Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant[1–4]

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ABSTRACT

Scientific data pertaining to vitamin D supplementation during lactation are scarce. The daily recommended intake for vitamin D during lactation has been arbitrarily set at 400 IU/d (10 μg/d). This recommendation is irrelevant with respect to maintaining the nutritional vitamin D status of mothers and nursing infants, especially among darkly pigmented individuals. Our objective was to examine the effect of high-dose maternal vitamin D_2 supplementation on the nutritional vitamin D status of mothers and nursing infants. Fully lactating women ($n = 18$) were enrolled at 1 mo after birth to 1 of 2 treatment arms, ie, 1600 IU vitamin D_2 and 400 IU vitamin D_3 (prenatal vitamin) or 3600 IU vitamin D_2 and 400 IU vitamin D_3, for a 3-mo study period. High-dose (1600 or 3600 IU/d) vitamin D_2 supplementation for a period of 3 mo safely increased circulating 25-hydroxyvitamin D [25(OH)D] concentrations for both groups. The antirachitic activity of milk from mothers receiving 2000 IU/d vitamin D increased by 34.2 IU/L, on average, whereas the activity in the 4000 IU/d group increased by 94.2 IU/L. Nursing infant circulating 25(OH)D_2 concentrations reflected maternal intake and the amount contained in the milk. With limited sun exposure, an intake of 400 IU/d vitamin D would not sustain circulating 25(OH)D concentrations and thus would supply only limited amounts of vitamin D to nursing infants in breast milk. A maternal intake of 2000 IU/d vitamin D would elevate circulating 25(OH)D concentrations for both mothers and nursing infants, albeit with limited capacity, especially with respect to nursing infants. A maternal intake of 4000 IU/d could achieve substantial progress toward improving both maternal and neonatal nutritional vitamin D status. *Am J Clin Nutr* 2004;80(suppl):1752S–8S.

KEY WORDS Vitamin D, human milk, lactation, 25-hydroxyvitamin D

INTRODUCTION

Thirty-five years ago, nutritional rickets was thought to be disappearing (1). Many reports since then, however, indicated that this is not the case (2–5). Most cases reported in the past decade involved darkly pigmented infants who had been breast-fed exclusively. Hypovitaminosis D among breast-fed infants also is a severe problem in sunlight-rich environments such as the Middle East (6). This hypovitaminosis D occurs because sun exposure is extremely limited for both mothers and infants and dietary supplementation at the current daily recommended intake (DRI) of 400 IU/d is inconsequential.

In the past, human milk was thought to be an adequate source of antirachitic activity for neonates and growing infants. Even before the discovery of vitamin D, McCollum et al (7) and Park (8) stated that rickets was attributable to the lack of sunlight and a dietary factor X. They observed that factor X was found in "good breast milk" and cod liver oil and that, although rickets did develop among breast-fed children, it was rarely as severe as that among artificially fed infants. Those investigators did not know that the source of vitamin D in the mother's milk was the mother's exposure to the sun, which cutaneously generated large amounts of vitamin D. Early attempts to quantify the antirachitic potential in human milk were crude and yielded little information.

In the 1980s, the antirachitic activity of human milk was defined with sensitive assays to be 20–70 IU/L (9, 10). Furthermore, almost all of the activity was attributable to vitamin D and 25-hydroxyvitamin D [25(OH)D]. Those studies also demonstrated that dietary maternal vitamin D supplementation and ultraviolet light exposure increased the vitamin D content of human milk (11, 12). Specker et al (13) determined that the antirachitic content of human milk was lower among African American than white mothers. If a lactating mother has limited sun exposure and/or limited vitamin D intake (such as occurs with the current 400 IU DRI), then the vitamin D content of her milk is poor, especially if she has darker pigmentation.

Studies among normal adults demonstrated that oral vitamin D intakes of up to 10 000 IU/d for a period of 5 mo are safe, with no evidence of toxicity (14, 15). We hypothesized that vitamin D intakes up to 10 times the DRI among lactating women not only would elevate maternal nutritional vitamin D status to safe healthy levels but also would supply the nursing infants, through milk transfer, with adequate vitamin D to prevent hypovitaminosis D. The hypothesis was investigated in this study.

