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Safety Aspects of a Randomized Clinical Trial of Maternal and Infant Vitamin D Supplementation by Feeding Type Through 7 Months Postpartum

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Abstract

Background: The safety of higher dose vitamin D (vitD) supplementation in women who change from exclusive or full breastfeeding to combination feeding or who continue supplementation after cessation of breastfeeding is unknown.
Objective: Compare vitD supplementation safety of 6,400 to 400 IU/day and 2,400 IU/day using specific laboratory parameters in postpartum women and their infants through 7 months postpartum by feeding type.
Design: In this randomized controlled trial, mothers (exclusively breastfeeding or formula-feeding) were randomized at 4–6 weeks' postpartum to 400, 2,400, or 6,400 IU $vitD_3$ (cholecalciferol)/day for 6 months. Breastfeeding infants in 400 IU group received oral 400 IU $vitD_3$/day; infants in 2,400 and 6,400 IU groups received placebo. Maternal safety parameters (serum vitD, 25-hydroxy-vitamin D [25(OH)D; calcidiol], calcium, phosphorus, intact PTH; urinary calcium/creatinine ratios; and feeding type/changes) were measured monthly; infant parameters were measured at months 1, 4, and 7. Sufficiency was defined as 25(OH)D >50 nmol/L. Feeding type was defined as exclusive/full, combination, or formula-feeding. Data were analyzed using SAS 9.4.
Results: Four hundred nineteen mother-infant pairs were randomized into the three treatment groups and followed: 346 breastfeeding and 73 formula-feeding pairs. A dose of 6400 IU/day safely and significantly increased maternal vitD and 25(OH)D from baseline in all mothers regardless of feeding type ($p < 0.0001$) and was superior to the 400 and 2,400 IU groups in achieving vitD sufficiency with no other differences in safety parameters by treatment or feeding type. Infants in the 2,400 IU group were more likely vitD-deficient than the other groups; otherwise, there were no infant safety parameter differences.
Conclusions: While 6,400 IU/day was more effective than 400 or 2,400 IU/day in achieving maternal vitD sufficiency in all feeding groups, the groups did not differ on other safety parameters. Similarly, infant safety parameters did not differ by treatment group or feeding status.
Clinical Trial Registration: FDA IND Number: 66,346; ClinicalTrials.gov Number: NCT00412074.

Keywords: vitamin D, cholecalciferol, lactation, infant, postpartum, RCT

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Introduction

MATERNAL VITAMIN D (vitD) supplementation with 6,400 IU/day alone during full lactation is effective in improving the breastfeeding infant's vitD status.[1] Prior reports focused on the effectiveness of supplementation rather than safety.[1,2] There remains a concern of the safety of higher dose vitD supplementation in women who change from exclusive or full breastfeeding to combination feeding or who continue supplementation after cessation of breastfeeding.[3] To date, a comparison of the safety of sustained high-dose supplementation with vitD in women who are fully breastfeeding with those providing a combination of breast milk and formula (combination feeding) or who are exclusively formula feeding has not been reported; and hence, the focus of this report. It was hypothesized that high-dose vitD supplementation women compared to control postpartum women—lactating and nonlactating—would have improved vitD status with similar safety parameters throughout the study period. The results of this safety aspect of the National Institute of Child Health and Human Development (NICHD)-sponsored vitD supplementation during lactation randomized controlled trial are reported here.

Materials and Methods

Study sites

As previously described, the study was conducted at the Medical University of South Carolina (MUSC; latitude 32.78°N) and the University of Rochester (U of R; latitude 43.15°N).[1,2] Approval for this two-site study was granted by: (1) MUSC's Institutional Review Board for Human Subjects HR #16536 and the Clinical and Translational Research Center (CTRC; Protocol #752); and (2) the U of R's Institutional Review Board for Human Subjects (#14460) and the CTRC (Protocol #1129). The study was registered via ClinicalTrials.gov #NCT00412074.

Study design

This was a randomized, double blind, comparative effectiveness, and safety trial of three doses of vitD supplementation (400, 2,400, and 6,400 IU $vitD_3$/day) in breastfeeding mothers and their infants and nonlactating control pairs that met the criteria for an optimized nutrient study.[4] The 2,400 IU arm ended due to safety concerns of increased vitD deficiency in the infants in February 2009 (with safety results of the three groups up to that point presented in Supplementary Tables S1–S4A, and B).[4] The efficacy results of this study were previously reported.[1] As part of the safety concern, a cohort of women who were exclusively formula-feeding were included in the study to assure safety if a woman continued high-dose vitD supplementation and had stopped breastfeeding.

Enrollment and randomizaton of breastfeeding mothers and their infants. Following written informed consent, mothers were randomized using block randomization of 10 to 1 of three vitD supplementation regimens: Group 1: Control, 400 IU (10 μg) $vitD_3$/day (0 IU $vitD_3$ placebo and one prenatal vitamin containing 400 IU $vitD_3$); Group 2: 2,400 IU (60 μg) $vitD_3$/day (2,000 IU $vitD_3$/day and one prenatal vitamin containing 400 IU $vitD_3$); and Group 3: 6,400 IU (160 μg) $vitD_3$/day (6,000 IU $vitD_3$ and one prenatal vitamin containing 400 IU $vitD_3$). The breastfeeding infants were given a liquid suspension (one drop per day) of $vitD_3$ or placebo (Bio-D-Mulsion, Biotics Research Corp, Rosenberg, TX): those infants in Group 1 received 400 IU $vitD_3$ and those in Groups 2 and 3 received placebo (containing 0 IU $vitD_3$).

Enrollment of exclusively formula-feeding mothers and infants. Mothers who chose to exclusively formula-feed were eligible for participation in the study at the Charleston site only. The formula-feeding women were randomized to either treatment group as outlined above. Their infants, who were receiving any commercially available infant formula containing 400 IU vitD/L, were not supplemented as part of the study.

