High-Dose Vitamin D₃ Supplementation in a Cohort of Breastfeeding Mothers and Their Infants: A 6-Month Follow-Up Pilot Study

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ABSTRACT

Objective: To examine the effect of high-dose maternal vitamin D_3 (vitD) supplementation on the nutritional vitD status of breastfeeding (BF) women and their infants compared with maternal and infant controls receiving 400 and 300 IU vitD/day, respectively.

Design: Fully lactating women ($n = 19$) were enrolled at 1–month postpartum into a randomized-control pilot trial. Each mother received one of two treatments for a 6–month study period: 0 or 6000 IU $vitD_3$ plus a prenatal vitamin containing 400 IU $vitD_3$. The infants of mothers assigned to the control group received 300 IU $vitD_3$/day; those infants of mothers in the high-dose group received 0 IU (placebo). Maternal serum and milk vitD and 25(OH)D were measured at baseline then monthly; infant serum vitD and 25(OH)D were measured at baseline, and months 4 and 7. Urinary calcium/creatinine ratios were measured monthly in both mothers and infants. Dietary and BF history and outdoor activity questionnaires were completed at each visit. Changes in skin pigmentation were measured by spectrophotometry. Data were analyzed using chi-square, *t*-test, and analysis of variance (ANOVA) on an intent-to-treat basis.

Results: High-dose (6400 IU/day) $vitD_3$ safely and significantly increased maternal circulating 25(OH)D and vitD from baseline compared to controls ($p < 0.0028$ and 0.0043, respectively). Mean milk antirachitic activity of mothers receiving 400 IU vitD/day decreased to a nadir of 45.6 at visit four and varied little during the study period (45.6–78.6 IU/L), whereas the mean activity in the 6400 IU/day group increased from 82 to 873 IU/L ($p < 0.0003$). There were no differences in circulating 25(OH)D levels of infants supplemented with oral vitD versus infants whose only source of vitD was breast milk.

Conclusion: With limited sun exposure, an intake of 400 IU/day vitamin D_3 did not sustain circulating maternal 25(OH)D levels, and thus, supplied only extremely limited amounts of vitamin D to the nursing infant via breast milk. Infant levels achieved exclusively through maternal supplementation were equivalent to levels in infants who received oral vitamin D supplementation. Thus, a maternal intake of 6400 IU/day vitamin D elevated circulating 25(OH)D in both mother and nursing infant.

INTRODUCTION

WITH THE STEADY and significant rise in nutritional rickets in breastfed infants, mainly in the African-American population, more attention has been given to assessing the vitamin D status of breastfed infants. In so doing, the marginal transfer of vitamin D in mothers' milk has been identified as the cause of hypovitaminosis in these infants.[1–5] The deficiency state is created by limited sun exposure in both mother and infant and the minimal con-

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tribution of dietary supplementation at the current adequate intake (AI) of 200 IU vitamin D/day in the mother.[6,7]

The premise that breast milk is deficient in vitamin D, and hence, the breastfeeding infant is deficient, has prompted health organizations (including the American Academy of Pediatrics) to recommend universal oral vitamin D supplementation of all breastfeeding infants to diminish the modern-day risk of vitamin D deficiency in this group.[8–10] Yet, despite these recommendations, concerns about adherence to this recommendation have been noted.[11]

Historically, the vitamin D status of the lactating mother and her breastfeeding infant has been viewed as separate. Scientific data pertaining to vitamin D supplementation during lactation in the human subject are extremely scarce. The paucity of data partially results from concerns about vitamin D toxicity of daily intakes $\geq$2000 IU, rendering maternal supplementation to increase vitamin D levels in mother's milk as a nonviable treatment modality until recently. An additional issue is that the dietary recommended intake for vitamin D has not been made. In 1997, the Institute of Medicine reported the adequate intake (AI) for vitamin D (and not the DRI) as 200 IU vitamin D/day for a lactating adult,[12] the same dose historically given to infants. When treating the infant alone, such therapy does not improve the vitamin D status of the mother, and in so doing, the vitamin D content (or antirachitic activity) of her milk remains low.

