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Maternal Vitamin D Supplementation to Improve the Vitamin D Status of Breast-fed Infants: A Randomized Controlled Trial

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Abstract

Objective: To determine whether a single monthly supplement is as effective as a daily maternal supplement in increasing breast milk vitamin D to achieve vitamin D sufficiency in their infants.

Patients and Methods: Forty mothers with exclusively breast-fed infants were randomized to receive oral cholecalciferol (vitamin D_3) 5000 IU/d for 28 days or 150,000 IU once. Maternal serum, breast milk, and urine were collected on days 0, 1, 3, 7, 14, and 28; infant serum was obtained on days 0 and 28. Enrollment occurred between January 7, 2011, and July 29, 2011.

Results: In mothers given daily cholecalciferol, concentrations of serum and breast milk cholecalciferol attained steady levels of 18 and 8 ng/mL, respectively, from day 3 through 28. In mothers given the single dose, serum and breast milk cholecalciferol peaked at 160 and 40 ng/mL, respectively, at day 1 before rapidly declining. Maternal milk and serum cholecalciferol concentrations were related (r=0.87). Infant mean serum 25-hydroxyvitamin D concentration increased from 17±13 to 39±6 ng/mL in the single-dose group and from 16±12 to 39±12 ng/mL in the daily-dose group (*P*=.88). All infants achieved serum 25-hydroxyvitamin D concentrations of more than 20 ng/mL.

Conclusion: Either single-dose or daily-dose cholecalciferol supplementation of mothers provided breast milk concentrations that result in vitamin D sufficiency in breast-fed infants.

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For editorial comment, see page 1350

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Vitamin D is essential for calcium absorption and skeletal growth, and deficiency of vitamin D can cause nutritional rickets. Although considered a historical disease after the advent of vitamin D fortification of foods, rickets persists in the United States, typically in unsupplemented, exclusively breast-fed infants.[1-4] The US Centers for Disease Control and Prevention has expressed concern regarding its prevalence.[5] Beyond skeletal effects, hypovitaminosis D has been associated with infectious, metabolic, neoplastic, and immune disorders.[6-8] The prevalence of vitamin D deficiency among infants may be as high as 43% to 70%, depending on the definition of vitamin D deficiency and the latitude of the population studied.[9-11]

Vitamin D can be ingested or cutaneously synthesized by UV light exposure. Because the American Academy of Pediatrics (AAP) recommends no direct UV light exposure during the first 6 months of life, infants are expected to rely entirely on dietary sources.[12] The US Food and Drug Administration requires that infant formula be fortified with 40 to 100 IU of vitamin D per 100 kcal, which corresponds to 270 to 677 IU/L.[13] Breast-feeding has many health advantages compared with formula feeding.[14] The US Healthy People 2010 targeted a goal for 75% of infants to breast-feed for their first 6 months.[15] Breast milk usually contains much less vitamin D than does infant formula, with values of 20 to 80 IU/L.[16-18] Recognizing the high prevalence of vitamin D deficiency in exclusively breast-fed infants and the low concentrations of vitamin D in breast milk, the AAP recommends that exclusively breast-fed infants receive 400 IU of supplemental vitamin D per day. However, adherence to this recommendation has been poor, with

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only 5% to 36% of exclusively breast-fed infants receiving supplemental vitamin D.[2,19,20] Poor adherence is the major determinant of vitamin D deficiency in breast-fed infants.[2]

Daily and intermittent vitamin D supplementation dosing regimens have been used. High-dose monthly regimens in adults and children improve vitamin D status without short-term toxicity.[21-25] Lactating mothers supplemented with sufficient doses of oral vitamin D had enriched milk vitamin D concentrations.[26-28] The parent compound cholecalciferol (vitamin D_3) is the major vitamin D metabolite that crosses from maternal serum into breast milk. The quantity of the downstream metabolites—25-hydroxyvitamin D (25[OH]D) and 1,25-dihydroxyvitamin D ($1,25[OH]_2D$)—in human milk is negligible because of the avid binding of 25(OH)D to vitamin D-binding protein and the low serum concentrations of $1,25(OH)_2D$.[29] Because of the short half-life of cholecalciferol, it may have to be replenished daily to be effective.[30]

We compared the effect of daily-dose vs single-dose cholecalciferol supplementation of lactating mothers on breast milk cholecalciferol concentrations and vitamin D status of their infants. We hypothesized that daily supplementation would be superior to monthly supplementation in improving infant vitamin D status because consistently elevated cholecalciferol concentrations in the mother with daily dosing would sustain improved breast milk vitamin D status.