METHODS

Subjects

Fully lactating mothers within 1 month after birth were eligible for inclusion in the study if they planned to continue full breast-feeding for the next 3 mo. The subjects were randomly divided

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into 2 groups. Exclusion criteria included preexisting type 1 or type 2 diabetes mellitus, hypertension, parathyroid disease, and uncontrolled thyroid disease.

Study design

This was a randomized controlled trial of lactating mothers. Each subject served as her own control; the vitamin D status at 1 mo was compared with values at 3 additional time points. Mothers were randomized to 1 of 2 vitamin D supplementation regimens. Groups 1 and 2 received 1600 and 3600 IU/d vitamin D_2, respectively, in an oral suspension. Both groups received additional multivitamin capsules containing 400 IU vitamin D_3 and were instructed to take them daily. Subjects also were instructed to limit sun exposure (for mother and infant). Furthermore, mothers were instructed to limit formula intake by the infant.

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, vitamin D_2, vitamin D_3, 25(OH)D_2, and 25(OH)D_3 concentrations. Infant serum was monitored for vitamin D_2, vitamin D_3, 25(OH)D_2, 25(OH)D_3, calcium, and phosphorus concentrations. Mother's urine was monitored for calcium/creatinine ratio. Vitamin D antirachitic activity in mother's milk was assessed through measurement of vitamin D_2, vitamin D_3, 25(OH)D_2, and 25(OH)D_3 concentrations in the milk and conversion of findings into biological activity values with reference data from biological activity assays (12).

Vitamin D_2 was used as a specific tracking agent for maternal dosing because the contribution of vitamin D_2 from other sources would be unlikely or minimal. By using vitamin D_2 in this study, we could precisely define the increase in and/or transfer of vitamin D compounds from the mother to her infant without confounding factors such as extradietary intake and sun exposure.

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 D_2, vitamin D_3, 25(OH)D_2, and 25(OH)D_3 were determined with HPLC and radioimmunoassay techniques, as described previously (16–18).

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 25(OH)D_2, 25(OH)D_3, and total circulating 25(OH)D concentrations and milk antirachitic activity with time (in months). Data were analyzed with SAS software (SAS Institute, Cary, NC), with Student's *t* test, chi-square test, and one-way analysis of variance methods.

RESULTS

A convenience sample of 64 lactating women was enrolled, of whom 29 had stopped breast-feeding by the first visit. Thirty-five women came for the first visit; of those, 18 women (13 white women and 5 African American women), 9 in each group, continued breast-feeding and completed the 3-mo study period. There were 3 African American women in group 1 (2000 IU/d vitamin D) and 2 in group 2 (4000 IU/d vitamin D). The groups did not differ according to age, ethnicity profile, insurance status, number of pregnancies, pregnancy interval, infant sex, birth weight, or gestational age (**Table 1**).

Dietary intakes of vitamin D up to 10 times the DRI for lactating women for a period of 3 mo resulted in no adverse events associated with vitamin D. Serum calcium concentrations all remained in the normal range, and no observation of hypercalcuria was noted (data not shown). This is not surprising, because circulating vitamin D and 25(OH)D concentrations never exceeded normal levels (**Figures 1** and **2**). There was a significant interaction of vitamin D concentrations with time in both groups. Although subjects in group 1 (who received 1600 IU/d vitamin D_2 and 400 IU/d vitamin D_3) did not exhibit increases in circulating concentrations of vitamin D_3 from baseline to 3 mo (from 1.0 ± 0.4 to 1.2 ± 1.2 ng/mL, mean ± SEM; $P < 0.7$), they did exhibit increases in circulating vitamin D_2 concentrations (from <0.5 to 2.2 ± 0.7 ng/mL; $P < 0.01$). In this group, circulating 25(OH)D_3 concentrations actually decreased during this time period (from 27.2 ± 3.2 to 18.7 ± 1.7 ng/mL; $P < 0.02$), whereas circulating 25(OH)D_2 concentrations increased (from 0.4 ± 0.1 to 17.4 ± 1.0 ng/mL; $P < 0.0001$). The total circulating 25(OH)D concentrations in this patient group increased from 27.6 ± 3.3 to 36.1 ± 2.3 ng/mL ($P < 0.05$).