During the past 5 years, reports of prolonged supplementation with 50 times the current AI (10,000 IU/day) for up to 5 months has been shown to be safe in adult men and nonlactating women.[13,14] To the authors' knowledge, only three prospective studies have been performed to evaluate vitamin D dosing in lactating women.[7,15,16] The first study involved supplementation of lactating mothers in Finland with either 1000 or 2000 IU vitamin D/day for a period of 15 weeks.[17] The rise in circulating 25(OH)D levels during this period of supplementation was 16 and 23 ng/mL for the 1000 and 2000 IU dose groups, respectively. A recent study performed in the authors' laboratory involved supplementing lactating mothers with 2000 and 4000 IU vitamin D/day for a period of 3 months.[7] These data also demon-

strated a rise in circulating maternal 25(OH)D levels. This rise was not as pronounced as observed in the earlier study,[16] most likely because vitamin D_2 instead of vitamin D_3 was utilized as the oral supplement. A recent publication clearly shows that vitamin D_2 is inferior to vitamin D_3 at maintaining circulating 25(OH)D levels in humans.[17]

Using the Heaney regression model,[13] a 400 IU/day intake of vitamin D_3—the standard dose in a prenatal vitamin prescribed to lactating women in the United States—will increase circulating 25(OH)D by 2.8 ng/mL following 5 months of supplementation in a healthy, nonlactating adult. It is estimated that ~20% of maternal vitamin D is transferred through milk to the exclusively breastfed infant.[6] Thus, based on the regression model, 400 IU/day maternal supplement will do little to sustain the nutritional vitamin D status of the mother or her nursing infant. The antirachitic content of human milk is quite variable and is affected by season, maternal vitamin D intake, form of vitamin D taken (D_2 or D_3), and race.[6] Cancela et al.[18] reported that circulating 25(OH)D levels in breastfed infants were 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.[19] As noted, maternal supplementation with 1000 IU vitamin D/day had little effect on either *maternal* or *nursing infants'* circulating 25(OH)D values, but a regimen of 2000 IU/day improved the nursing infants' vitamin D status significantly.[16]

Based on the authors' previous pilot study and the findings of Vieth et al.[14,20] and Heaney et al.[13,21] it was hypothesized that high-dose vitamin D_3 supplementation of 6400 IU/day would greatly improve the vitamin D status in both lactating women and their breastfeeding infants and that this form of supplementation would be superior to maternal supplementation with the current standard of 400 IU/day and comparable to infant oral vitamin D_3 supplementation of 300 IU/day. The validity of this hypothesis was tested in a randomized, placebo-controlled pilot trial involving fully lactating mothers and their infants over a 6-month study period, the results of which are presented here.

METHODS

Subjects

Approval for this study was granted by the Medical University of South Carolina's (MUSC) Institutional Review Board for Human Subjects, HR #11345 and the General Clinical Research Center (GCRC; Protocol #694). Fully lactating mothers[22] within 1 month postpartum were eligible for inclusion in the study if they planned to continue full breastfeeding for the next 6 months. The subjects were randomly divided into two groups. Exclusion criteria included preexisting type I or II diabetes, hypertension, parathyroid disease, and uncontrolled thyroid disease. Subjects were compensated for their participation with gift cards given at the end of each visit.

Study design

This was a randomized, double-blind, placebo-controlled trial of lactating mothers. After written informed consent, mothers were randomized to one of two vitamin D supplementation regimens: Group 1: 400 IU vitamin D_3/day (0 IU vitamin D_3, placebo and 1 prenatal vitamin containing 400 IU vitamin D_3), or Group 2: 6400 IU vitamin D_3/day (6000 IU vitamin D_3 and 1 prenatal vitamin containing 400 IU vitamin D_3). The mothers also were provided with a liquid supplement to give to their nursing infants. Mothers in Group 1 were instructed to give their infant 0.5 mL (300 IU vitamin D_3)/day. Those mothers in Group 2 who were receiving 6400 IU vitamin D_3/day also were instructed to give to their infants 0.5 mL/day (0 IU vitamin D_3, placebo). In addition to the two group comparisons, each subject also served as her own control, establishing each mother's vitamin D status (circulating vitamin D and 25(OH)D levels) at 1 month then compared monthly at six additional time points. The vitamin D status of the infants in the two groups was compared at baseline and months 4 and 7.

