IU/d, 20 times the AI and twice the safe limit put forth by the IOM in 1997[40] but currently put forth as the UL.[62] Thus, our study is the first one to test this current UL in pregnant women.

The only known avenue of vitamin D toxicity is manifested through hypercalcemia and hypercalciuria,[76] neither of which was observed in our ramdomized, controlled trial (RCT). In fact, our Data and Safety Monitoring Committee concluded that not a single adverse event in this RCT could be attributed to vitamin D intake. Hypervitaminosis D is largely arbitrarily defined as circulating levels of 25(OH)D that exceed 375 nmol/L (150 ng/mL), a level we never attained with our dosing regimen. As has been observed in other human supplementation studies, the conversion of vitamin D to 25(OH)D appears to be controlled.[77] Further, it has been known for decades that during pregnancy $1,25(OH)_2D_3$ levels become extremely elevated.[78,79] This increase in circulating $1,25(OH)_2D_3$ levels has in particular been attributed to an increase in the serum vitamin D–binding protein (VDBP) that would regulate the amount of "free" $1,25(OH)_2D_3$ available in the circulation.[79] While this rise in VDBP during pregnancy has been shown to be 46% to 103%, depending on the assay employed,[80] it cannot account, however, for the nearly three- to fourfold increase in circulating $1,25(OH)_2D_3$ observed in our study. Bikle and colleagues[81] clearly demonstrated that free $1,25(OH)_2D_3$ levels are increased during pregnancy despite the significant increase in VDBP levels. We were unable to measure "free" circulating levels of $1,25(OH)_2D_3$ in our subjects, but our data agree with those of Bikle and colleagues in that no relationship was observed during pregnancy between circulating VDBP and "total" circulating $1,25(OH)_2D_3$.[81] New data from our study suggest that a circulating 25(OH)D level of approximately 100 nmol/L (40 ng/mL) is required to optimize production of $1,25(OH)_2D_3$ during human pregnancy through renal and/or placental production of the hormone (Figs. 2*C* and 3*B*). It is also of great interest that production of circulating $1,25(OH)_2D_3$ in the fetus is linked directly to circulating 25(OH)D.[10]

Clearly, vitamin D metabolism is greatly altered during pregnancy, and pregnancy itself is the primary driver for these extraordinary circulating $1,25(OH)_2D_3$ levels. From our data, it is evident that production of $1,25(OH)_2D_3$ is really not under the control of the classic regulators of calcium, phosphorus, and PTH. The dramatic rise in maternal circulating $1,25(OH)_2D_3$ following conception is remarkable for many reasons: By 12 weeks of gestation, maternal circulating $1,25(OH)_2D_3$ levels are already triple those of a nonpregnant female (Fig. 2*C*). From that point in gestation, the $1,25(OH)_2D_3$ levels rise much higher and are driven by substrate—25(OH)D—availability (Fig. 3*B*). This substrate dependence of $1,25(OH)_2D_3$ production is never observed in normal human physiology driven by classic calcium homeostasis.[10,82,83]

Another remarkable factor in pregnant women is how they can attain supraphysiologic levels of $1,25(OH)_2D_3$, sometimes exceeding 700 pmol/L in our study, and never exhibit hypercalciuria or hypercalcemia. These tremendous circulating levels of $1,25(OH)_2D_3$ during pregnancy are possibly of placental origin or from the renal 1-α-hydroxylase that would have to be uncoupled from feedback control and for reasons other than maintaining calcium homeostasis. The second scenario is most likely because women with nonfunctional renal 1-α-hydroxylase and normal placental function fail to increase circulating $1,25(OH)_2D_3$ during pregnancy.[84] The increased levels of $1,25(OH)_2D_3$ may be due to

