

The Effect of High-Dose Vitamin D Supplementation on Serum Vitamin D Levels and Milk Calcium Concentration in Lactating Women and Their Infants

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ABSTRACT

Objective: Improve vitamin D status in lactating women and their recipient infants, and measure breast milk calcium concentration [Ca] as a function of vitamin D regimen.

Design/Methods: Fully breastfeeding mothers were randomized at 1 month postpartum to 2000 ($n = 12$) or 4000 ($n = 13$) IU/day vitamin D for 3 months to achieve optimal vitamin D status [serum 25(OH)D ≥ 32 ng/mL (80 nmol/L)]. Breast milk [Ca], maternal and infant serum 25(OH)D and serum Ca, and maternal urinary Ca/Cr ratio were measured monthly.

Results: Mothers were similar between groups for age, race, gestation, and birth weight. 25(OH)D increased from 1 to 4 months in both groups (mean \pm SD): $+11.5 \pm 2.3$ ng/mL for group 2000 ($p = 0.002$) and $+14.4 \pm 3.0$ ng/mL for group 4000 ($p = 0.0008$). The 4000 IU/day regimen was more effective in raising both maternal and infant serum levels and breast milk antirachitic activity than the 2000 IU/day regimen. Breast milk [Ca] fell with continued lactation through 4 months in the 2000 and 4000 IU groups. Decline in breast milk [Ca] was not associated with vitamin D dose ($p = 0.73$) or maternal 25(OH)D ($p = 0.94$). No mother or infant experienced vitamin D-related adverse events, and all laboratory parameters remained in the normal range.

Conclusion: High-dose vitamin D was effective in increasing 25(OH)D levels in fully breastfeeding mothers to optimal levels without evidence of toxicity. Breast milk [Ca] and its decline in both groups during 1 to 4 months were independent of maternal vitamin D status and regimen. Both the mother and her infant attained improved vitamin D status and maintained normal [Ca].

INTRODUCTION

VITAMIN D DEFICIENCY in infants and children, specifically nutritional rickets, was thought to be disappearing.¹ However, hypovitaminosis D is more prevalent than suspected in both children and adults.²⁻⁹ Hypovi-

taminosis D occurs because sun exposure is extremely limited for both mothers and infants and dietary supplementation at the current adequate intake (AI) of 400 IU/d is inconsequential. Vitamin D levels also vary according to race and degree of pigmentation, season, and latitude.¹⁰⁻¹³

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Vitamin D₃ is primarily derived from a photosynthetic mechanism in skin. This cutaneous generation of vitamin D₃ begins when UVB (UVB) range of the spectrum 290 to 315 nm reaches the skin converting 7-dehydrocholesterol (7-DHC) to previtamin D₃, which is transformed to vitamin D₃ by thermally induced isomerization.^{14–18}

Vitamin D₃ is poorly distributed in natural foodstuffs and is primarily found in oily fish such as salmon, sardines and mackerel, fish oils, egg yolks, butter, and liver.¹⁹ Food sources, most commonly milk, orange juice, cereals, and breads, often are fortified with low concentrations of vitamin D. Additional sources of vitamin D come from vitamin supplementation and plant sterol ergosterol (D₂).

Once vitamin D enters the circulation, either through epidermal transfer or intestinal absorption, it associates with vitamin D-binding protein (DBP), a 58-kDa globular protein that binds vitamin D or its metabolites with various affinities based on the number and position of polar functional groups and/or methyl groups.²⁰ The initial step in the metabolic activation of vitamin D is the enzyme-catalyzed insertion of an OH group at carbon 25. This oxidation is primarily an hepatic microsomal function²¹ and produces 25(OH)D, the most abundant circulating form of vitamin D.^{22,23} Because of its relatively long $t_{1/2}$ as compared with vitamin D (1 to 2 days) and 1,25(OH)₂D₃ (12 to 24 hours), circulating 25(OH)D₃ is the best indicator of nutritional vitamin D status.²⁴ The primary site of systemic regulation of vitamin D metabolism is the kidney. 1,25(OH)₂D₃ and 24,25-dihydroxyvitamin D₃ are produced by cytochrome P₄₅₀-mixed function oxidases in the mitochondria of the proximal tubules.²⁵ 1,25(OH)₂D₃ is the most active, and thus, the hormonal form of vitamin D. Its mechanisms of action include increasing intestinal calcium absorption in the small intestine, increasing urinary calcium reabsorption, and increasing bone mineralization.