Sample size calculation

This study was conducted using a proof of concept design utilized routinely in the pharmaceutical industry to demonstrate the clinical efficacy of a new drug or therapy using a small number of patients. This design has a primary focus of providing key information to make rapid and effective decisions based on Phase I/IIa data. For this specific application, the authors' goal was to determine whether vitamin D supplementation of 6400 IU would result in circulating values of 25(OH)D exceeding 90 ng/mL (an upper limit of normal chosen to be well within safety parameters) in eight healthy, fully lactating mothers compared with the reference group receiving the standard dose of 400 IU vitamin D_3/day. All outcome markers were ratio-scaled and normally distributed.

Block randomization

Mothers were randomized to one of the two treatment groups using Proc Plan in SAS® (SAS Institute, Inc., Cary, NC). This program allowed for the input of number of strata and estimated sample size. Specifically, a list of random assignments was generated stratified by ethnicity. At the time of enrollment, the study coordinator accessed the randomization web page from the General Clinical Research Center (GCRC) web site developed uniquely for this investigation. After completing general registration procedures, selecting the appropriate ethnic group, and successfully supplying a password, the patient was assigned to one of two groups. Only the Research Pharmacy and the Data Coordinating Center were notified via the computer program that a patient had been newly enrolled, of the group assignment, and general registration information.

Study protocol

Visit frequency. Each lactating mother and her breastfeeding infant came to the General Clinic Research Center (GCRC) at MUSC monthly starting at 1 month postpartum for a total of seven study visits.

Completion of questionnaires. Questionnaires regarding sociodemographic information, baseline health status, and medical history were completed at the first visit. One week prior to each visit, including the first visit, each mother was asked to complete questionnaires regarding physical activity, sunlight exposure, type of clothing worn, and provide a breastfeeding his-

tory log. An interim health history questionnaire for both mother and infant also was completed at each visit by the study coordinator, discussing type and frequency of acute illnesses such as respiratory, gastrointestinal, and other viral and/or bacterial illnesses. A review of medications and doctor's visits was obtained at that time. In addition, a detailed infant breastfeeding history was completed by the mother during the week before the scheduled visit and reviewed by the study coordinator at the study visit. Any supplementation with formula or other foods was noted and quantified.

Maternal dietary intake. Each mother completed a Block 1998.2 Food Frequency Questionnaire (FFQ) at the second visit to ascertain her generalized eating pattern, with specific calculation of calcium and vitamin D intake (Block Dietary Systems, Berkeley, CA). This questionnaire was chosen over food diaries or 3-day food records for the following reasons: (a) The questionnaire does not rely on memory as much as other validated methods. (b) Its readability is designed for lower literacy populations. (c) It accurately estimates individual and population-wide average daily intakes of all macronutrients and micronutrients. This tool has been validated and reproduced against other dietary intake tools.[23–28] Each completed FFQ form was sent to the processing center (Berkeley, CA).

Anthropomorphic measures. At each GCRC study visit, maternal weight was obtained; maternal height was measured at visit one. Infant weight (kg), length (cm), and head circumference (cm) were obtained at each monthly visit.

Blood, urine, and milk samples. Maternal blood, urine, and milk samples were collected at each visit. Infant urine also was collected monthly; however, to minimize venipuncture in the young infants, infant blood samples were obtained at baseline (month 1) and then at months 4 and 7.