PATIENTS AND METHODS

Study Population

Healthy, nonpregnant, lactating women aged 18 years or older who were exclusively breast-feeding a single, healthy infant aged between 1 and 6 months were eligible. We recruited via advertisements and letters to postpartum mothers. Exclusion criteria included (1) travel south of 35°N latitude during or 30 days preceding the study interval; (2) recent or planned indoor tanning; (3) taking medications that affect vitamin D metabolism (ie, steroids, anticonvulsants, and barbiturates), (4) nursing multiple infants, (5) taking more than 1000 mg of elemental calcium supplements, (6) maternal cholecalciferol supplementation more than 400 IU (dose in prenatal vitamins) or any infant cholecalciferol supplementation, (7) infant weight less than 1.67 kg (greater than minimal risk for 5 mL blood draw), (8) baseline 25(OH)D concentration of more than 70 ng/mL (175 nmol/L) in mother or infant, (9) baseline hypercalcemia or hyperphosphatemia in mother or infant, (10) history of nephrolithiasis, and (11) any serious infant health problem. Before this study, we performed a pilot study in 40 nonpregnant, nonlactating women to characterize the pharmacokinetics of daily-dose (5000 IU) and single-dose (150,000 IU) cholecalciferol. We observed no evidence of hypercalcemia or adverse effects over a 28-day interval (unpublished data, M Meekins et al, 2011).

Procedures

Mother-infant pairs were enrolled between January 7, 2011, and July 29, 2011, in Rochester, Minnesota (44°N latitude). Vital signs, weight, and height/length were recorded for mothers and infants. Maternal and infant blood was collected by venipuncture, women collected their breast milk via a breast pump or self-expression, and maternal urine was collected. Because measurements of vitamin D metabolites do not differ between whole milk and milk whey,[16] no attempt was made to distinguish foremilk and hindmilk collection. Infants were allowed to comfort nurse or administered an oral sucrose solution (Sweet-Ease, Philips Healthcare) during venipuncture to reduce distress. Serum calcium, phosphorus, and 25(OH)D concentrations in mothers and their infants were measured before enrollment to determine eligibility.

Participant pairs were randomized in blocks of 4 to maternal administration of oral cholecalciferol either 150,000 IU once or 5000 IU/d for 28 days. The randomization schedule was secured by the research pharmacy, and allocation was concealed until the participant pair was enrolled. One 5000 IU capsule or three 50,000 IU capsules (BioTech Pharmacal, Inc) were dispensed and ingested under supervision. Based on high-performance liquid chromatography analysis of the cholecalciferol content by the manufacturer, the 3 lots of 5000-IU capsules used in this study contained 5404, 5705, and 5764 IU per capsule. The 2 lots of 50,000-IU capsules used in this study contained 55,668 and 58,033 IU per capsule. Mothers in the 5000-IU/d group were dispensed their remaining medication and asked to record adherence in a

medication diary. The diary was examined at each visit and collected with the medication container on study completion. Each participant was instructed to make no dietary changes, avoid additional vitamin D ingestion, and use sunscreen.

Laboratory Measurements

Serum cholecalciferol, 25(OH)D, $1,25(OH)_2D$, calcium, and phosphorus levels were measured in mothers on days 0, 1, 3, 7, 14, and 28 and in their infants on days 0 and 28. Maternal urine calcium and creatinine levels and breast milk cholecalciferol and 25(OH)D levels were measured on days 0, 1, 3, 7, 14, and 28. Biochemical analyses were performed on all samples in a single batch to avoid interassay variation. Serum and urine calcium, phosphorus, and creatinine levels were measured with standard methods. Serum and breast milk vitamin D and its metabolites were measured by using isotope-dilution liquid chromatography–tandem mass spectrometry (ThermoFisher Scientific and Applied Biosystems-MDS Sciex).[31] All assays for $25(OH)D_3$ and $25(OH)D_2$ were standardized against National Institute of Standards and Technology reference material. C3-epi-25(OH)D, a metabolite of uncertain biologic significance, was measured in all children at baseline. Only 2 patients had C3-epi-25OHD levels of greater than 20% of total 25(OH)D levels. Because concentrations of vitamin D_2 and its metabolites were very low ($\leq$1.6 ng/mL [4.16 nmol/L]) or undetectable in all subjects, the absence of subscripts designates cholecalciferol (vitamin D_3) and its metabolites. Cholecalciferol was extracted from breast milk by using 210 μL of isopropyl alcohol. The extract was injected into the mass spectrometer by using online extraction and liquid chromatography. The intra-assay and inter-assay precisions for cholecalciferol were 8.0% and 6.1%, respectively. The recovery and linearity validation parameters were 104% and 100%, respectively. The limit of detection and the limit of quantitation were 0.96 and 7.0 ng/mL, respectively.