Evidence from randomized controlled trials strongly suggests that 25(OH)D doses exceeding 1000 IU vitamin D/day (preferably 2000 to 10,000 IU/day) are required to achieve a robust normal concentration of circulating 25(OH)D.^{26–28} These are much higher levels than the current 400 IU/day AI for adults. His-

torically, circulating 25(OH)D levels in the range of 18 to 80 ng/mL were considered normal.²⁹ In recent years, investigators have turned to the use of biomarkers or functional endpoints to more clearly define adequacy of circulating 25(OH)D levels with respect to calcium homeostatic function of vitamin D. These biomarkers include the functional indicators parathyroid hormone (PTH), calcium absorption, and bone mineral density (BMD).^{30–37} A significant inverse relationship exists between circulating 25(OH)D and PTH; with secondary hyperparathyroidism as a key hallmark of poor nutritional vitamin D status in the elderly.^{34–36} Secondary hyperparathyroidism in this population may subside when circulating 25(OH)D levels reach ≈ 32 ng/mL (80 nmol/L).³⁴ Likewise, when circulating 25(OH)D drops below 32 ng/mL, calcium absorption is impaired.³² Similarly, circulating levels of 25(OH)D of at least 32 ng/mL are required to optimize BMD.³⁷ Thus, based on the preceding discussion, the lower end of normal circulating vitamin D levels should be ≥ 32 ng/mL (80 nmol).^{30,31}

Manifestations of vitamin D deficiency include rickets, osteomalacia, and osteoporosis. New evidence points to chronic vitamin D deprivation and increased risks for malignancies, myopathies, depression, falls in the elderly, and immune dysfunction/autoimmune disease (diabetes type 1, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis).^{37–47}

Lactating women often are vitamin D deficient, resulting in low vitamin D concentrations in breast milk and subsequently in their infants. Marginal vitamin D status of mothers and breastfeeding infants even in sunny climates is underscored in the authors' recent data.⁴⁸ Hypovitaminosis D in the breastfed infant also is a severe problem in sun-rich environments such as the Middle East because of extremely limited sun exposure and dietary supplementation.⁴⁹

The antirachitic content of human milk is quite variable and is affected by season, maternal vitamin D intake, and race. In the 1980s antirachitic activity of human milk from mothers receiving 400 IU vitamin D/day was defined with sensitive assay technology to be 20

to 70 IU/L.^{50–52} Further, almost all the activity was attributable to vitamin D and 25(OH)D. These studies also demonstrated that dietary maternal vitamin D supplementation and ultraviolet (UV) light exposure increased the vitamin D content of human milk.^{51,53,54}

Cancela et al.⁵⁵ have reported that circulating 25(OH)D levels in breastfed infants are directly related to the vitamin D content of mother's milk. Available evidence indicates that if the vitamin D status of the lactating mother is adequate, her nursing infant will maintain a "minimally normal" nutritional vitamin D status.^{54,56–58}

The calcium concentration ([Ca]) in human milk normally decreases during the first 3 months of lactation.^{59,60} There is concern that high-dose vitamin D supplementation will increase serum and milk [Ca]. Hypervitaminosis D is characterized by hypercalcemia, hypercalciuria, and extraskeletal calcifications. However, there have been no cases of hypercalcemia or hypercalciuria in studies supplementing with 1000 to 10,000 IU of vitamin D/day.^{26,27,56,57,61} An opposite concern was raised when, in an earlier reported study, it was found that with increased vitamin D dose to lactating mothers, there was less than predicted BMD loss.⁶² Do mothers who are replete in vitamin D, who are found to have less than predicted BMD loss, have lower breast milk [Ca] because of inhibition of calcium mobilization from bone?

The objectives of this study were to: (a) improve vitamin D status in lactating women and their recipient infants; and (b) measure breast milk [Ca] as a function of vitamin D supplementation regimen. The authors' hypothesis states that [Ca] in human milk is independent of maternal vitamin D supplementation and status when achieving optimal vitamin D status in the mother.

MATERIALS AND METHODS

Subjects

Lactating mothers, within 1 month after birth, were recruited for inclusion in the study if they planned to fully breastfeed for the next 3 months. The subjects were randomly divided

into two groups. Exclusion criteria included preexisting type 1 or 2 diabetes mellitus, hypertension, parathyroid disease, and uncontrolled thyroid disease. The project was approved by the Medical University of South Carolina's Institutional Review Board (IRB) for Human Subjects (HR#10424). All subjects gave written informed consent and received gift cards after each visit.