Compared with group 1, higher concentrations of total circulating 25(OH)D were observed for subjects in group 2 (who received 3600 IU/d vitamin D_2 and 400 IU/d vitamin D_3). In group 2, circulating vitamin D_3 concentrations increased slightly (from 0.9 ± 0.4 to 2.8 ± 1.0 ng/mL; $P < 0.06$), whereas circulating vitamin D_2 concentrations increased from 0.6 ± 0.1 to 6.6 ± 2.4 ng/mL ($P < 0.04$) (Figure 2). As for group 1, circulating 25(OH)D_3 concentrations decreased (from 32.0 ± 2.5 to 18.9 ± 3.0 ng/mL; $P < 0.0007$), whereas 25(OH)D_2 concentrations increased (from 1.8 ± 1.0 to 25.0 ± 2.5 ng/mL; $P < 0.0001$). Total circulating 25(OH)D concentrations increased from 32.9 ± 2.4 to 44.5 ± 3.9 ng/mL ($P < 0.04$).

Comparisons between groups 1 and 2 revealed no significant differences at baseline for any of the vitamin D indices for mothers and infants. Although 25(OH)D_3 concentrations did not differ between the groups during the study period, there were significant differences between the 2 groups with respect to 25(OH)D_2 concentrations ($P < 0.01$), with higher concentrations in the 4000 IU group.

The changes in milk antirachitic activity observed during the study period are presented in **Figure 3**. Subjects who received a total of 2000 IU/d vitamin D for 3 mo exhibited increases in milk activity from 35.5 ± 3.5 to 69.7 ± 3.0 IU/L ($P < 0.0001$), whereas the increase observed in the 4000 IU/d group was from 40.4 ± 3.7 to 134.6 ± 48.3 IU/L ($P < 0.0001$). This elevation was attributable to increases in milk concentrations of both vitamin D_2 and 25(OH)D_2 (data not shown).

The increases in circulating 25(OH)D_2, 25(OH)D_3, and total 25(OH)D concentrations for the infants at baseline and after 3 mo of nursing from mothers receiving 2000 or 4000 IU/d vitamin D are presented in **Figures 4** and **5**. As with the mothers, there was a significant interaction of vitamin D concentrations with time for both infant groups. Infants of mothers ingesting 2000 IU/d exhibited increases in circulating 25(OH)D_3 and 25(OH)D_2 concentrations, from 7.9 ± 1.1 to 21.9 ± 4.7 ng/mL ($P < 0.02$) and from <0.5 to 6.0 ± 1.0 ng/mL ($P < 0.0007$),

TABLE 1
Sociodemographic data and clinical factors for subjects as a function of study group

Maternal and infant factors[1]	2000 IU group	4000 IU group
Maternal	$n = 9$	$n = 9$
Age (y) (mean ± SD)	29.0 ± 6.0	30.8 ± 5.2
Race (no.)		
African American	3 (33.3%)	2 (22.2%)
White	6 (66.6%)	7 (77.8%)
Insurance status (no.)		
Private	5 (55.6%)	6 (66.7%)
Indigent/Mcdicaid	4 (44.4%)	3 (33.3%)
Parity (median)	0.5	1.0
Gravidity (median)	2.0	2.0
Compliance with vitamin D supplement (%) (mean ± SD)		
Returned month 1	10.3 ± 9.0	8.4 ± 8.9
Returned month 2	12.1 ± 9.0	10.4 ± 14.4
Returned month 3	10.2 ± 9.9	7.6 ± 7.4
Infant	$n = 9$	$n = 8$
Birth weight (g) (mean ± SD)	3526.6 ± 497.5	3368.8 ± 552.1
Gestational age (wk) (mean ± SD)	39.1 ± 1.5 (range: 37–41)	38.4 ± 1.7 (range: 36–41)

[1] No significant differences were found between the 2 groups with respect to sociodemographic data and maternal/infant clinical factors.

respectively. Total circulating 25(OH)D concentrations increased from 7.9 ± 1.1 to 27.8 ± 3.9 ng/mL ($P < 0.02$).