Ethics

The study was approved by the Institutional Review Board of Mayo Clinic, and all participants provided written informed consent for themselves and their infants.

Statistical Analyses

The primary outcome was infant vitamin D status, as measured by the serum 25(OH)D concentration. Although there is no universal agreement regarding the definition of sufficiency, we considered deficiency as less than 12 ng/mL (<30 nmol/L), insufficiency as 12 to 20 ng/mL (30-50 nmol/L), and sufficiency as more than 20 ng/mL (>50 nmol/L), which is consistent with the Institute of Medicine's (IOM's) conclusion that levels of more than 20 ng/mL meet the physiologic needs of 97.5% of the healthy population.[32] We calculated our sample size on the basis of the hypothesis that the single-dose group would have fewer days of detectable cholecalciferol in breast milk. Assuming an α value of 0.05 and a power of 80%, 17 subjects per group would be sufficient to detect a single SD difference in the number of days of detectable breast milk cholecalciferol. Assuming the SD of the number of days of detectable breast milk cholecalciferol in each group is 4, we would be able to detect a difference of 4 days between groups. Allowing for a 15% dropout rate, we chose a 40 patient-pair target.

Seasonal effects were examined by comparing patients enrolled in January to March and April to July. Data were handled with Excel 2003 (Microsoft Corp) and analyzed with JMP 9.0.1 software (SAS Institute Inc). A paired *t* test was used to compare normally distributed continuous variables with baseline values within subjects. The Student *t* test was used to compare continuous variables between the 2 treatment groups. The nonparametric Wilcoxon test was used to compare variables with unequal variances. Pearson correlation and multiple linear regression analyses were used to identify independent determinants of vitamin D status. *P* values of less than .05 were considered significant.

RESULTS

A total of 42 mother-infant pairs completed the initial study visit; 2 mother-infant pairs were excluded for maternal hyperphosphatemia (5.2 mg/dL [1.7 nmol/L]) and maternal hypercalcemia (11.0 mg/dL [2.8 mmol/L]). The remaining 40 pairs were randomized, with 20 per group, and all completed the study.

The 2 study groups were similar (Table 1). Infant ages ranged from 4 to 28 weeks at enrollment. Mean ± SD baseline serum 25(OH)D values were 29.0±8.3 ng/mL in mothers (range,

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10-44 ng/mL) and 16.6±12.5 ng/mL in infants (range, 2-55 ng/mL). At baseline, 7 mothers (18%) and 27 infants (68%) had serum 25(OH)D concentrations of less than 20 ng/mL; 1 mother (3%) and 18 infants (45%) had serum 25(OH)D concentrations of less than 12 ng/mL. Baseline 25(OH)D concentrations in mothers and their infants were positively correlated (r=0.40; *P*=.01), with infant concentration being approximately 60% of the maternal concentration. Baseline 25(OH)D concentrations were greater in mothers enrolled in April to July than in January to March (31.2±9.1 ng/mL vs 26.1±6.2 ng/mL; *P*=.05) and in infants (21.3±12.8 ng/mL vs 10.4±9.1 ng/mL; *P*=.005). Likewise, baseline serum cholecalciferol concentrations were greater in mothers enrolled in April to July (5.2±5.3 ng/mL) than in January to March (1.6±1.3 ng/mL; *P*=.004). Mean baseline breast milk cholecalciferol concentrations were below the limit of quantitation of 7 ng/mL. Therefore, mean breast milk cholecalciferol values below the limit of quantitation have been designated as less than 7 ng/mL in Table 2. Baseline breast milk cholecalciferol was related to serum cholecalciferol (r=0.38; *P*=.02). Baseline maternal 25(OH)D values were not related to age (*P*=.08) or body mass index (calculated as the weight in kilograms divided by the height in meters squared) (*P*=.39). Infant 25(OH)D values were unrelated to infant age (*P*=.95), weight (*P*=.80), or gestational age at birth (*P*=.52).

Maternal serum 25(OH)D values increased in both groups from baseline to day 28 (Table 2). The single-dose group had significantly greater maternal 25(OH)D concentrations than did the daily-dose group on days 1, 3, and 7 but not on days 14 and 28 (Figure 1, A); the incremental change in 25(OH)D concentration was significantly greater in the single-dose group on days 1, 3, 7, and 14 but not on day 28. In the single-dose group, maternal 25(OH)D values peaked on day 3 and the maximum value observed in any mother was 72 ng/mL. By day 28, the increase in 25(OH)D value between baseline and day 28 was 11.9±4.2 ng/mL in the single-dose group and 15.0±5.7 ng/mL in the daily-dose group (*P*=.06). None of the mothers' serum 25(OH)D concentrations remained less than 20 ng/mL on day 28 (daily-dose group, 43.9±11.8, range, 22-71 ng/mL; single-dose group, 41.2±8.9, range, 26-60 ng/mL). In the single-dose group, maternal serum cholecalciferol concentrations peaked on day 1 and were greater than baseline values in both groups through day 28 (Figure 1, B). Breast milk cholecalciferol concentrations mirrored serum concentrations, with peak values approximately 25% of serum values on day 1 in the single-dose group (Figure 1, C). Breast milk 25(OH)D was undetectable in all samples. Maternal serum $1,25(OH)_2D$ concentrations remained relatively stable over the 28-day interval (Table 2).