Subjects

Inclusion criteria. Exclusively breastfeeding mothers or formula-feeding mothers and their singleton infants ≥35 weeks of gestation at birth (receiving either exclusive breast milk or formula at the time of study entry[5,6]) within 4-to-6 weeks' postpartum in good general health were eligible for inclusion in the study. Women who chose to begin combination or exclusively formula-feeding at or after 4 months postpartum were eligible to continue their participation in the study.

Exclusion criteria. Exclusion criteria included any preexisting maternal health problem, twin/multiple pregnancy, or if the infant was <35 weeks of gestation at birth and with a health problem. Infants receiving combination feedings (defined as feedings of <90% breast milk feedings and formula feedings) at the onset of the trial were not eligible to participate.

Definition of feeding type. Feeding type was defined *a priori* as exclusively or fully breastfeeding (referred to as breastfeeding hereafter), combination feeding (both breast milk/breastfeeding and formula were given to the infant on a daily basis), or exclusive formula-feeding (referred to as formula-feeding hereafter).

Outcome measures

Anthropometric measures. (1) Maternal: at each study visit, maternal weight was obtained; maternal height was measured at visit 1 (V1 at 1 month postpartum) and used to determine monthly BMI, which was calculated as follows: weight (kg)/[height (m)]^2. (2) Infant: each infant's weight (kg), length (cm) using a length board, and head circumference (cm) were measured according to standard clinical pediatric practice at V1 and each monthly visit.

Laboratory safety measurements

(1) Maternal and infant total circulating 25-hydroxyvitamin D (25(OH)D) and vitD (parent compound) were measured using high-performance liquid chromatography (HPLC) and radioimmunoassay techniques, respectively, as previously described.[7] Deficiency was defined *a priori* as total circulating 25(OH)D <50 nmol/L (<20 ng/mL).[7] The inter- and intra-assay coefficient of variation was ≤10%.

Given the limitation of reagents for the measurement, the total circulating concentration of the parent compound vitD was measured in a subset of sequential mothers and infants using HPLC as previously described.[8] The laboratory participated in Vitamin D External Quality Assessment Scheme (DEQAS) throughout the study period.[9]

(2) Maternal and infant circulating intact parathyroid hormone (iPTH) concentrations were measured by immune-radiometric assay as previously described and expressed as pmol/L (Diasorin Biotechnology Company, Stillwater, MN).[10]

(3) Measurement of Maternal and infant serum calcium and creatinine (mmol/L) and urinary calcium:creatinine ratios were measured using standard methodology and laboratory normative data at each site. Cross-validation between laboratories was performed for 5% of the samples (interassay variation 5.4%). Maternal values were measured monthly while infant values were reported at V1, visit 4 (V4 at 4 months' postpartum) and visit 7 (V7 at 7 months' postpartum); all values were reported as mmol/L. Maternal urinary calcium/creatinine ratios of 0.8 or higher resulted in notification to the Data and Safety Monitoring Board (DSMB). Infant ratios vary by postnatal age and are variable but decrease with advancing postnatal age.[1]

Adherence to protocol

Adherence to protocol was assessed through pill count for the mothers and weighing of the vitD bottle for the infant at each visit. The adherence rate was derived from the actual pill count or bottle weight over the expected pill count or weight, respectively, and expressed as a percentage of expected.

Deficiency safety measure and stopping of the 2,400 IU arm

As part of the study's data safety and monitoring plan, any breastfeeding infant with a total circulating 25(OH)D <20 ng/mL at V4 received open-label 400 IU $vitD_3$/day. Because of the safety measure in place at the start of clinical trial, and concern that infants of mothers in the 2,400 IU group would be more likely to have a total circulating 25(OH)D <20 ng/mL, an interim analysis was requested by the DSMB. This concern was based on prior pharmacokinetic studies during lactation.[2,11] As previously described,[1] the DSMB oversaw this interim analysis and deemed that the 2,400 IU arm of the study should be terminated due to disproportionate number of infants in that arm falling <20 ng/mL threshold for total circulating 25(OH)D adequacy. Results up to the ending of the 2,400 IU arm in February 2009 are reported separately (as Supplementary Tables S1–S4A and B) from the analyses of the two arms that continued until the completion of the trial.

Statistical analysis

Sample size calculation. The primary study was powered with site included in models to account for variability between sites to assess whether the expected increase in vitD in the experimental group was greater than that of the control group beyond random variability.[1] These calculations were conducted with 80% power, two-sided test, and a type I error of 0.05. For the assessment of safety, the approach was a noninferiority design since it was assumed that serum calcium, serum creatinine, urinary calcium: creatinine ratio, and the iPTH would not differ between groups (assumption of equal means). The midlevel value of the normal ranges of these markers was taken as the assumed values of what would be observed in the control group. Our sample size was sufficient to conduct an assessment with 99% power.

Statistical analyses. Statistical analyses were performed using SAS software version 9.4 (SAS, Cary, NC). Descriptive statistics were used to characterize and compare laboratory values between treatment groups at baseline. Analysis of variance was used to test for differences among treatment groups for normally distributed variables (i.e., weight, BMI, dietary calcium, dietary phosphorus, dietary vitD, gestational age, birth weight, and 25(OH)D). The Kruskal–Wallis test was used for analyses of variables that were not normally distributed (i.e., interpregnancy interval, gravidity, parity, and health rating). The percent of women and infants by treatment group with 25(OH)D <50 nmol/L was compared at V1, V4, and V7.[1] Continuous variables were summarized using means, standard deviations for normally distributed measures; medians and interquartile ranges for nonparametric measures, as appropirate, and categorical variables were summarized using frequencies and percentages.

Regression methods to model 25(OH)D status (linear and logistic) included variables that showed a significant association with the laboratory values in the bivariate analyses as well as site. Generalized linear mixed-effect models were used to measure the relationship between treatment (400, 2,400, or 6,400 IU), visit (which corresponded to the month postpartum), and treatment by time interaction while adjusting for other covariates, predicting each safety outcome (serum calcium, serum creatinine, iPTH concentration, and urine calcium: creatinine ratio), and accounting for repeated measures per person. The other covariates in the model included baseline maternal 25(OH)D concentration, race/ethnicity (non-Hispanic African American, Hispanic, and white/Caucasian), feeding type (exclusive breastfeeding, combination-feeding or formula-feeding), and BMI. In addition, the respectivate baseline measurement for each outcome was also included in the models. Significance was set *a priori* as $p < 0.05$.