the methylation of the catabolic *CYP24A1* placental gene.[85] It is possible that calcitonin may be a contributor to this process in that calcitonin rises during pregnancy,[86] is known to stimulate the renal *1-α-hydroxylase* gene independent of calcium levels,[87,88] and also protects by opposing hypercalcemia.[89] Another possible stimulator of 1-α-hydroxylase during pregnancy is prolactin.[90] If prolactin were a major contributor, however, the effect should continue into lactation, which we do not see, and would be accompanied by elevated circulating $1,25(OH)_2D_3$ levels, which also are not seen.[91] Further, the physiologic function of this altered vitamin D metabolism may be related to increased reliance on innate immune function during pregnancy, as well as decreased adaptive immune responses,[7,8,10,92] protecting the newborn from respiratory infection and subsequent wheezing[93,94] and possibly epigenetic alterations in invariant natural killer (NK) T cells, which can lead to increased autoimmune disease prevalence.[95,96] As supported by this and prior studies, it is important to remember that for cord blood to attain a 25(OH)D level of 50 nmol/L, the maternal 25(OH)D level would need to be at least 80 nmol/L.[97]

Our data also suggest that a circulating level of approximately 75 nmol/L (30 ng/mL) of 25(OH)D is required to normalize calcium excretion into the urine. Interestingly, this value is virtually identical to the value obtained by Heaney and colleagues with respect to the equilibration of intestinal calcium absorption.[98] This increased level of circulating 25(OH)D in the pregnant woman also appears to reduce circulating PTH, especially in black subjects. It is also important to compare our study results with respect to two recent reports dealing with vitamin D supplementation during pregnancy.[62,99] The IOM report recommends a vitamin D intake of 400 to 600 IU/d and states that this level can be obtained solely from the diet. Further, this intake level would be sufficient to meet their circulating 25(OH)D target of 20 ng/mL (50 nmol/L).[62] Even using this conservative 25(OH)D level, the IOM recommendation would have left more than 50% of our total cohort and more than 80% of black women in the cohort deficient at study entry. The Endocrine Society's recommendation of a daily vitamin D intake of 1500 to 2000 IU and target 25(OH)D level of greater than 30 ng/mL (75 nmol/L)[99] is more sound advice yet is still conservative compared with our study results. It must be pointed out that the purpose of the IOM report was to guide food manufacturers and fortifiers and is not intended to guide clinical practice.[62] On the other hand, clinical practice guidance is precisely the purpose of the Endocrine Society's recommendations.[99]

This study has certain limitations. This study was conducted at a southern latitude, and therefore, the vitamin D requirements of women living at more northern latitudes could be greater. While women with preexisting hypertension and diabetes were excluded from the study, these women may be at greater risk of vitamin D deficiency and therefore may receive the greatest benefit from vitamin D supplementation of 4000 IU/d. Because of safety concerns, women were not allowed to remain in the study if their total circulating 25(OH)D level rose above 225 nmol/L. There were three women who attained this threshold, none of whom had any associated hypercalciuria or hypercalcemia. Lastly, owing to safety concerns that surrounded the use of 4000 IU of vitamin D supplementation during pregnancy, the study was designed to begin supplementation starting at the twelfth week of gestation, beyond the period of early organogenesis. Hence we cannot ensure the safety before the twelfth week of gestation. With regard to vitamin D intake during pregnancy, it is interesting that our study largely confirms the observations of Obermer in England more than 60 years ago.[100] Obermer's suggestions largely were ignored because of greatly flawed associations between vitamin D and SAS syndrome.[65,66,101] The data in our paper put us back on the path suggested by Obermer with respect to vitamin D intake during pregnancy. Additional studies will be necessary to ascertain safety of 4000 IU/d of vitamin D supplementation before the twelfth week of gestation.

Conclusions

In summary, starting at 12 to 16 weeks of gestation, vitamin D supplementation with 4000 IU/d was most effective in achieving vitamin D sufficiency throughout pregnancy, 1 month prior to delivery, and at delivery in a diverse group of women and their neonates without increased risk of toxicity. These findings suggest that the current vitamin D EAR and RDA for pregnant women issued in 2010 by the IOM[62] should be raised to 4000 IU of vitamin D per day so that all women, regardless of race, can attain optimal nutritional and hormonal vitamin D status throughout pregnancy.

Disclosures

BWH serves as a consultant for Diasorin, Inc. (Stillwater, MN, USA). All the other authors state that they have no conflicts of interests.