By day 28, serum 25(OH)D concentration had a nearly identical increase in the infants of both groups (Figure 2). The increase in infant 25(OH)D concentration was inversely related to the baseline 25(OH)D concentration (r=−0.67; *P*<.001). By day 28, all infants achieved a serum 25(OH)D concentration of more than 20 ng/mL (range, 23-70 ng/mL). The increase in the infants' 25(OH)D concentration was not related to their mothers' increase in 25(OH)D concentration (r=0.07; *P*=.64). Neither the infants' final value nor the increase in 25(OH)D concentration was related to peak breast milk cholecalciferol values in either group. Assuming that day 14 breast milk cholecalciferol concentration represents an average measure of infant vitamin D intake, we found that infant 25(OH)D values at day 28 were not significantly related

TABLE 1. Baseline Characteristics of Study Patients[a,b]

Characteristic	Daily dose 5000 IU (n=20)	Single dose 150,000 IU (n=20)
Maternal age (y)	30.3±2.9	30.1±4.0
Infant age (wk)	13.7±7.3	11.0±5.6
Infant gestation at birth (wk)	39.9±1.3	39.5±0.9
Maternal race (% white)	95	95
Infant sex (% female)	60	60
Maternal weight (kg)	72.7±10.6	67.6±12.1
Maternal height (cm)	165.6±5.5	163.8±4.2
Maternal BMI (kg/m^2)	26.5±4.0	25.2±4.7
Infant weight (kg)	6.0±3.7	5.7±1.0
Infant length (cm)	61.7±3.7	60.5±4.3
Maternal serum 25(OH)D (ng/mL)[c]	28.8±9.2	29.3±7.5
Infant serum 25(OH)D (ng/mL)[c]	16.9±12.9	16.4±12.4
Enrollment date, No. (%)		
January-March	9 (45)	8 (40)
April-July	11 (55)	12 (60)

[a]25(OH)D = 25-hydroxyvitamin D; BMI = body mass index.
[b]Values are presented as mean ± SD unless indicated otherwise.
[c]To convert to nmol/L, multiply by 2.5.

TABLE 2. Comparison of Metabolic Responses to Daily-Dose or Single-Dose Vitamin D Supplementation Regimens[a,b]