Infants of mothers receiving 4000 IU/d exhibited increases in circulating 25(OH)D_3 and 25(OH)D_2 concentrations from 12.7 ± 3.4 to 18.8 ± 4.1 ng/mL ($P < 0.2$, NS) and from 0.8 ± 0.4 to 12.0 ± 1.4 ng/mL ($P < 0.0001$), respectively. Total circulating 25(OH)D concentrations increased from 13.4 ± 3.3 to 30.8 ± 5.0 ng/mL ($P < 0.01$). Compared with infants in the 2000 IU group, infants in the 4000 IU group exhibited higher 25(OH)D_2 concentrations at the end of the study period ($P < 0.003$).

DISCUSSION

Rickets was nearly eradicated in the United States with vitamin D fortification of milk in the 1930s (1). However, recent reports showing a resurgence of rickets in infancy focused renewed attention on the vitamin D status of infants and their mothers (2–5). The reported cases occurred almost exclusively among African American infants who were breastfed (2, 3). Why has this been the case? There are 2 major reasons for this affliction. The first is sun paranoia, which is constantly reinforced by

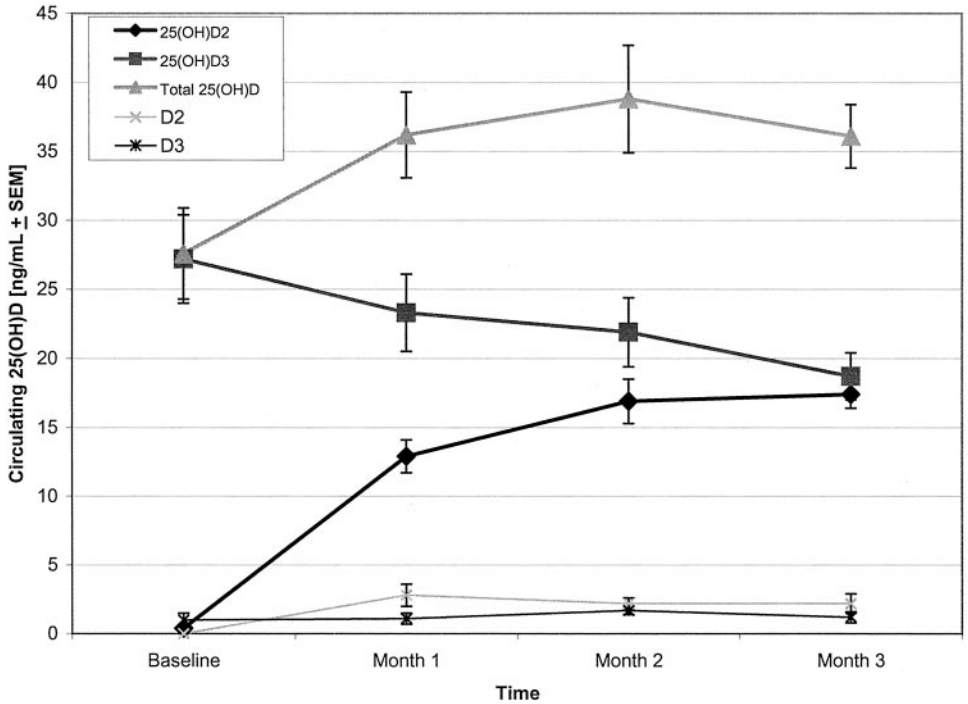

FIGURE 1. Circulating vitamin D concentrations (mean ± SEM) over time among lactating mothers receiving 1600 IU/d vitamin D_2 and 400 IU/d vitamin D_3 ($n = 9$).