Study design

This was a prospective, double-blinded, randomized controlled trial of high-dose vitamin D supplementation of fully breastfeeding mothers (detailed in a previously published manuscript).⁴⁸ Randomization was performed using the SAS Proc Plan (SAS Institute, Cary, NC). Individuals were randomly assigned to one of two groups balanced by ethnicity. Each subject served as her own control; the vitamin D status at 1 month was compared with values at three additional time points. Mothers were randomized to receive either 2000 or 4000 IU vitamin D supplementation per day. Groups 1 and 2 received 1600 and 3600 IU per day vitamin D₂, respectively, in an oral suspension. Both groups received additional multivitamin capsules containing 400 IU vitamin D₃ and were instructed to take them daily. Subjects also were instructed to limit sun exposure (for mother and infant). Furthermore, mothers were instructed to fully breastfeed, and thus, to limit formula intake by the infant. Detailed daily activity, sun exposure, and dietary intake (infant and maternal) diaries were kept for each mother/infant pair.

Blood, urine, and milk samples were obtained from the mothers at months 1, 2, 3, and 4 of lactation. Infant blood was collected at months 1 and 4 (beginning and end of the study). Maternal serum was monitored for total calcium and 25(OH)D concentrations. Infant serum was monitored for 25(OH)D, total calcium and phosphorus concentrations. The calcium/creatinine ratio of the mother's urine was monitored.

Vitamin D₂ was used as a specific tracking agent for maternal dosing because the contribution of vitamin D₂ from other sources would be unlikely or minimal. By using vitamin D₂ in

this study, the increase in and/or transfer of vitamin D compounds from the mother to her infant could be precisely defined without confounding factors such as extradietary intake and sun exposure.⁴⁸ Detailed vitamin D analyses were previously published on the preliminary pilot study data.⁴⁸

Laboratory measurements

Total blood calcium and urinary calcium and creatinine concentrations were determined in the General Clinical Research Center at the Medical University of South Carolina. Circulating and milk concentrations of vitamin D₂, vitamin D₃, 25(OH)D₂, and 25(OH)D₃ were determined with high-powered liquid chromatography and radioimmunoassay techniques, as described previously.^{23,48,50,63} Human milk [Ca] as a function of maternal vitamin D status and dose (2000 versus 4000 IU/day) was measured monthly by atomic absorption spectroscopy.⁶⁴

Statistical methods

Groups 1 and 2 were compared at entrance into the study for determination of potential

differences with respect to sociodemographic and baseline clinical characteristics. The main variables of interest were maternal and infant serum 25(OH)D and calcium concentrations, breast milk [Ca], and maternal urinary Ca/Cr ratio with time (in months). Statistical analyses were performed using SAS software with student's *t*-test, chi-square test, and one-way analysis of variance methods. Significance was set at $p < 0.05$ *a priori*.

RESULTS

A convenience sample of 64 lactating women was enrolled into the study, of whom 29 had stopped breastfeeding by the first visit. Thirty-five (35) women came for the first visit; of those, 25 women continued to fully breastfeed throughout the study period. This sample of 25 women (16 white and 9 African-American women), 12 in the 2000 IU vitamin D/day group and 13 in the 4000 IU vitamin D/day group, completed the 3-month study period. There were five African-American women in group 1 (2000 IU vitamin D/day) and four in group 2 (4000 IU vitamin

TABLE 1. CHARACTERISTICS AND CLINICAL PARAMETERS OF THE 2000 IU/DAY VITAMIN D GROUP VERSUS THE 4000 IU/DAY VITAMIN D GROUP

Characteristics and clinical parameters	Gr 2000 IU/d (n = 12)	Gr 4000 IU/d (n = 13)	p-Value
Maternal age (yrs)	30.6 ± 4.6	29.6 ± 4.9	0.6
Maternal race			
African American	5 (41.7%)	4 (30.8%)	0.6
White	7 (58.3%)	9 (69.2%)	
Birth weight (g)	3464 ± 663	3457 ± 522	0.9
Range	(2315–4765)	(2570–4165)	
Gestational age (weeks)	38.8 ± 1.8	38.8 ± 1.6	0.9
Maternal circulating total 25(OH)D (mg/dL)	0.002*	0.0008*	0.03**
Baseline	22.4 ± 8.8	28.5 ± 8.6	
After 3-month supplementation	33.9 ± 6.5	43.0 ± 11.6	
Infant circulating total 25(OH)D (mg/dL)	0.001*	0.001*	0.01**
Baseline	7.8 ± 1.1	13.4 ± 3.3	
After 3-month supplementation	27.8 ± 3.9	30.8 ± 5.0	
Episodes of hypercalcemia			
Measured monthly × 4			
Mother	0/48	0/52	
Infant	0/48	0/52	
Episodes of hypercalciuria			
Measured monthly × 4			
Mother	0/48	0/52	

*Change in serum 25(OH)D concentrations from baseline to 3 months of vitamin D supplementation (within group comparison).