	Vitamin D 5000 IU daily dose (n=20)		Vitamin D 150,000 IU single dose (n=20)	
	Concentration	Increment	Concentration	Increment
Maternal serum				
Cholecalciferol (ng/mL)[b]				
Day 0	2.6±1.4	NA	4.7±6.0	NA
Day 1	10.6±3.8[c]	8.0±3.0[c]	160.0±38.8[c,d]	155.2±37.8[c,d]
Day 3	14.7±3.7[c]	12.1±3.0[c]	57.9±15.7[c,d]	53.1±13.5[c,d]
Day 7	17.2±4.6[c]	14.5±4.0[c]	17.6±7.6[c]	12.9±6.1[c]
Day 14	18.5±6.2[c]	16.0±5.8[c]	9.5±5.1[c,d]	4.8±3.7[c,d]
Day 28	18.3±5.2[c]	15.7±4.9[c]	7.0±6.3[c,d]	2.1±2.9[c,d]
25(OH)D (ng/mL)[b]				
Day 0	28.8±9.2	NA	29.3±7.5	NA
Day 1	30.7±9.7	1.9±3.5[c]	43.3±9.7[c,d]	14.0±3.7[c,d]
Day 3	31.8±8.9[c]	2.7±3.7[c]	50.0±10.2[c,d]	21.2±4.0[c,d]
Day 7	34.3±8.6[c]	5.0 ±3.6[c]	47.5±9.6[c,d]	18.2±4.9[c,d]
Day 14	38.6±10.3[c]	9.7±3.4[c]	45.3±9.5[c]	16.0±4.7[c,d]
Day 28	43.9±11.8[c]	15.0±5.7[c]	41.2±8.9[c]	11.9±4.2[c]
$1,25(OH)_2D$ (pg/mL)[b]				
Day 0	52.0±10.7	NA	60.4±24.9	NA
Day 1	58.6±12.0[c]	6.2±20.2	64.2±17.6	6.0±10.2[c]
Day 3	58.2±12.2	−0.3±22.3	59.6±16.8	7.0±14.0
Day 7	56.8±13.2	0.2±0.6	61.0±9.0	2.2±1.2[c,d]
Day 14	55.0±11.3	6.9±21.4	67.1±15.2[d]	3.9±13.3
Day 28	57.5±12.6	4.0±25.4	65.1±16.9	7.0±14.5
Calcium (mg/dL)[b]				
Day 0	9.6±0.3	NA	9.6±0.4	NA
Day 1	9.6±0.4	−0.01±0.38	9.7±0.4	0.07±0.37
Day 3	9.6±0.4	0.04±0.42	9.7±0.5	0.08±0.36
Day 7	9.6±0.5	0.04±0.43	9.6±0.4	0.01±0.33
Day 14	9.7±0.4	0.13±0.34	9.8±0.5	0.13±0.32
Day 28	9.7±0.4	0.19±0.41	9.7±0.4	0.14±0.39
Phosphorus (mg/dL)[b]				
Day 0	4.1±0.6	NA	4.2±0.5	NA
Day 1	4.2±0.7	0.18±0.48	4.4±0.5	0.17±0.58
Day 3	4.5±0.7[c]	0.45±0.57[c]	4.5±0.5[c]	0.27±0.37[c]
Day 7	4.3±0.6	0.22±0.45[c]	4.3±0.5	0.11±0.47
Day 14	4.3±0.7	0.27±0.51[c]	4.3±0.6	0.10±0.51
Day 28	4.3±0.6	0.23±0.58	4.2±0.4	0.0±0.40
Maternal breast milk				
Cholecalciferol (ng/mL)[b,e]				
Day 0	<7.0	NA	<7.0	NA
Day 1	<7.0	NA	39.7±16.2[c,d]	NA
Day 3	8.0±3.7[c]	NA	24.6±8.9[c,d]	NA
Day 7	7.2 ±4.8	NA	11.2±4.7[c]	NA
Day 14	8.6±5.4[c]	NA	<7.0	NA
Day 28	7.7±3.7[c]	NA	<7.0	NA
Maternal urine calcium/creatinine (mg/g)[b]				
Day 0	72.0±52.6	NA	74.6±46.6	NA
Day 1	72.5±57.2	0.5±47.6	98.4±65.0	23.8±48.9[c]
Day 3	77.8±64.0	5.8±46.9	87.7±77.2	13.1±65.0
Day 7	72.9±62.8	−0.6±58.9	104.9±71.9[c]	30.3±55.8[c]
Day 14	94.2±104.5	22.2±74.2	100.1±44.2[c]	25.5±38.9[c]
Day 28	106.7±98.4[c]	34.7±71.8[c]	114.2±70.3[c]	39.6±71.5[c]

Continued on next page

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TABLE 2. Continued

	Vitamin D 5000 IU daily dose (n=20)		Vitamin D 150,000 IU single dose (n=20)	
	Concentration	Increment	Concentration	Increment
Infant serum				
25(OH)D (ng/mL)				
Day 0	16.9±12.9	NA	16.3±12.4	NA
Day 28	39.2±6.3[c]	22.2±10.6[c]	38.7±11.7[c]	22.4±5.6[c]
Calcium (mg/dL)				
Day 0	11.0±0.3	NA	11.0±0.3	NA
Day 28	10.9±0.3	−0.09±0.39	10.9±0.3	−0.05±0.36
Phosphorus (mg/dL)				
Day 0	6.1±0.6	NA	6.5±0.5	NA
Day 28	6.0±0.5	−0.08±0.41	6.2±0.5	−0.33±0.54[c]

[a]25(OH)D = 25-hydroxyvitamin D; 1,25(OH)$_2$D = 1, 25-dihydroxyvitamin D; NA = not applicable.
[b]To convert values for calcium to mmol/L, multiply by 0.25. To convert values for phosphorus to mmol/L, multiply by 0.32. To convert values for cholecalciferol to nmol/L, multiply by 2.60. To convert values for 25(OH)D to nmol/L, multiply by 2.50. To convert values for 1,25(OH)$_2$D to pmol/L, multiply by 2.40. To convert values for 24,25(OH)$_2$D to nmol/L, multiply by 2.40. To convert values for calcium/creatinine ratio to millimoles/millimoles, multiply by 0.0028.
[c]$P<.05$ for comparison with baseline values.
[d]$P<.05$ for comparison with daily-dose group.
[e]Mean breast milk cholecalciferol values below the limit of quantitation are designated as <7.0 ng/mL.

to day 14 breast milk cholecalciferol concentrations (r=0.29; $P=.07$).