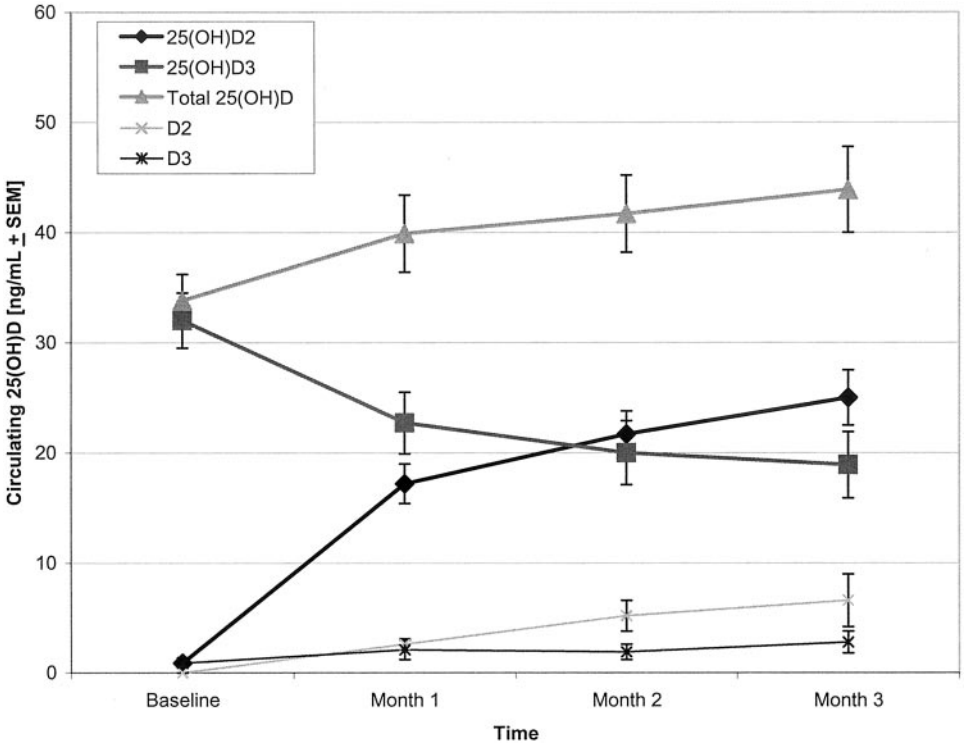

FIGURE 2. Circulating vitamin D concentrations (mean ± SEM) over time among lactating mothers receiving 3600 IU/d vitamin D_2 and 400 IU/d vitamin D_3 ($n = 9$).

the medical profession and the media. We are told to avoid the sun totally or to wear sunscreen. In fact, the American Academy of Pediatrics (19) recommends that newborn infants receive no sun exposure during the first 6 mo of life. By following these recommendations, we disrupt an important endocrine pathway that has evolved over millions of years, ie, the solar-driven production of vitamin D_3 in our skin. This lack of solar exposure is especially acute among darkly pigmented individuals, because