Results

Allocation of participants by treatment group

The allocation of participants following enrollment is found in Figure 1 (CONSORT diagram) for the exclusively breastfeeding mother/infant pairs and exclusively formula-feeding mother/infant pairs. Maternal and infant sociodemographic and clinical characteristics for 400 and 6,400 IU groups at study entry are summarized in Table 1. Baseline characteristics did not differ between the groups by treatment. Furthermore, there were no significant differences in baseline characteristics between the treatment groups on any of the measures analyzed before discontinuation of the 2,400 IU group (Supplementary Table S1). The attrition rate for the

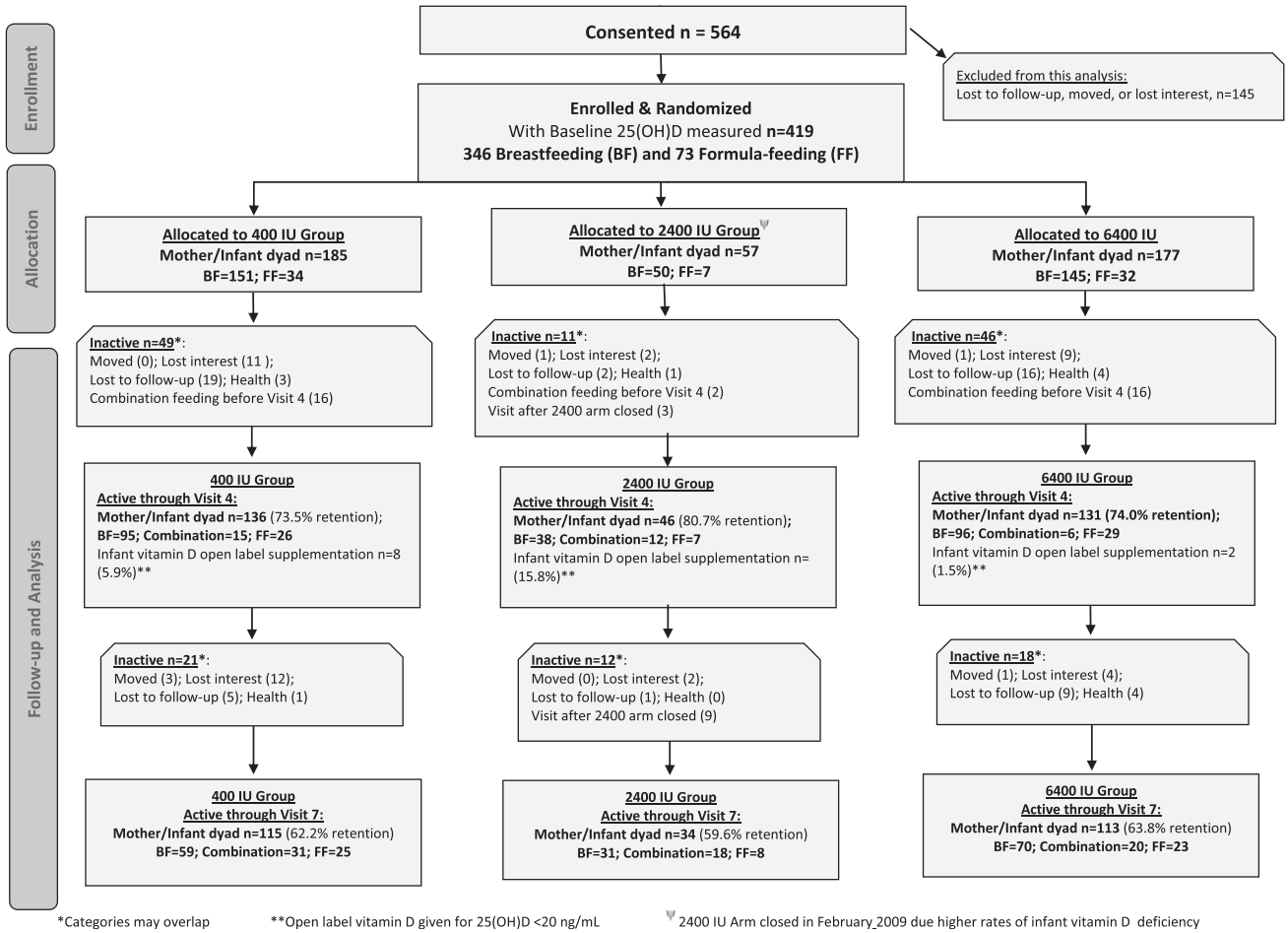

FIG. 1. Flow diagram of maternal and infant vitD supplementation study through 7 months postpartum. 25(OH)D, 25-hydroxy-vitamin D; vitD, vitamin D.

two remaining arms through completion of the study did not differ (see Supplementary Data).

Maternal and infant vitD status and safety parameters by treatment group and feeding type

The effect of treatment on maternal and infant safety laboratory measurements at V1, V4, and V7 for breastfeeding and formula-feeding mothers before discontinuing the 2,400 IU group did not show any differences in safety parameters (Supplementary Table S2). For the two remaining treatment groups—400 and 6,400 IU/day, maternal and infant safety parameters were compared at completion of the study.