Adherence to supplementation was 100% in the single-dose group and 98% in the daily-dose group. No significant changes in serum calcium values occurred in either group. The highest serum calcium value was 11.1 mg/dL (2.8 mmol/L) on day 28 in the daily-dose group, with a simultaneous urine calcium/creatinine ratio of 48 mg/g (0.13 mmol/mmol). Compared with baseline values, urinary calcium excretion significantly increased by day 28 in the daily-dose group and by day 7 in the single-dose group, with the incremental change being significant compared with baseline on day 1 as well in the single-dose group. Urine calcium/creatinine ratios greater than the upper limit of the accepted reference range (220 mg/g) were observed in 4 mothers in the daily-dose group and in 3 mothers in the single-dose group. The maximum urine calcium/creatinine ratio of 441 mg/g occurred in the daily-dose group at day 14. Three mothers had urine calcium/creatinine ratios of more than 220 mg/g on day 28. Urine calcium/creatinine ratios were unrelated to serum 1,25(OH)$_2$D values (r=−0.10). Adverse events during the study included upper respiratory tract infections (4), diarrhea (3), headache (3), and cellulitis (1) in mothers and bronchiolitis (1) and altered stool pattern (1) in infants. None of these events was attributed to vitamin D.

DISCUSSION

Contrary to our hypothesis, we found that either daily-dose or single-dose cholecalciferol maternal supplementation provides breast milk concentrations that result in infant 25(OH)D concentrations of more than 20 ng/mL over a 28-day period. We observed a mild increase in urinary calcium excretion but no hypercalcemia or adverse symptoms attributable to vitamin D.

We confirmed a high prevalence of poor vitamin D status in unsupplemented, breast-fed infants, with two-thirds of the infants having 25(OH)D concentrations of less than 20 ng/mL at baseline. Alarmingly, 45% had serum 25(OH)D concentrations of less than 12 ng/mL, putting them at risk for nutritional rickets.[8] In neighboring Iowa, 70% of exclusively breast-fed infants at age 3.5 months had 25(OH)D concentrations of less than 11 ng/mL during winter.[11] Although there is no universally agreed upon definition of sufficiency, the IOM has concluded that levels of more than 20 ng/mL meet the physiologic needs of 97.5% of the healthy population.[32]

Our study confirms the beneficial effect of maternal cholecalciferol supplementation on the vitamin D status of breast-fed infants. Milk from daily supplemented mothers can provide