Acknowledgments

We would like to acknowledge the hundreds of women who participated in this study, without whose participation this study would not have been possible; our dedicated study coordinator team: Judy Shary, MS, Pamela G Smith, RN, Martha Murphy, BS, Betty Bivens, RA, and Deanna Fanning, RN, who truly made the study possible; our Data Safety and Monitoring Committee, without whose due diligence this study could not have taken place; and lastly, our data management team, Melanie Floyd and Frilatha Bandlamuri, BS, MS, for their meticulous care of the data.

This study was funded by National Institute of Children's Health and Human Development Grant Number R01 HD47511, NIH Grant Number RR01070, and the South Carolina Clinical and Translational Research (SCTR) Institute, with an academic home at the Medical University of South Carolina, and NIH/NCRR Grant Number UL1 RR029882.

Authors' roles: Study design: All the authors contributed to study design. Study conduct: All the authors contributed to study conduct. Data collection: All the authors contributed to data collection. Data analysis: BHW, TCH, ME, and CLW did the data analysis. Data interpretation: All the authors contributed to data interpretation. Drafting manuscript: BWH, TCH, and CLW drafted the manuscript. Revising manuscript content: All the

authors contributed to revising the manuscript content. Approving final version of manuscript: All the authors approved the final version of the manuscript. TCH and ME take responsibility for the integrity of the data analysis.