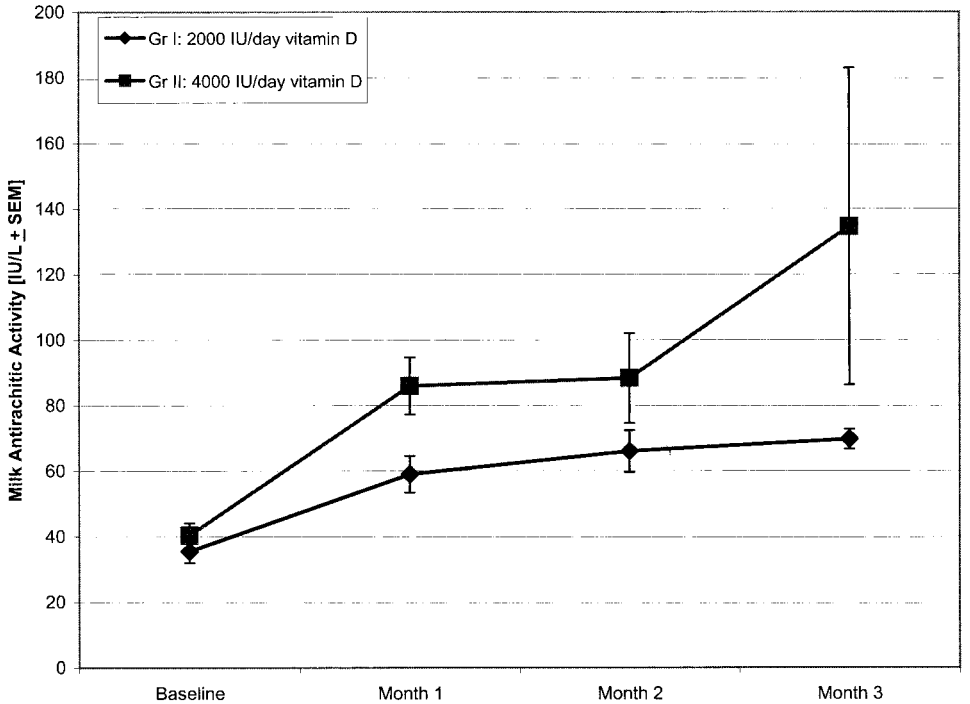

FIGURE 3. Milk antirachitic activity (mean ± SEM) over time among lactating mothers receiving 2000 or 4000 IU/d vitamin D ($n = 18$). Whole-milk samples were evaluated for vitamin D antirachitic activity through measurement of vitamin D_2, vitamin D_3, $25(OH)D_2$, and $25(OH)D_3$ concentrations in the milk; values were converted to biological activity with reference data from biological activity assays.

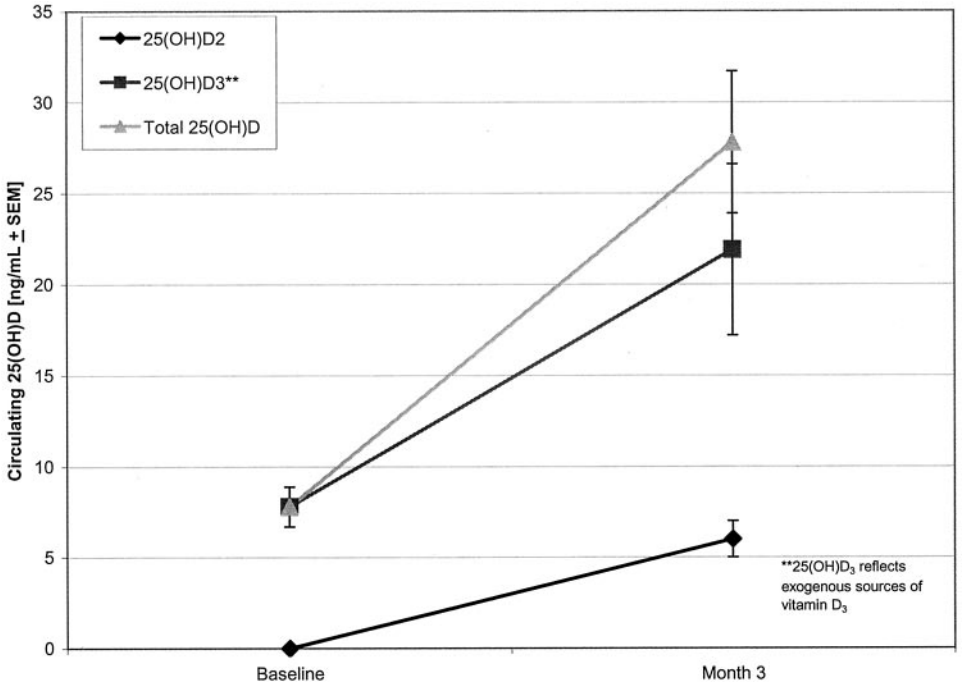

FIGURE 4. Circulating 25(OH)D concentrations (mean ± SEM) over time among nursing infants of mothers receiving 1600 IU/d vitamin D_2 and 400 IU/d vitamin D_3 ($n = 9$).

they require many times the exposure to generate vitamin D_3 in the epidermis (20).