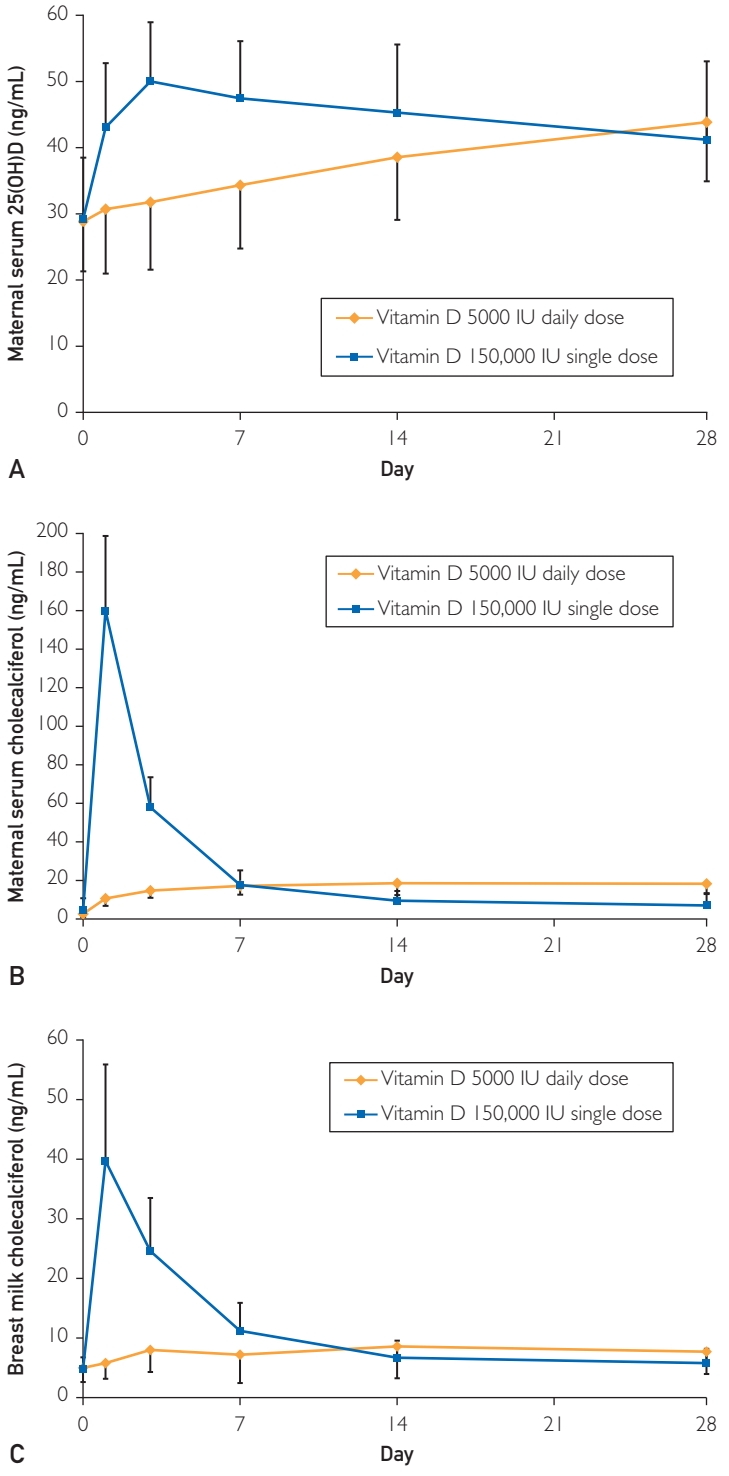

FIGURE 1. Maternal vitamin D metabolite values with 2 vitamin D supplementation regimens: serum 25-hydroxyvitamin D (25[OH]D) (A), serum cholecalciferol (B), and breast milk cholecalciferol (C). Error bars represent SD.

infants with sufficient vitamin D if the cholecalciferol dose is high enough.[26-28,33] In Finland, daily supplementation of lactating mothers with cholecalciferol 2000 IU, but not 1000 IU, improved infant vitamin D status to values similar to those obtained by daily infant supplementation with 400 IU.[32] Supplementation of 9 lactating mothers with cholecalciferol at 6400 IU/d for 6 months resulted in infant 25(OH)D concentrations of 36 and 46 ng/mL after 1 and 6 months, respectively, similar to values in infants supplemented with 400 IU/d.[28] Maternal 25(OH)D concentrations rose from 34 ng/mL at baseline to 47 and 59 ng/mL at 1 and 6 months, respectively—slightly higher than values we observed with 5000 IU/d or values obtained in prior 4000 IU/d studies.[26,27] No adverse effects were reported, and maternal urinary calcium excretion was similar to that of mothers taking a standard prenatal vitamin (cholecalciferol 400 IU) over 6 months.

We extend these findings by demonstrating that a single dose of cholecalciferol in lactating mothers is as effective as daily dosing for improving the vitamin D status of their breast-fed infants. Currently, adherence to vitamin D supplementation of breast-fed infants is poor.[20] As a public health measure, intermittent cholecalciferol supplementation could theoretically be administered to lactating mothers during well child visits to improve adherence.[34] This supplementation strategy could prove particularly useful in countries where nutritional rickets and maternal vitamin D deficiency is prevalent. However, the optimal interval of intermittent doses needed to ensure adequate cholecalciferol concentrations in mothers' breast milk to maintain sufficiency in their infants is unknown.

Breast milk cholecalciferol concentrations of 8 ng/mL (the mean 28-day value in the daily-dose group) correspond to 320 IU/L (1 μg = 40 IU) and approximate the 400 IU/L infant formula target. Previous reports of "antirachitic activity" of breast milk were attributed to the contribution of both cholecalciferol and 25(OH)D in breast milk.[17,35,36] However, by using tandem mass spectrometry, we did not detect 25(OH)D in breast milk. Previous studies of breast milk 25(OH)D concentrations have reported values below 1 ng/mL, which was below the lower limit of detection of our assay. Such low concentrations of 25(OH)D are unlikely to have added an important

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amount of antirachitic activity to the cholecalciferol that was measurable in the breast milk.

The breast milk antirachitic activity of mothers supplemented with 400 to 800 IU/d has been reported as 33 to 68,[37] 47 to 50,[17] and 38±11 IU/L,[26] insufficient to meet the needs of exclusively breast-fed infants. The antirachitic activity of milk from 9 mothers receiving ergocalciferol (vitamin D_2) at 4000 IU/d for 3 months increased to 135 IU/L, which is less than half of the value that we observed with 5000 IU/d, possibly related to our use of cholecalciferol (not ergocalciferol).[26] Nine lactating mothers supplemented with cholecalciferol at 6400 IU/d for 6 months had mean antirachitic activity of 873 IU/L in breast milk.[28] Regulated conversion of cholecalciferol to 25(OH)D likely provides a built-in safety measure. This is consistent with our observation of an inverse relationship between the increase in 25(OH)D concentration and the initial 25(OH)D concentration in infants.