In Table 2, a comparison by treatment at the three main timepoints—V1, V4, and V7 is provided for exclusively breastfeeding mothers and infants. There were no statistically significant differences at baseline on any of the vitD and safety parameters. At both V4 and V7, maternal total circulating 25(OH)D was greater in the 6,400 IU group versus the 400 IU group ($p < 0.0001$). There was a dose effect on the parent compound vitD by treatment with highest concentrations in mothers in the 6,400 IU group versus the 400 IU group ($p < 0.0001$). Maternal iPTH at V4 and V7 differed statistically between the groups ($p = 0.04$ and 0.004, respectively), with lowest values in the 6,400 IU group, but all values were within the normal range. Serum calcium and creatinine and urinary calcium:creatinine ratios did not differ at any of the study visits by treatment group with one exception: at V4 only, there was a modest increase in the ratio in the 6,400 IU group (0.3) compared to 400 IU group (0.2), but within the safety parameters of the study. In exclusively breastfeeding infants, 25(OH)D concentration was slightly higher in the 400 IU group compared to the 6,400 IU group at V4 ($p = 0.03$) only. In the subset of infants whose vitD (parent compound) was measured, there were no statistically significant differences at V4 or V7. When analyzed by treatment group, the infants did not differ on any of the safety measures.

For the exclusively formula-feeding mothers (Table 3), there were no statistically significant differences in 25(OH)D concentration at baseline, but by V4, there was a higher maternal 25(OH)D concentration in the 6,400 IU group compared to the 400 IU group that was sustained at V7 ($p < 0.006$). Similarly, in the sequential subset of the women who were tested, there was a dose effect on the parent compound vitD by treatment with highest concentrations in the mothers assigned to the 6,400 IU group compared to the 400 IU group at V7 ($p < 0.003$). Maternal iPTH, serum calcium, serum creatinine, and urinary calcium:creatinine ratio did not

Table 1. Baseline Maternal and Infant Sociodemographic and Clinical Characteristics of 400 and 6,400 IU Groups ($N=362$)*

	Treatment	
	400 IU n = *185*	*6,400 IU* n = *177*
Baseline characteristics at time of enrollment[a]	n *(% of treatment)*	n *(% of treatment)*
Site		
MUSC	116 (62.7)	102 (57.6)
Rochester	69 (37.3)	75 (42.4)
Feeding at baseline		
Breastfeeding	151 (81.6)	145 (81.9)
Formula-feeding (at MUSC site only)	34 (18.4)	32 (18.1)
Maternal race/ethnicity		
African American	55 (29.7)	58 (32.8)
Hispanic	62 (33.5)	58 (32.8)
White/Caucasian	68 (36.8)	61 (34.4)
Maternal education		
Less than High School	43 (23.4)	22 (14.7)
High School	38 (20.7)	48 (27.1)
College	103 (56.0)	103 (58.2)
Maternal employment		
Not full-time employed	72 (38.9)	58 (32.8)
Full-time employed	113 (61.1)	119 (67.2)
Maternal insurance status		
Private insurance	66 (35.7)	75 (42.4)
Medicaid \ no insurance	119 (64.3)	102 (57.6)
Maternal BMI		
BMI $\geq 30\,kg/m^2$	65 (36.5)	58 (33.9)
BMI $<30\,kg/m^2$	113 (63.5)	113 (66.1)
Season at initial visit		
April–September	97 (52.4)	94 (53.4)
October–March	88 (47.6)	82 (46.6)
	n *Mean ± SD*	n *Mean ± SD*
Days postpartum	184 36.8 ± 7.7	174 36.4 ± 7.6
BMI	135 29.0 ± 6.9	130 27.9 ± 5.2
Maternal Smart-Probe 400 Site: Forearm[b]	184 52.3 ± 9.9	177 52.4 ± 9.9
Maternal dietary calcium (mg/day)	54 967 ± 414	50 979 ± 544
Maternal dietary phosphorus (mg/day)	54 1,384 ± 560	50 1,377 ± 719
Maternal dietary vitD (IU/day)	54 172.9 ± 123.2	50 152.8 ± 111.3
	n *Median, 25–75 interquartile range*	n *Median, 25–75 interquartile range*
Maternal interpregnancy interval (months)	133 24.0, 12–43	126 24.0, 12–48
Maternal gravidity	184 2.0, 1–3	177 2.0, 1–3
Maternal parity	184 2.0, 1–3	177 2.0, 1–2
Maternal health rating, 0–10[c]	184 10.0, 8–10	176 10.0, 8–10
Infant characteristics	n *Mean ± SD*	n *Mean ± SD*
Infant birth weight (g)	184 3337 ± 519	177 3391 ± 442
Infant gestational age at birth (weeks)	184 39.3 ± 1.4	175 39.4 ± 1.0

[a]Total number for each characteristic varies based on available data from the 400 and 6,400 IU arms of the study. The 2,400 IU group was not included in this analysis.

[b]Maternal forearm skin pigmentation score derived using SmartProbe 400 (IMS, Portland, ME), a skin spectrophotometer that derives a score of 0–100 score, with absolute black equal to 0 and absolute white equal to 100, for a given region of the body. Forearm was chosen as the most representative of maximal sun exposure resulting in pigment darkening as a surrogate for sunlight exposure.

[c]Maternal health rating was derived from question asked each mother using a Likert scale of 0–10, with 0 being the worse health and 10 being the best health.

*Chi-square analyses were used to test for differences in frequencies between treatment groups; Student's *t*-test analyses were used to test for differences in means between treatment groups; Mann–Whitney tests were used to test for differences in median between treatment groups.

BMI, body mass index; MUSC, Medical University of South Carolina; vitD, vitamin D.

differ between the groups at baseline or at subsequent visits. Formula-feeding infants did not differ on any of vitD and safety parameters at any time point except for iPTH at V7 with lower levels in 6,400 IU group compared to 400 IU group ($p=0.03$); however, their iPTH was not influenced by maternal vitD supplementation.

In multiple regression analyses that included baseline 25(OH)D, race/ethnicity, feeding type, visit, and treatment in the model, feeding type was not significant for any maternal safety parameter at any time point. Feeding type was significant for infant serum calcium (with higher mean values for breastfeeding infants) and serum creatinine (with lower mean values for breastfeeding infants) (data not shown).

Infant growth parameters by feeding type and treatment group

Breastfeeding infants by treatment group did not differ at baseline or across subsequent visits on measured growth parameter. Specifically, for the infants who had initially been fully breastfeeding to V4 and then continued on in the study with some breastfeeding, weight, head circumference, and length did not differ by treatment group (Supplementary Table S3).