**Difference in rise of serum 25(OH)D between the 2000 IU/day vitamin D group and the 4000 IU/day vitamin D group (between-group comparison).

D/day). The groups did not differ according to maternal age, race, birth weight, gestational age (Table 1), supplemental formula (minimal), or sun exposure (data not shown). All data points were obtained except for serum values in one infant whose mother refused blood draws.

Mean maternal and infant total circulating 25(OH)D levels had statistically significant increases during the 3 months of high-dose vitamin D supplementation. The increases in circulating 25(OH)D concentrations for mothers and infants at baseline and after 3 months of nursing from mothers receiving 2000 or 4000 IU/day vitamin D also are presented in Table 1. There was a significant interaction of vitamin D concentrations with time in both groups. Mothers in group 1 (who received 1600 IU/day vitamin D₂ and 400 IU/day vitamin D₃) exhibited increases in total circulating concentrations of 25(OH)D from baseline to 3 months ($p = 0.002$). In group 2 (mothers who received 3600 IU/day vitamin D₂ and 400 IU/day vitamin D₃), total circulating 25(OH)D concentrations also increased significantly during the 3 months of supplementation ($p = 0.0008$). Compared with mothers in the 2000 IU group, mothers in the 4000 IU group exhibited higher 25(OH)D concentrations at the end of the study period ($p = 0.03$).

As with the mothers, there was a significant increase of vitamin D concentrations with time for both infant groups. Compared with infants in the 2000 IU group, infants in the 4000 IU group exhibited higher 25(OH)D concentrations at the end of the study period ($p = 0.01$).

Dietary intakes of vitamin D up to 10 times the recommended intake for lactating women for a period of 3 months resulted in no adverse events associated with vitamin D. Maternal and infant serum calcium concentrations all remained in the normal range. There were no episodes of maternal hypercalciuria. This is not surprising, because circulating vitamin D and 25(OH)D concentrations never exceeded normal levels.

Breast milk [Ca] (mg/dL) in lactating women during vitamin D supplementation over the 3-month study period is presented in Figure 1. Mean breast milk [Ca] in mothers supplemented with 2000 and 4000 IU/day vitamin D

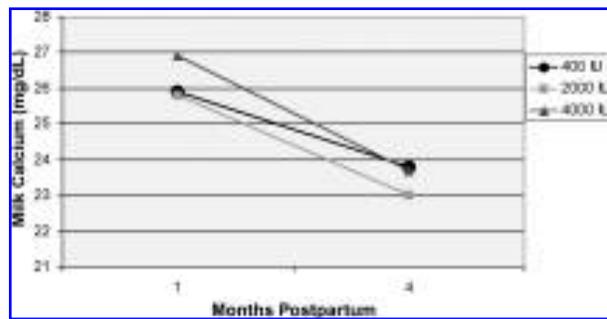


FIG. 1. Milk calcium concentration in lactating women during vitamin D supplementation.

were plotted, as were the normative data for breast milk [Ca] in historical controls (400 IU/day vitamin D).⁵⁹ Similar to previous reports,⁵⁹ [Ca] declined in both groups during 1 to 4 months ($p = 0.001$). The decline in breast milk [Ca] was not associated with the vitamin D dose ($p = 0.73$) or maternal 25(OH)D concentration ($p = 0.94$). No differences were seen among the three groups. As stated, no mother or infant experienced vitamin D-related adverse events and all laboratory parameters remained in the normal range.

DISCUSSION

Recent reports demonstrating an increased prevalence of rickets in infancy have focused renewed attention on the vitamin D status of infants and their mothers.^{1-4,65,66} Avoidance of sun and the inadequate AI value for vitamin D among lactating women are largely responsible. The lack of solar exposure causes a disruption in an important endocrine pathway that has evolved over millions of years: solar driven production of vitamin D₃ in skin. Fair-skinned individuals can generate $\approx 20,000$ IU of vitamin D₃ 24 hours after just 10 to 15 minutes of total body exposure to sunlight.^{12,67} Darkly pigmented individuals are at greatest risk given that they require many times the exposure to generate vitamin D₃ in the skin.⁶⁸

The dietary recommended intake for vitamin D in adults is inadequate and needs to be revised. The AI was arbitrarily set based on approximately what is found in one teaspoon of cod liver oil. Interestingly, the AI of 400 IU of vitamin D per day is the same for a 3.5-kg in-

fant, a 15-kg child, and a 90-kg adult as well as for pregnant and lactating women. Using a vitamin D intake regression model, Heaney et al.²⁷ estimate a 400 IU/day intake would increase maternal circulating 25(OH)D concentrations by only 2.8 ng/mL after 5 months of supplementation.