References

1. Hatun S, Ozkan B, Orbak Z, Doneray H, Cizmecioglu F, Toprak D, Calikoglu AS. Vitamin D deficiency in early infancy. J Nutr. 2005; 135(2):279–82.
2. Ladhani S, Srinivasan L, Buchanan C, Allgrove J. Presentation of vitamin D deficiency. Arch Dis Child. 2004;9:781–4.
3. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics. 2008;122(5): 1142–52.
4. Yorifuji J, Yorifuji T, Tachibana K, Nagai S, Kawai M, Momoi T, Nagasaka H, Hatayama H, Nakahata T. Craniotabes in normal newborns: the earliest sign of subclinical vitamin D deficiency. J Clin Endocrinol Metab. 2008;93(5):1784–8.
5. Anatoliotaki M, Tsilimigaki A, Tsekoura T, Schinaki A, Stefanaki S, Nicolaidou P. Congenital rickets due to maternal vitamin D deficiency in a sunny island of Greece. Acta Paediatr. 2003;92(3):389–91.
6. Hewison M. Vitamin D and the Immune System: New Perspectives on an Old Theme. Endocrinology & Metabolism Clinics of North America. 2010;39(2):365–79.
7. Liu PT, Schenk M, Walker VP, Dempsey PW, Kanchanapoomi M, Wheelwright M, Vazirnia A, Zhang X, Steinmeyer A, Zügel U, Hollis BW, Cheng G, Modlin RL. Convergence of IL-1β and VDR Activation Pathways in Human TLR2/1-Induced Antimicrobial Responses. PLoS ONE. 2009;4(6):e5810.
8. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zugel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006;311(5768):1770–3.
9. Adams JS, Ren S, Liu PT, Chun RF, Lagishetty V, Gombart AF, Borregaard N, Modlin RL, Hewison M. Vitamin d-directed rheostatic regulation of monocyte antibacterial responses. J Immunol. 2009; 182(7):4289–95.
10. Walker VP, Zhang X, Rastegar I, Liu PT, Hollis BW, Adams JS, Modlin RL. Cord blood vitamin D status impacts innate immune responses. J Clin Endocrinol Metab. 2011;96(6):1835–43.
11. Svoren BM, Volkening LK, Wood JR, Laffel LM. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. J Pediatr. 2009; 154(1):132–4.
12. Boucher BJ, Mannan N, Noonan K, Hales CN, Evans SJ. Glucose intolerance and impairment of insulin secretion in relation to vitamin D deficiency in east London Asians. Diabetologia. 1995; 38(10):1239–45.
13. Maghbooli Z, Hossein-Nezhad A, Karimi F, Shafaei AR, Larijani B. Correlation between vitamin D(3) deficiency and insulin resistance in pregnancy. Diabetes Metab Res Rev. 2008;24(1):27–32.
14. Fuleihan E, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, Choucair M, Arabi A, Vieth R. Effect of vitamin D replacement on musculoskeletal parameters in school children: A randomized controlled trial. J Clin Endocrinal Metab. 2006;91:405–12.
15. Adams JS. Vitamin D as a defensin. J Musculoskelet Neuronal Interact. 2006;6(4):344–6.
16. Plotnikoff G, Quigley J. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. Mayo Clin Proc. 2003;78:1463–70.
17. Boyan BD, Sylvia VL, Dean DD, Schwartz Z. Membrane mediated signaling mechanisms are used differentially by metabolites of vitamin D(3) in musculoskeletal cells. Steroids. 2002;67(6):421–7.
18. Zittermann A, Schleithoff SS, Koerfer R. Vitamin D insufficiency in congestive heart failure: why and what to do about it? Heart failure reviews. 2006;11(1):25–33.
19. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr. 2006;83(4):754–9.
20. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS. Vitamin D Deficiency and Risk of Cardiovascular Disease. Circulation. 2008; 117(4):503–11.
21. Cui X, McGrath JJ, Burne THJ, Mackay-Sim A, Eyles DW. Maternal vitamin D depletion alters neurogenesis in the developing rat brain. International Journal of Developmental Neuroscience. 2007;25(4): 227–32.
22. Brown J, Bianco J, McGrath J, Eyles D. 1,25-Dihydroxyvitamin D-3 induces nerve growth factor, promotes neurite outgrowth and inhibits mitosis in embryonic rat hippocampal neurons. Neurosci Lett. 2003;343:139–43.
23. Eyles D, Brown J, MacKay-Sim A, McGrath J, Feron F. Vitamin D3 and brain development. Neuroscience. 2003;118(3):641–53.
24. Eyles D, Smith S, Kinobeb R, Hewison M, McGrath J. Distribution of the vitamin D receptor and 1α-hydroxylase in human brain. Journal of Chemical Neuroanatomy. 2005;29:21–30.
25. Halhali A, Tovar AR, Torres N, Bourges H, Garabedian M, Larrea F. Preeclampsia is associated with low circulating levels of insulin-like growth factor 1 and 1,25-dihydroxyvitamin D in maternal and umbilical cord compartments. J Clin Endocrinol. 2000;85(5):1828–33.
26. Robinson CJ, Wagner CL, Hollis BW, Baatz JE, Johnson DD. Maternal vitamin D and fetal growth in early-onset severe preeclampsia. Am J Obstet Gynecol. 2011.
27. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal Vitamin D Deficiency Increases the Risk of Preeclampsia, 2007; vol. 92, pp 3517–22.
28. Merewood A, Mehta SD, Chen TC, Bauchner H, Holick MF. Association between vitamin D deficiency and primary cesarean section. J Clin Endocrinol Metab. 2009;94(3):940–5.
29. Mahomed K, Gulmezoglu AM. Vitamin D supplementation in pregnancy (Cochrane Review) Cochrane Database of Systematic Reviews. Chicester, UK: John Wiley & Sons, Ltd; 1999.
30. Mallet E, Gugi B, Brunelle P, Henocq A, Basuyau J, Lemeur H. Vitamin D supplementation in pregnancy: a controlled trial of two methods. Obstet Gynecol. 1986;68:300–4.
31. Brooke OG, Brown IRF, Bone CDM, Carter ND, Cleeve HJW, Maxwell JD, Robinson VP, Winder SM. Vitamin D supplements in pregnant Asian women: Effects on calcium status and fetal growth. Br Med J. 1980;1:751–4.
32. Maxwell J, Ang L, Brooke O, Brown I. Vitamin D supplements enhance weight gain and nutritional status in pregnant Asians. Br J Obstet Gynaecol. 1981;88:987–91.
33. Ala-Houhala M, Koskinen T, Terho A, Koivula T, Visakorpi J. Maternal compared with infant vitamin D supplementation. Arch Dis Child. 1986;61:1159–63.
34. Marya R, Rathee S, Lata V, Mudgil S. Effects of vitamin D supplementation in pregnancy. Gynecol Obstet Invest. 1981;12:155–61.
35. Delvin EE, Salle BL, Glorieux FH, Adeleine P, David LS. Vitamin D supplementation during pregnancy: effect on neonatal calcium homeostasis. J Pediatr. 1986;109(2):328–34.
36. Brooke OG, Butters F, Wood C. Intrauterine vitamin D nutrition and postnatal growth in Asian infants. Brit Med J. 1981;283:1024.