The second reason for hypovitaminosis D among breastfed infants is the inadequate DRI value for vitamin D among lactating women. In fact, the DRI of 200–400 IU/d, which was arbitrarily set, is inadequate for the adult population in general (21). The DRI for vitamin D clearly needs to be revised. For example, white individuals exposed (total body) to 10–15 min of peak sunlight in the summer endogenously produce and release into the circulation ~20 000 IU of vitamin D_3 (22, 23). It is ironic that the 1989 US Nutrition Guidelines state that 2000 IU/d vitamin D may be harmful (24). This guideline is in error and is based on

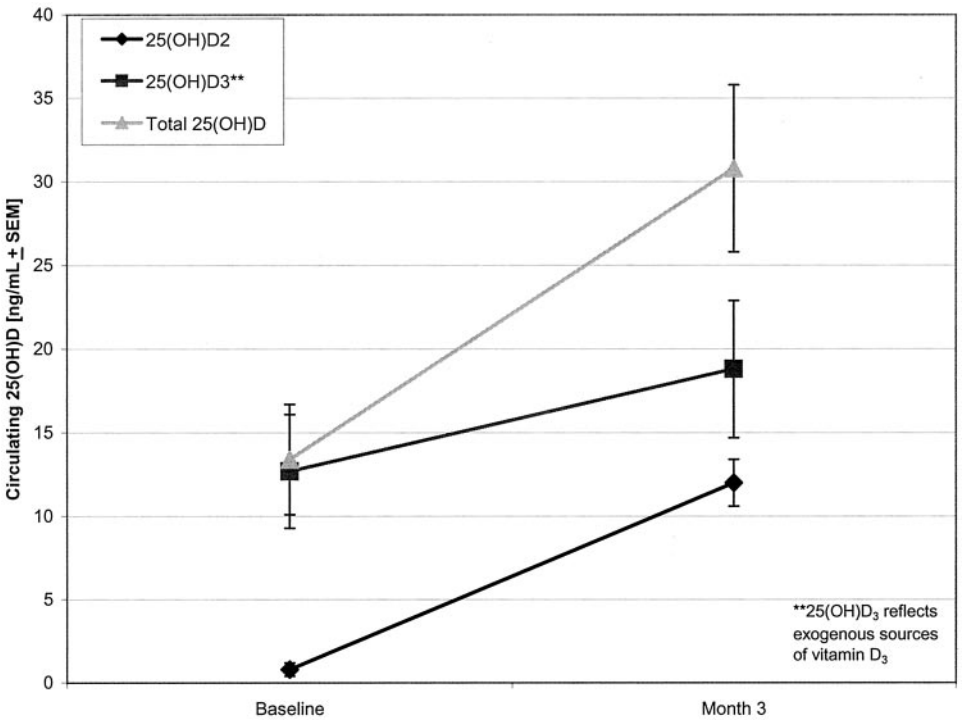

FIGURE 5. Circulating 25(OH)D concentrations (mean ± SEM) over time among nursing infants of mothers receiving 3600 IU/d vitamin D_2 and 400 IU/d vitamin D_3 ($n = 8$).

faulty scientific findings (25). In fact, other work showed that vitamin D intakes up to 10 000 IU/d produced no ill effects and elevated circulating 25(OH)D concentrations to the upper limits of the normal range (26).

To our knowledge, no prospective study has been performed to evaluate supplementation with 400 IU/d vitamin D for lactating mothers. In other words, we have no idea what effect the DRI has on the nutritional status of lactating mothers or their nursing infants. However, with the vitamin D intake regression model described by Heaney et al (15), a 400 IU/d intake would increase maternal circulating 25(OH)D concentrations by only 2.8 ng/mL after 5 mo of supplementation. This underscores how irrelevant a 400 IU/d intake is for adults.

We have been able to identify only 3 prospective studies that examined vitamin D supplementation during lactation (27–29). Greer and Marshall (27) 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 mo. It should be noted, however, that the circulating 25(OH)D concentrations among the breastfeeding infants declined as the study progressed, as noted in our own study. This decline occurred despite a maternal vitamin D intake of ~700 IU/d. A Finnish study showed that maternal supplementation with 1000 IU/d vitamin D resulted in a "minimal" increase in circulating 25(OH)D concentrations among nursing infants (28). The same investigators performed a similar study with 2000 IU/d maternal supplementation and found that the nursing infants' vitamin D status improved significantly (29). The increase in maternal circulating 25(OH)D concentrations during the 4-mo study period averaged 23 ng/mL.