This study demonstrated that high-dose vitamin D supplementation was effective in raising the 25(OH)D levels in fully breastfeeding mothers to optimal levels (≥ 32 ng/mL) without evidence of toxicity. While maintaining normal serum calcium concentrations, both mother and infant attained improved vitamin D status. In addition, the concentration of calcium in the breast milk of mothers receiving high-dose vitamin D supplementation did not differ from historic controls, with a gradual decline noted over the 3-month study period.

The authors have been able to identify only three prospective studies that examined vitamin D supplementation during lactation.^{56–58} Greer and Marshall⁵⁸ found that exclusively breastfed white infants nursed during the winter in a northern climate maintained a “minimally normal” vitamin D status for a period of 6 months. However, circulating 25(OH)D concentrations did decline despite a maternal intake of ≈ 700 IU/day. A Finnish study showed that maternal supplementation with 1000 IU/day vitamin D resulted in a “minimal” increase in circulating 25(OH)D concentrations among nursing infants.⁵⁶ A similar study was later performed by the same investigators using 2000 IU/day maternal supplementation and found that nursing infants’ vitamin D status improved significantly.⁵⁷

In the present study, similar to previous reports,^{59,60} it was demonstrated that the calcium concentration in breast milk declines with advancing lactation duration through 4 months. Calcium concentration and its overall decline in breast milk samples over time were independent of maternal vitamin D status and regimen. Calcium appears to be very tightly controlled. Regardless of low or high calcium intakes, studies indicate that calcium absorption does not appear to increase during lactation.^{69–73} Rather, during lactation, bone resorption and increased urinary reabsorption are thought to be responsible for providing calcium in breast milk.^{72–76}

Calcium homeostasis is independent of maternal calcium intake. The process has been attributed to hormonal changes during and after lactation. Hypoestrogenemia and amenorrhea resulting from suppression of the hypothalamic-pituitary-gonadal axis while lactating results in bone resorption.⁷¹ Once breastfeeding ceases or menstruation resumes, 1,25(OH)₂D levels rise.^{69,71,76,77} However, in studies that have looked at breast milk calcium, maternal vitamin D status was not factored into the analyses. In lactating women, it remains unknown how maternal calcium gut absorption and urinary resorption vary as a function of maternal vitamin D status.

Because the main source of calcium into breast milk seems to be from increased maternal bone resorption, concerns arose with the findings that women receiving high-dose vitamin D supplementation had less than predicted bone mineral density loss.⁶² However, instead of lower than normal calcium concentrations in breast milk, the calcium concentrations in breast milk from mothers supplemented with high-dose vitamin D had a decline similar to that historically reported in women receiving the current AI of 400 IU vitamin D/day. Thus, mothers had improved BMD while giving the same amount of calcium to their infants. One might speculate that the maintained bone mineral density was a result of improved efficiency of calcium absorption from the gut and enhanced urinary calcium resorption. Further testing is necessary to determine the mechanism of action of preserved bone mineral content of lactating mothers who have achieved optimal vitamin D status.

CONCLUSION

In conclusion, high-dose vitamin D was effective in increasing the total 25(OH)D levels in fully breastfeeding mothers to optimal levels (≥ 32 ng/mL) without evidence of toxicity. The current AI of 400 IU/day of vitamin D for lactating mothers is inadequate for the vitamin D nutritional status of mothers and nursing infants. Maternal vitamin D intakes ≥ 4000 IU/day appear to be safe and provide sufficient vitamin D to ensure adequate nutritional vitamin D status

for both mother and nursing infants. Calcium concentration and its overall decline in milk samples over time were independent of maternal vitamin D status and regimen and were similar to previously reported normative data. Both mother and the recipient infant attained improved vitamin D status and maintained normal serum calcium levels without increased calcium loss from mother into her milk.

Ongoing studies are underway to compare vitamin D status in lactating women and their infants during supplementation of the mothers with the AI of 400 IU/day vitamin D to those of higher doses (6400 IU/day) of vitamin D supplementation. The calcium concentration in breast milk, maternal serum, and infant serum are being studied during vitamin D supplementation to evaluate calcium delivery to the breastfed infant. Studies to measure intestinal calcium absorption and the characteristics of breast milk with respect to calcium content must be undertaken during high-dose vitamin D supplementation to ensure that calcium delivery to the breastfed infant is preserved.

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