37. Datta S, Alfaham M, Davies D, Dunstan F, Woodhead S, Evans J, Richards B. Vitamin D deficiency in pregnant women from a non-European ethnic minority population: an interventional study. Br J Obstetr Gynaecol. 2002;109:905–8.
38. Arora P, Arora RS. Vitamin D supplementation for non-Western pregnant women: the British experience. Am J Clin Nutr. 2007; 85(4):1164–5.
39. Yu C, Sykes L, Sethi M, Teoh T, Robinson S. Vitamin D deficiency and supplementation during pregnancy. Clinical Endocrinology. 2009;70(5):685–90.
40. Food and Nutrition Board. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, DC: National Academy Press; 1997.
41. Hollis B. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr. 2005;135:317–22.
42. Block G, DiSogra C. Validation, self-administered, in low-income white, African-American and Hispanic women. Alexandria, VA: Food and Nutrition Service, U.S. Department of Agriculture; 2001.
43. Block G, Thompson FE, Hartman AM, Larkin FA, Guire KE. Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1-year period. J Am Diet Assoc. 1992; 92:686–93.
44. Mares-Perlman JA, Klein BEK, Klein R, Ritter LL, Fisher MR, Freudenheim JL. A diet history questionnaire ranks nutrient intakes in middle-aged and older men and women similarly to multiple food records. J Nutr. 1993;123:489–501.
45. Coates RJ, Eley JW, Block G, Gunter EW, Sowell AL, Grossman C. An evaluation of a food frequency questionnaire for assessing dietary intake of specific carotenoids and vitamin E among low-income black women. Am J Epidemiol. 1991;134:658–71.
46. Sinha R, Patterson BH, Mangels AR, Levander OA, Gibson T, Taylor PR, Block G. Determinants of plasma vitamin E in health males. Cancer Epidemiology Biomarkers and Prevention. 1993;2: 473–9.
47. Sobell J, Block G, Koslowe P, Tobin J, Andres R. Validation of a retrospective questionnaire assessing diet 10–15 years ago. Am J Epidemiol. 1989;130:173–87.
48. Heaney R, Davies K, Chen T, Holick M, Barger-Lux M. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr. 2003;77:204–10.
49. Appelgren KE, Nietert PJ, Hulsey TC, Hollis BW, Wagner CL. Analyzing adherence to prenatal supplement: does pill count measure up? Int J Endocrinol. 2010;2010:631971.
50. Hollis BW. Detection of vitamin D and its major metabolites. In: Feldman D, Glorieux F, Pike J, eds. Vitamin D. New York, NY: Academic Press; 2005: 932–50.
51. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD. Determination of vitamin D status by radioimmunoassay with a 125I-labeled tracer. Clin Chem. 1993;39:529–33.
52. Laboratories MC. Laboratory Reference Data. Rochester, MN: Mayo Clinic; 2003.
53. Hollis BW, Wagner CL, Kratz A, Sluss PM, Lewandrowski KB. Normal serum vitamin D levels. Correspondence. N Engl J Med. 2005; 352:515–6.
54. Hollis BW, Wagner CL. Assessment of dietary vitamin D requirements during pregnancy and lactation. Am J Clin Nutr. 2004;79: 717–26.
55. Hollis BW, Wagner CL. Vitamin D requirements during lactation: High-dose maternal supplementation as therapy to prevent hypovitaminosis D in both mother and nursing infant. Amer J Clin Nutr. 2004;80S:1752S–8S.