Table 2. Breastfeeding Maternal and Infant Biochemical Safety Measurements at Visits 1, 4, and 7 by 400 and 6,400 IU groups ($N=296$)

	400 IU (n = *151*)	*6,400 IU* (n = *145*)	p-*Value**
Maternal			
Serum 25(OH)D (nmol/L), mean ± SD			
Visit 1 ($n=296$)	77.2 ± 31.8	83.5 ± 34.8	0.1
Visit 4 ($n=210$)	78.5 ± 28.7	146.5 ± 47.3	<0.0001
Visit 7 ($n=178$)	73.7 ± 29.5	148.4 ± 51.6	<0.0001
Serum D_3 (nmol/L), median (25–75 IQR)			
Visit 1 ($n=175$)	1.6 (1.5–6.4)	1.7 (1.5–5.6)	0.9
Visit 4 ($n=125$)	2.0 (1.5–8.4)	105.5 (66.5–123.2)	<0.0001
Visit 7 ($n=96$)	2.1 (1.5–5.0)	129.4 (72.0–160.5)	<0.0001
Serum calcium (mmol/L), mean ± SD			
Visit 1 ($n=283$)	2.3 ± 0.1	2.3 ± 0.1	0.9
Visit 4 ($n=210$)	2.4 ± 0.08	2.4 ± 0.08	0.7
Visit 7 ($n=175$)	2.3 ± 0.09	2.3 ± 0.08	0.4
Serum creatinine (mmol/L), mean ± SD			
Visit 1 ($n=295$)	0.06 ± 0.01	0.06 ± 0.01	0.8
Visit 4 ($n=210$)	0.06 ± 0.01	0.06 ± 0.01	0.7
Visit 7 ($n=178$)	0.06 ± 0.01	0.06 ± 0.01	0.3
Urinary calcium: creatinine ratio, mean ± SD			
Visit 1 ($n=294$)	0.2 ± 0.2	0.2 ± 0.2	0.3
Visit 4 ($n=210$)	0.2 ± 0.2	0.3 ± 0.3	0.05
Visit 7 ($n=178$)	0.3 ± 0.2	0.3 ± 0.3	0.1
iPTH (pmol/L), median (25–75 IQR)			
Visit 1 ($n=294$)	3.0 (2.0–4.2)	2.9 (2.0–4.2)	0.8
Visit 4 ($n=210$)	2.7 (2.1–3.4)	2.3 (1.7–3.1)	0.04
Visit 7 ($n=176$)	3.3 (2.2–4.3)	2.6 (2.0–3.4)	0.004
Infant			
25(OH)D (nmol/L), mean ± SD			
Visit 1 ($n=279$)	40.8 ± 30.9	44.6 ± 27.5	0.3
Visit 4 ($n=199$)	117.8 ± 52.1	104.2 ± 32.3	0.03
Visit 7 ($n=152$)	114.8 ± 34.7	107.1 ± 35.5	0.2
D_3 (nmol/L), median (25–75 IQR)			
Visit 1 ($n=71$)	1.5 (1.5–1.5)	1.5 (1.5–1.4)	0.9
Visit 4 ($n=51$)	18.8 (2.8–41.5)	11.6 (2.3–23.4)	0.2
Visit 7 ($n=43$)	24.6 (3.9–47.7)	13.3 (3.6–29.8)	0.2
Serum calcium (mmol/L), mean ± SD			
Visit 1 ($n=212$)	2.6 ± 0.09	2.6 ± 0.1	0.2
Visit 4 ($n=150$)	2.6 ± 0.09	2.6 ± 0.1	0.8
Visit 7 ($n=115$)	2.6 ± 0.1	2.6 ± 0.08	0.4
Serum creatinine (mmol/L), mean ± SD			
Visit 1 ($n=222$)	0.02 ± 0.005	0.02 ± 0.006	0.6
Visit 4 ($n=160$)	0.02 ± 0.004	0.02 ± 0.005	0.3
Visit 7 ($n=121$)	0.02 ± 0.004	0.02 ± 0.004	0.9
Urinary calcium: creatinine ratio, mean ± SD			
Visit 1 ($n=246$)	1.4 ± 1.1	1.4 ± 0.9	0.5
Visit 4 ($n=193$)	1.4 ± 0.9	1.4 ± 0.9	0.8
Visit 7 ($n=133$)	1.0 ± 0.7	1.1 ± 0.8	0.2
iPTH (pmol/L), median (25–75 IQR)			
Visit 1 ($n=168$)	2.4 (1.8–3.5)	2.2 (1.5–3.1)	0.2
Visit 4 ($n=131$)	1.8 (1.3–2.5)	1.9 (1.3–2.5)	0.6
Visit 7 ($n=105$)	2.0 (1.6–2.6)	2.0 (1.4–2.7)	0.8

Numbers for each laboratory test vary based on blood sample volume obtained at given visit and number of mothers and infants who were still active in study at time of visit.

For measurement of parent compound vitD, this was measured only in a sequential subset of mothers and infants.

*Student's *t*-test analyses were used to test for differences in mean laboratory values between treatment groups at each visit. Nonparametric analyses were used to test for differences in median laboratory value (D_3 and iPTH) between treatment groups at each visit.

25(OH)D, 25-hydroxy-vitamin D; iPTH, intact parathyroid hormone.

Rates of deficiency in mothers and infants over time

At study entry, there were no significant differences in the percentages of mothers with 25(OH)D concentrations <50 nmol/L threshold by entrance feeding type (Table 4). By V4, there were significant differences by treatment most dramatically seen in the exclusively breastfeeding group of women: there were consistently more women in the 400 IU group compared to the 6,400 IU group with a 25(OH)D concentration <50 nmol/L. This also was noted with the combination and exclusively formula-feeding mothers.