Because of the latter observations and the recent work of Vieth et al (14) and Heaney et al (15), we decided to initiate this study to determine the effects of high-dose maternal vitamin D supplementation on the nutritional vitamin D status of mothers and their nursing infants. It is well known that increases in maternal vitamin D intake or solar exposure increase the antirachitic activity of human milk (11–13). It is unknown how this increase in milk concentrations affects the actual circulating concentrations of 25(OH)D among nursing infants. Our study investigated this question by treating lactating mothers with 2000 or 4000 IU/d vitamin D for a period of 3 mo. This supplement included 1600 IU/d vitamin D_2 for one group and 3600 IU/d vitamin D_2 for the other group. Both groups received 400 IU/d vitamin D_3 from their prenatal vitamin supplement. We used vitamin D_2 as the major supplement because we could differentially track its metabolism and milk transfer independent of solar or dietary confounders, as noted with vitamin D_3. In other words, if vitamin D_2 metabolites appeared in the nursing infant's circulation, they could have arrived only through milk transfer from the mother.

Supplementation with high-dose vitamin D for mothers resulted in increases in circulating 25(OH)D concentrations that were completely attributable to increased 25(OH)D_2 concentrations (Figures 1 and 2). This increase was more pronounced among mothers who received 3600 IU/d vitamin D_2. A similar profile was observed for circulating vitamin D_2 (Figures 1 and 2). It is of interest that, in both groups, circulating 25(OH)D_3 concentrations decreased although the mothers were receiving 400 IU/d vitamin D_3. This observation reinforces the uselessness of a 400 IU DRI for adults. It is important to note that, while the mothers received 4000 IU/d vitamin D for a period of 3 mo, maternal 25(OH)D concentrations were elevated to and remained in a normal healthy range. Again, no adverse side effect was observed.

The initial antirachitic activity in the milk from the mothers in our study was not different from previously reported values (10, 13, 16). The increase in maternal circulating vitamin D and 25(OH)D concentrations resulted in elevation of the antirachitic activity of the mothers' milk (Figure 3). This increase was completely attributable to increased concentrations of vitamin D_2 and 25(OH)D_2 in the milk (data not shown). A maternal daily intake of 4000 IU/d vitamin D increased the antirachitic activity of milk by ~100 IU/L. The increase in milk antirachitic activity was directly responsible for increasing the circulating 25(OH)D_2 concentrations observed among the nursing infants (Figures 4 and 5). This increase in circulating 25(OH)D_2 concentrations among the nursing infants was a direct reflection of the vitamin D_2 intake of the mothers.

What can be inferred from the data in the current study? First, we need to address sun exposure or, rather, the lack of it. Individuals are constantly told not to receive direct sun exposure or to wear sunscreen if they do. By following this advice, we are eliminating the initial step in an important endocrine system that can easily generate 10 000–20 000 IU/d vitamin D (22, 23). How do we compensate for this? It is not likely with an intake of 400 IU/d vitamin D. Other confounding factors include dark skin pigmentation and northern latitudes, which inhibit cutaneous vitamin D_3 production (30, 31). We are rapidly becoming completely dependent on dietary supplementation as a means to ensure adequate vitamin D concentrations, and 400 IU/d is far from adequate. Second, maternal intake of 400 IU/d vitamin D does not elevate the nutritional status of mothers or nursing infants. This is indicated by the occurrence of hypovitaminosis D and rickets among breastfed infants (2–4). Maternal intake of 2000 IU/d vitamin D elevates maternal 25(OH)D concentrations, but the amount passed on to nursing infants through the milk is still inadequate to elevate the infants' circulating 25(OH)D concentrations satisfactorily. Maternal intake of 4000 IU/d increases maternal circulating concentrations to a degree that enough vitamin D enters the milk to produce significant effects on the infants' circulating 25(OH)D concentrations.

We conclude that the current DRI of 400 IU/d vitamin D for lactating mothers is irrelevant to the vitamin D nutritional status of mothers and nursing infants. Maternal vitamin D intakes of $\geq$ 4000 IU/d appear to be safe and to provide sufficient vitamin D to ensure adequate nutritional vitamin D status for both mothers and nursing infants. Additional detailed studies will be required to determine the optimal vitamin D supplementation regimen for lactating women.

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