56. Hollis BW, Kamerud JQ, Kurkowski A, Beaulieu J, Napoli JL. Quantification of circulating 1,25-dihydroxyvitamin D by radioimmunoassay with a ^{125}I-labeled tracer. Clin Chem. 1996;42:586–92.
57. Vieth R, Ladak Y, Walfish P. Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. J Clin Endocrinal Metab. 2003;88:185–91.
58. Vieth R, Chan PC, MacFarlane GD. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. Am J Clin Nutr. 2001;73(2):288–94.
59. Pitkin RM. Endocrine regulation of calcium homeostasis during pregnancy. Clin Perinatol. 1983;10(3):575–92.
60. System S. SAS Software, 9.22 ed. SAS Institute, Inc., Cary, NC. 2010.
61. Gillings D, Koch G. The application of the principle of intention-to-treat in the analysis of clinical trials. Drug Inf J. 1991;25:411–24.
62. Food and Nutrition Board. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Vitamin D and Calcium. Washington, DC: National Academy Press; 2010.
63. Pitkin RM. Nutritional influences during pregnancy. Med Clin North Am. 1977;61(1):3–15.
64. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. Am J Clin Nutr. 2007;85(2):614S–20.
65. Black J, Bonham-Carter J. Association between aortic stenosis and facies of severe infantile hypercalcemia. Lancet. 1963;2:745–9.
66. Friedman WF, Mills LS. The production of ''elfin facies'' and abnormal dentition by vitamin D during pregnancy: relationship to the supravalvular aortic stenosis syndrome. Proc Soc Pediatr Res. 1967;37:80.
67. Taussig HB. Possible injury to the cardiovascular system from vitamin D. Ann Intern Med. 1966;65:1195–200.
68. Morris CA, Mervis CB. William's syndrome and related disorders. Ann Dev Genomics Human Genet. 2000;1:461–84.
69. Aravena T, Castillo S, Carrasco X, Mena I, Lopez J, Rojas JP, Rosembert C, Schroter C, Aboitiz F. Williams syndrome: clinical, cytogenetical, neurophysiological and neuroanatomic study. Rev Med Child. 2002;130:631–7.
70. Taylor A, Stern P, Bell N. Abnormal regulation of circulating 25-hydroxyvitamin D on the Williams Syndrome. N Engl J Med. 1982; 306:972–5.
71. Garabedian M, Jacqz E, Guillozo H, Grimberg R, Guillot M, Gagnadoux MF, Broyer M, Lenorr G, Balsan S. Elevated plasma 1,25-dihydroxyvitamin D concentrations in infants with hypercalcemia and an elfin facies. N Engl J Med. 1985;312:948–52.
72. Knudtzon J, Aksnes L, Akslen LA, Aarskog D. Elevated 1,25-dihydroxyvitamin D and normocalcaemia in presumed familial Williams Syndrome. Clin Genet. 1987;32:369–74.
73. Wozikowska J, Wojtanowska H, Kozuszko K, Slowik J, Blazewska K, Balo G. A case of idopathic hypercalcemia, hypersensitivity to vitamin D_3. Wiad Lek. 1992;45:229–32.
74. McTaggart SJ, Craig J, MacMillan J, Burke JR. Familial occurrence of idiopathic intantile hypercalcemia. Pediatr Nephrol. 1999;13:668–71.
75. Mathias RS. Rickets in an infant with Williams Syndrome. Pediatr Nephrol. 2000;14:489–92.
76. Vieth R. The mechanisms of vitamin D toxicity. Bone Miner. 1990; 11(3):267–72.
77. Heaney RP, Armas LA, Shary JR, Bell NH, Binkley N, Hollis BW. 25-Hydroxylation of vitamin D3: relation to circulating vitamin D3 under various input conditions. Am J Clin Nutr. 2008;87(6):1738–42.
78. Kumar R, Cohen WR, Silva P, Epstein FH. Elevated 1,25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. J Clin Invest. 1979;63(2):342–4.