Table 3. Maternal and Infant Biochemical Measurements at Visits 1, 4, and 7 by 400 and 6,400 IU Groups in Exclusively Formula-Feeding Mother/Infant Dyads ($n=66$)

	400 IU (n = *34*)	*6,400 IU* (n = *32*)	p-*Value**
Maternal			
25(OH)D (nmol/L), mean ± SD			
Visit 1 ($n=66$)	68.6 ± 31.1	56.6 ± 24.2	0.09
Visit 4 ($n=56$)	72.6 ± 37.0	96.3 ± 55.5	0.07
Visit 7 ($n=50$)	65.2 ± 28.2	109.2 ± 71.6	0.006
D_3 (nmol/L), median (25–75 IQR)			
Visit 1 ($n=42$)	1.5 (1.5–1.5)	1.5 (1.5–1.5)	0.8
Visit 4 ($n=28$)	1.5 (1.5–2.7)	1.5 (1.4–40.9)	0.4
Visit 7 ($n=25$)	1.5 (1.5–1.5)	20.5 (1.5–44.3)	0.003
Serum calcium (mmol/L), mean ± SD			
Visit 1 ($n=66$)	2.3 ± 0.06	2.3 ± 0.09	0.3
Visit 4 ($n=56$)	2.3 ± 0.08	2.3 ± 0.06	0.4
Visit 7 ($n=49$)	2.3 ± 0.07	2.3 ± 0.09	0.7
Serum creatinine (mmol/L), mean ± SD			
Visit 1 ($n=66$)	0.06 ± 0.01	0.06 ± 0.01	0.9
Visit 4 ($n=56$)	0.06 ± 0.01	0.06 ± 0.01	0.9
Visit 7 ($n=49$)	0.06 ± 0.01	0.06 ± 0.01	0.07
Urinary calcium: creatinine ratio, mean ± SD			
Visit 1 ($n=65$)	0.1 ± 0.1	0.1 ± 0.2	0.7
Visit 4 ($n=56$)	0.2 ± 0.1	0.2 ± 0.2	0.7
Visit 7 ($n=$)	0.2 ± 0.2	0.2 ± 0.2	0.9
iPTH (pmol/L), median (25–75 IQR)			
Visit 1 ($n=66$)	3.4 (2.9–4.4)	3.8 (3.3–5.3)	0.2
Visit 4 ($n=56$)	3.5 (3.0–4.3)	3.4 (2.4–4.0)	0.3
Visit 7 ($n=49$)	3.3 (2.4–4.3)	3.2 (2.2–4.1)	0.8
Infant			
25(OH)D (nmol/L), mean ± SD			
Visit 1 ($n=65$)	65.4 ± 19.9	64.5 ± 17.2	0.8
Visit 4 ($n=56$)	93.9 ± 28.8	97.6 ± 25.9	0.6
Visit 7 ($n=49$)	96.5 ± 27.2	93.5 ± 24.6	0.8
D_3 (nmol/L), median (25–75 IQR)			
Visit 1 ($n=16$)	10.1 (1.5–20.7)	16.3 (1.5–20.3)	0.9
Visit 4 ($n=10$)	13.0 (8.7–41.1)	26.2 (4.9–47.4)	0.6
Visit 7 ($n=12$)	27.9 (14.4–85.7)	12.9 (9.2–25.8)	0.2
Serum calcium (mmol/L), mean ± SD			
Visit 1 ($n=46$)	2.5 ± 0.08	2.5 ± 0.05	0.04
Visit 4 ($n=39$)	2.6 ± 0.07	2.6 ± 0.07	0.5
Visit 7 ($n=34$)	2.5 ± 0.1	2.5 ± 0.09	0.9
Serum creatinine (mmol/L), mean ± SD			
Visit 1 ($n=50$)	0.02 ± 0.008	0.02 ± 0.006	0.7
Visit 4 ($n=40$)	0.02 ± 0.006	0.02 ± 0.006	0.7
Visit 7 ($n=35$)	0.02 ± 0.004	0.02 ± 0.006	0.09
Urinary calcium: creatinine ratio, mean ± SD			
Visit 1 ($n=57$)	0.9 ± 0.6	0.9 ± 0.4	0.8
Visit 4 ($n=53$)	0.8 ± 0.5	0.7 ± 0.5	0.5
Visit 7 ($n=46$)	0.9 ± 0.7	0.8 ± 0.8	0.7
iPTH (pmol/L), median (25–75 IQR)			
Visit 1 ($n=35$)	3.8 (2.5–5.0)	3.4 (2.1–4.2)	0.5
Visit 4 ($n=30$)	2.8 (2.0–3.5)	1.9 (1.6–2.6)	0.1
Visit 7 ($n=30$)	2.2 (1.7–3.1)	1.6 (1.3–1.9)	0.03

Numbers for each laboratory test vary based on blood sample volume obtained at given visit and number of mothers and infants who were still active in study at time of visit.

For measurement of parent compound vitD, this was measured only in a sequential subset of mothers and infants.

*Student's *t*-test analyses were used to test for differences in mean laboratory values between treatment groups at each visit. Nonparametric analyses were used to test for differences in median laboratory value (D_3 and iPTH) between treatment groups at each visit.

Table 4. Percent of Women and Infants by Treatment Group with 25(OH)D <50 nmol/L at Visits 1, 4, and 7 Substratified by Feeding Type

	25(OH)D <50 nmol/L by treatment, n *(%)*		
Feeding type	*400 IU* n = *185*	*6,400 IU* n = *177*	p-*Value*
Mothers			
Visit 1			
Exclusive breastfeeding	27 (17.9)	21 (14.5)	0.4
Exclusive formula-feeding	10 (29.4)	13 (40.6)	0.3
Visit 4			
Exclusive breastfeeding	11 (11.6)	0 (0.0)	0.0003
Combination feeding	7 (46.7)	0 (0.0)	0.06
Exclusive formula-feeding	6 (23.1)	5 (17.2)	0.6
Visit 7			
Exclusive breastfeeding	10 (17.0)	0 (0.0)	0.0003
Combination feeding	4 (17.4)	0 (0.0)	0.1
Exclusive formula-feeding	8 (32.0)	5 (21.7)	0.4
Infants			
Visit 1			
Exclusive breastfeeding	100 (69.4)	83 (61.5)	0.2
Exclusive formula-feeding	6 (18.2)	6 (18.8)	0.9
Visit 4			
Exclusive breastfeeding	8 (8.7)	3 (3.4)	0.2
Combination feeding	0 (0.0)	0 (0.0)	n/a
Exclusive formula-feeding	1 (3.9)	0 (0.0)	0.5
Visit 7			
Exclusive breastfeeding	2 (3.6)	2 (3.3)	1.0
Combination feeding	0 (0.0)	0 (0.0)	n/a
Exclusive formula-feeding	1 (4.0)	0 (0.0)	1.0

n/a, not applicable.