During the 28-day study interval, both cholecalciferol-dosing regimens appeared safe. However, the mild increase in renal calcium excretion raises concern for an increased risk of nephrolithiasis. The mechanism for the increased calcium excretion is unclear. Parathyroid hormone–related peptide produced by the lactating breast and low estradiol concentrations contribute to mobilization of calcium from bone.[29,38] Calcium lost in breast milk also contributes to a negative calcium balance during lactation that is not prevented by calcium supplementation.[39] We did not observe a significant increase in $1,25(OH)_2D$ concentrations to suggest that cholecalciferol increased intestinal calcium absorption, and urinary calcium excretion was unrelated to $1,25(OH)_2D$ concentrations. In a large population study, nephrolithiasis was not associated with serum 25(OH)D values.[40]

In 2011, the IOM updated vitamin D supplementation guidelines. Adequate intake for infants younger than 1 year was set at 400 IU/d, in line with the AAP recommendations and the goals of supplementation. The recommended dietary allowance for adults, including lactating mothers, was listed at 600 IU/d. The doses of vitamin D used in this study were selected to adequately fortify the mother's milk to meet the requirement of the breast-fed infant and were not intended for long-term supplementation beyond the period of lactation. The IOM listed a tolerable upper intake level of 4000 IU/d and 10,000 IU/d as the "no observed adverse effect level" with no reports of adverse events for supplementation regimens below this dosage.[32]

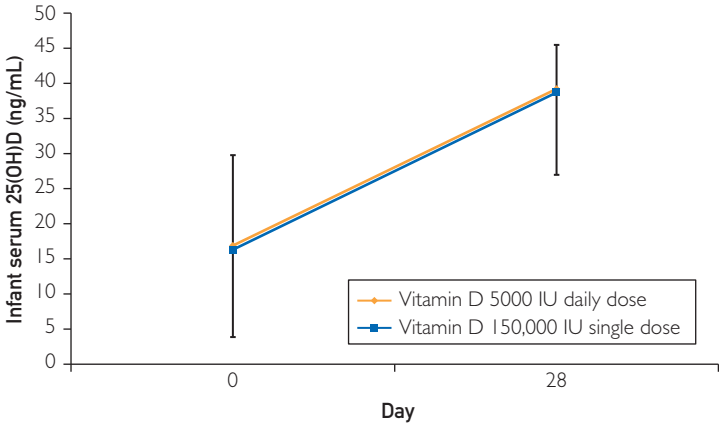

FIGURE 2. 25-Hydroxyvitamin D (25[OH]D) concentrations in breast-fed infants in response to 2 vitamin D supplementation regimens of their mothers. Error bars represent SD. The upper error bars are the SD for the 5000 IU/d group, and the lower error bars are the SD for the 150,000 IU single-dose group.

Our study has several limitations. Although we attempted to minimize UV light exposure, we cannot exclude endogenous vitamin D production. We did not measure the dietary contribution of vitamin D intake, but this should be small compared with supplement doses. We did not include a control group with no maternal cholecalciferol supplementation, because our aim was to determine whether daily supplementation was more advantageous than a single dose. The limited duration of the study does not allow us to determine the effect of continued cholecalciferol supplementation beyond 28 days or to document long-term safety. Continuous cholecalciferol supplementation with up to 11,000 IU/d produces a stable serum cholecalciferol concentration in approximately 3 weeks.[41] However, cholecalciferol supplementation of lactating mothers with 6400 IU/d produced a continued upward trend in breast milk antirachitic activity at 6 months, but the increase in infant 25(OH)D concentrations did not differ from that of infants supplemented with cholecalciferol at 300 IU/d.[28] Further research is needed to determine how frequently intermittent doses should be administered to prevent decreases in breast milk cholecalciferol concentrations below levels necessary to maintain adequate 25(OH)D in infants.

CONCLUSION

Either single-dose or daily-dose maternal cholecalciferol can provide breast milk concentrations that result in vitamin D sufficiency in their infants. Larger trials documenting the safety of cholecalciferol supplementation in lactating mothers need to be conducted before universally adopting this strategy for preventing vitamin D deficiency in infants.

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Abbreviations and Acronyms: **AAP** = American Academy of Pediatrics; **IOM** = Institute of Medicine; **25(OH)D** = 25-hydroxyvitamin D; **1,25(OH)$_2$D** = 1,25-dihydroxyvitamin D

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