79. Bouillon R, Van Assche FA, Van Baelen H, Heyns W, DeMoor P. Influence of the Vitamin D-binding protein on serum concentrations of 1,25$(OH)_2$D. J Clin Invest. 1981;67:589–96.
80. Haughton MA, Mason RS. Immunonephelometric assay of vitamin D-binding protein. Clin Chem. 1992;38(9):1796–801.
81. Bikle DD, Gee E, Halloran B, Haddad JG. Free 1,25-dihydroxyvitamin D levels in serum from normal subjects, pregnant subjects, and subjects with liver disease. J Clin Invest. 1984;74(6):1966–71.
82. Sowers MR, Wallace RB, Hollis BW. The relationship of 1,25-dihydroxyvitamin D and radial bone mass. Bone Miner. 1990; 10(2):139–48.
83. Gloth FM, Gundberg CM, Holllis BW, Haddad JG, Tobin JD. Vitamin D deficiency in homebound elderly persons. JAMA. 1995;274:1683–6.
84. Greer FR, Hollis BW, Napoli JL. High concentrations of vitamin D_2 in human milk associated with pharmacologic doses of vitamin D_2. J Pediatrics. 1984;105:61–4.
85. Novakovic B, Sibson M, Ng HK, Manuelpillai U, Rakyan V, Down T, Beck S, Fournier T, Evain-Brion D, Dimitriadis E, Craig JM, Morley R, Saffery R. Placenta-specific methylation of the vitamin D 24-hydroxylase gene: implications for feedback autoregulation of active vitamin D levels at the fetomaternal interface. J Biol Chem. 2009; 284(22):14838–48.
86. Stevenson JC, Hillyard CJ, MacIntyre I, Cooper H, Whitehead MI. A physiological role for calcitonin: protection of the maternal skeleton. Lancet. 1979;2(8146):769–70.
87. Shinki T, Ueno Y, DeLuca HF, Suda T. Calcitonin is a major regulator for the expression of renal 25-hydroxyvitamin D3-1alpha-hydroxylase gene in normocalcemic rats. Proc Natl Acad Sci U S A. 1999;96(14):8253–8.
88. Zhong Y, Armbrecht HJ, Christakos S. Calcitonin, a regulator of the 25-hydroxyvitamin D3 1alpha-hydroxylase gene. J Biol Chem. 2009;284(17):11059–69.
89. Zaidi M, Moonga BS, Abe E. Calcitonin and bone formation: a knockout full of surprises. J Clin Invest. 2002;110(12):1769–71.
90. Ajibade DV, Dhawan P, Fechner AJ, Meyer MB, Pike JW, Christakos S. Evidence for a role of prolactin in calcium homeostasis: regulation of intestinal transient receptor potential vanilloid type 6, intestinal calcium absorption, and the 25-hydroxyvitamin D(3) 1alpha hydroxylase gene by prolactin. Endocrinology. 2010;151(7):2974–84.
91. Carneiro RM, Prebehalla L, Tedesco MB, Sereika SM, Hugo M, Hollis BW, Gundberg CM, Stewart AF, Horwitz MJ. Lactation and bone turnover: a conundrum of marked bone loss in the setting of coupled bone turnover. J Clin Endocrinol Metab. 2010;95(4): 1767–76.
92. Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat Clin Pract Endocrinol Metab. 2008;4(2):80–90.
93. Camargo CA Jr, Ingham T, Wickens K, Thadhani R, Silvers KM, Epton MJ, Town GI, Pattemore PK, Espinola JA, Crane J. Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. Pediatrics. 2011;127(1):e180–7.
94. Belderbos ME, Houben ML, Wilbrink B, Lentjes E, Bloemen EM, Kimpen JL, Rovers M, Bont L. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. Pediatrics. 2011;127(6):e1513–20.
95. Yu S, Cantorna MT. Epigenetic reduction in invariant NKT cells following in utero vitamin D deficiency in mice. J Immunol. 2010.
96. van der Vliet H, von Blomberg B, Nishi N, Reijm M, Voskuyl A, van Bodegraven A, Polman C, Rustemeyer T, Lips P, van den Eertwegh A, Giaccone G, Scheper R, Pinedo H. Circulating V[alpha]24+ V[beta]11+ NKT cell numbers are decreased in a wide variety of diseases that are characterized by autoreactive tissue damage. Clinical Immunology. 2001;100(2):144–8.
97. Hollis BW, Pittard WB. Evaluation of the total fetomaternal vitamin D relationships at term: Evidence for racial differences. J Clin Endorcrinol Metab. 1984;59:652–7.
98. Heaney R, Dowell M, Hale C, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Amer College Nutr. 2003;22(2):142–6.
99. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911–30.
100. Obermer E. Vitamin-D requirements in pregnancy. Br Med J. 1947;2(4535):927.
101. Friedman WF. Vitamin D as a cause of the supravalvular aortic stenosis syndrome. Am Heart J. 1967;73:718–20.