With regard to infants, there were no differences in the frequency of infants with a 25(OH)D <50 nmol/L threshold. At study entry, the infants who were breastfeeding had more deficiency compared to the formula-fed infants, reflecting the lack of vitD supplementation (88%) in the majority of breastfeeding infants in this cohort during the first month after birth. By V4, the rates of deficiency in the breastfeeding infants had decreased, which was sustained to V7. There were a small number of infants in both groups (5.9% in the 400 IU group and 1.5% in the 6,400 IU group) who required open-label vitD supplementation at the fourth visit due to a total circulating 25(OH)D <50 nmol/L.

Mixed model regression analyses

Mixed models for predicting each maternal safety parameter were initially constructed to compare the 400, 2,400, and 6,400 IU groups before discontinuation of the 2,400 IU arm of the study. There were no treatment by time interaction effects for any of the safety measures for either the mothers or their infants (Supplementary Tables S4A, B). Mixed models were then created for those women who completed the study in the two remaining treatment groups 400 and 6,400 IU.

As shown in Table 5, using a mixed model approach, there were certain associations noted for each maternal safety measure independent of confounders. Time was negatively associated with maternal serum calcium concentration with a slight decline between V4 and V7. Treatment and treatment overtime interaction were not associated with maternal serum calcium concentration.

Maternal serum creatinine was not associated with maternal 25(OH)D concentration. In the model, treatment and treatment overtime interaction were not associated with maternal urinary calcium/creatinine ratios. Similarly, treatment was not associated with maternal iPTH, but treatment over time was associated ($p = 0.035$).

As shown in Table 6, analysis of infant safety parameters using a mixed model approach for each safety parameter at baseline, 25(OH)D concentration at baseline, 25(OH)D concentration at V4 and 7, race/ethnicity (African American, Hispanic, white/Caucasian), feeding type (exclusively breastfeeding, formula feeding or combination feeding), visit, treatment, and visit by treatment over time were not significant. Urinary calcium/creatinine ratio was positively associated with infant 25(OH)D concentration ($p = 0.028$). Treatment alone and treatment over time interaction were not associated with infant urinary calcium/creatinine ratios. Infant iPTH was inversely associated with infant 25(OH)D concentration ($p < 0.0001$) and with treatment ($p = 0.0092$), but all values were in the normal reported clinical range. Treatment over time interaction was not associated with infant iPTH ($p = 0.49$).

Overall, in both the maternal and infant mixed models, no significant treatment over time interaction effects existed for any maternal and infant safety parameters. Thus, the effect of the two treatments on safety during the intervention period did not differ on the parameters tested.

Discussion

In this vitD supplementation clinical trial of women who were exclusively breastfeeding or formula-feeding at baseline or later transitioned to combination feeding

Table 5. Mixed Models for Predicting Each Maternal Safety Parameter

Safety parameter	*Beta estimate*	*Beta standard error*	p-*Value*
1. Serum calcium			
Treatment 400 IU	Reference		
Treatment 6,400 IU	–0.00678	0.01093	
Treatment			0.9185
Visit 4	Reference		
Visit 7	–0.02656	0.007972	
Visit			0.0011
Treatment by visit interaction 400 IU	Reference		
Treatment by visit interaction 6,400 IU	0.01563	0.01110	
Treatment by visit interaction			0.1604
2. Serum creatinine			
Treatment 400 IU	Reference		
Treatment 6,400 IU	–0.00048	0.001224	
Treatment			0.3464
Visit 4	Reference		
Visit 7	–0.00144	0.000844	
Visit			0.0009
Treatment by visit interaction 400 IU	Reference		
Treatment by visit interaction 6,400 IU	–0.00119	0.001171	
Treatment by visit interaction			0.3095
3. Urinary calcium/creatinine ratio			
Treatment 400 IU	Reference		
Treatment 6,400 IU	0.03997	0.03242	
Treatment			0.1736
Visit 4	Reference		
Visit 7	0.009965	0.02440	
Visit			0.5428
Treatment by visit interaction 400 IU	Reference		
Treatment by visit interaction 6,400 IU	0.001259	0.03403	
Treatment by visit interaction			0.9705
4. Serum iPTH			
Treatment 400 IU	Reference		
Treatment 6,400 IU	–0.1752	0.2716	
Treatment			0.3240
Visit 4	Reference		
Visit 7	0.4041	0.2249	
Visit			0.0349
Treatment by visit interaction 400 IU	Reference		
Treatment by visit interaction 6,400 IU	–0.1315	0.3103	
Treatment by visit interaction			0.6725

Adjusted for baseline value, baseline 25-hydroxy vitD concentration, 25-hydroxy vitD concentration at visit 4 and visit 7, race, and feeding category.

Table 6. Mixed Models for Predicting Each Infant Safety Parameter

Safety parameter	*Beta estimate*	*Beta standard error*	p-*Value*
1. Serum calcium			
Treatment 400 IU	Reference		
Treatment 6,400 IU	–0.00651	0.01359	
Treatment			0.8038
Visit 4	Reference		
Visit 7	–0.02953	0.01289	
Visit			0.0318
Treatment by visit interaction 400 IU	Reference		
Treatment by visit interaction 6,400 IU	0.01856	0.01813	
Treatment by visit interaction			0.3083
2. Serum creatinine			
Treatment 400 IU	Reference		
Treatment 6,400 IU	–0.00074	0.000695	
Treatment			0.1700
Visit 4	Reference		
Visit 7	–0.001262	0.000664	
Visit			0.0118
Treatment 6,400 IU	Reference		
Treatment by visit interaction 6,400 IU	–0.00010	0.000921	
Treatment by visit interaction			0.9121
3. Urinary calcium/creatinine ratio			
Treatment 400 IU	Reference		
Treatment 6,400 IU	0.01785	0.1038	
Treatment			0.4641
Visit 4	Reference		
Visit 7	–0.2565	0.08826	
Visit			0.0017
Treatment by visit interaction 400 IU	Reference		
Treatment by visit interaction 6,400 IU	0.1013	0.1257	
Treatment by visit interaction			0.4218
4. Serum iPTH			
Treatment 400 IU	Reference		
Treatment 6,400 IU	–0.7058	0.2366	
Treatment			0.0124
Visit 4	Reference		
Visit 7	–0.1839	0.2144	
Visit			0.9402
Treatment by visit interaction 400 IU	Reference		
Treatment by visit interaction 6,400 IU	0.3453	0.2723	
Treatment by visit interaction			0.2122

Adjusted for baseline value, baseline 25-hydroxy vitD concentration, 25-hydroxy vitD concentration at visit 4 and visit 7, race, and feeding category.

(breastfeeding and formula-feeding) or exclusive formula-feeding, maternal supplementation with 6,400 IU vitD_3/day was superior to 400 and 2,400 IU/day in safely achieving maternal vitD sufficiency sustained to 7 months postpartum. As viewed by the DSMB overseeing the study, with the exception of the 2,400 IU group having a greater percentage of infants requiring open-label vitD, there were no instances of adverse events attributable to vitD supplementation.

Our findings are supported by other studies. In their observational study, Vieth Streym et al. explored concentrations of vitD_2 and D_3 and 25(OH)D in foremilk and hindmilk during the first 9 months of lactation in 107 women,[12] with improved vitD status in those supplemented but similar safety profiles in the women regardless of supplementation status. Niramitmahapanya et al.,[13] studied 68 lactating women in Thailand who were randomized to placebo versus 1,800 IU vitD_3, and showed a strong correlation between the change in maternal 25(OH)D and maternal milk 25(OH)D that were both correlated with the change in infant 25(OH)D with no significant differences in the safety marker urinary calcium/creatinine ratios at 6 weeks by treatment group, which is consistent with our findings. Saadi et al., studied 90 breastfeeding women in the UAE[14] with marked deficiency states, who were randomly assigned to 2,000 IU vitD_2 daily in oral capsule form ($n=45$) or 60,000 IU monthly (avg 2,000 IU/day) in oral tablet form ($n=45$) for 3 months, with significantly improved vitD status in some but not all, with no safety issues in any of the women.

Czech-Kowalska et al.[15] studied 174 healthy, lactating women and their term infants randomized to receive 1,200 IU/day vitD_3 or 400 IU/day for 6 months while breastfeeding. The infants themselves received 400 IU D_3/day. At baseline and 3 months, vitD status was similar between the groups, but by 6 months, mean 25(OH)D concentration was higher in the 1,200 IU/day group without safety issues. More recently, Dawodu et al. conducted a longitudinal, 6-month clinical trial of 190 mother-infant exclusively breastfeeding pairs living in Doha, Qatar,[16] with a study design similar to this trial: Mothers were randomized to receive either 600 or 6,000 IU vitD_3/day starting at $\sim$4 weeks postpartum. The infants of the mothers in the 600 IU group were randomized to receive 400 IU vitD_3/day while the infants in the 6,400 IU group were randomized to receive placebo. The investigators reported a significant improvement in maternal vitD status of the mothers in the 6,000 IU group with no difference in safety parameters between the two groups. The infants receiving 400 IU/day had a slightly higher mean 25(OH)D at the end of the study, but both groups achieved the threshold 25(OH)D concentration of at least 50 nmol/L in the majority of infants. There was a statistically significant and clinically relevant difference in maternal PTH, with a higher mean PTH in the 600 versus 6,000 IU group.

Additional data surrounding the safety and effectiveness of higher dose vitD supplementation during pregnancy and early lactation come from the work of March et al.,[17] Wall et al.,[18] and Thiele et al.[19] and in our earlier pregnancy and lactation studies,[1,2,11,20,21] where there were no safety issues ascribed to vitD supplementation. In the study by Wall et al.,[18] while 10% of the women participants met their definition of hypercalcemia, there were no statistically significant differences between the groups by treatment and urinary calcium/creatinine ratios also did not differ by treatment.[18]

This study is unique in that it extends the safety of higher dose vitD to those mothers who provide a combination of breast milk and formula to their infants or who exclusively formula feed. The other strengths of this study are the racially diverse patient population at two distinct geographical locations—Charleston, SC and Rochester, NY, and a sample size that is considerably larger than the prior published studies. Thus, the safety of higher dose vitD supplementation during lactation extends to those women who might choose to provide less breast milk and combination breast- and formula feed and to those women who might choose to stop breastfeeding, but continue higher dose vitD supplementation.

A limitation of the study is that the biological significance of vitD repletion via maternal breast milk versus direct infant supplementation is unknown. Future studies identifying the biological significance of maternal vitD repletion and sufficiency and its effect on the immune signature of maternal breast milk versus direct infant supplementation are underway. Another limitation of the study was that the formula-feeding mothers were studied at only one site, but this site was at a lower latitude with potentially higher risk of hypervitaminosis D with both UV exposure and vitD supplementation.

In conclusion, in this study where safety measures were followed during lactation as well as during the postpartum period in nonlactating women, the sustained safety of 6,400 IU vitD_3/day for 6 months was shown. Infants of mothers in the 6,400 IU vitD_3 were equivalent in their vitD status to infants receiving supplementation with 400 IU/day without safety issues.

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Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Disclosure Statement

The authors have no conflicts of interest relevant to this article to disclose.

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Supplmentary Material

Supplmentary Data
Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Table S4A and B

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