Prenatal vitamins and vitamin D tablets. The prenatal vitamins provided to the mothers in the study contained 400 IU vitamin D_3 (United Research Labs, Philadelphia). Vitamin D_3 tablets (0, 2000, and 4000) were manufactured by Tishcon Corporation (Westberry, NY) a Good-Manufacturing-Practice (GMP) facility that met FDA production guidelines. To achieve the correct dosage of vitamin D supplementation, each mother took one prenatal vitamin and two vitamin D study tablets daily (*Group 1*: two tablets containing 0 IU vitamin D_3 (placebo), identical in appearance to the other vitamin D tablets. *Group 2*: One tablet containing 2000 IU vitamin D_3 and one tablet containing 4000 IU vitamin D_3 for a total of 6000 IU vitamin D_3). The total vitamin D_3 intake of mothers in Groups 1 and 2 was 400 IU and 6400 IU/day, respectively.

Infant multivitamin preparation. The multivitamin liquid preparation (polyvitamin drops, Rx Choice®, High Tech Pharmacal, Corp., Amityville, NY) provided for the infants contained 300 IU vitamin D_3/0.5 cc dispensed via a tuberculin syringe. A placebo preparation identical in appearance, taste, and smell to the polyvitamin drops was manufactured by MUSC's research pharmacy. Each mother was instructed on how to properly administer the vitamin preparation to her infant by a physician or registered nurse.

Adherence to medication regimen. Adherence to the prescribed vitamin D supplementation regimen of 1 prenatal vitamin and the vitamin D supplement was measured by maternal self-report and pill counts at each follow-up visit. Adherence to the regimen was defined as ≥90% (# pills taken divided by anticipated number of pills to be consumed between study visits). Similarly, adherence with polyvitamin study drops prescribed for each infant was defined as ≥90% (starting volume minus volume of polyvitamin D remaining divided by anticipated volume of drops to be consumed between study visits).

Determination of skin pigmentation. Skin pigmentation changes were monitored monthly in mother and infant using the SmartProbe 400 (IMS, Inc., Milford, CT), a spectrophotometer device that measures degrees of pigmentation on a continuous scale from 0 to 100, 0 being absolute black and 100 being absolute white. Each mother had pigmentation measurements recorded from her exposed forearm, underarm, and stomach, with two readings averaged and recorded. Each infant had pigmentation measurements recorded from the forearm and upper thigh. Of note, mothers were instructed to

use sunscreen if outdoors for more than 15 minutes and avoid direct sunlight exposure of their infants during the first 6 months.

Laboratory measurements

Total calcium, phosphorus, vitamins D_3 and 25(OH)D_3 were measured in both maternal and infant blood samples by established methods.[29] Mother's milk was assessed for vitamin D antirachitic activity by measuring vitamin D_3 and 25(OH)D_3 concentrations in the milk, which was converted into biological activity using reference data from biological activity assays.[30]

Total blood calcium and urinary calcium and creatinine levels were measured by the Clinical Chemistry Laboratory of the GCRC at MUSC. Circulating and milk levels of vitamin D_3 and 25(OH)D_3 were determined by the investigators using high-performance liquid chromatography and radioimmunoassay techniques as described.[31–33]

Statistical methods

The analysis was undertaken with an "Intention to Treat" perspective in which all individuals randomized to one group were considered to be within that group throughout the analysis, irrespective of their adherence to the vitamin supplementation regimen.[34] Thus, Groups 1 and 2 were compared at entrance into the study to detect potential differences with regard to sociodemographic and baseline clinical characteristics. The main variables of interest were maternal and infant total circulating 25(OH)D and milk antirachitic activity over time in months. Data were analyzed with SAS software[35] using student's t-test, McNemar's chi-squared test, and repeated ANOVA measures. Repeated sampling provided the opportunity to examine the changes in blood chemistry by group over time. These trends also were examined by use of repeated measures analysis of variance.

RESULTS

A convenience sample of 19 fully lactating women (15 white, two Hispanic, and two black) was enrolled within the first month postpartum, 10 in Group 1 (400 IU vitamin D/day) and 9 in Group 2 (6400 IU vitamin D/day). The groups did not differ by age, ethnicity profile, insurance status, number of pregnancies, pregnancy interval, infant gender, birth weight, or gestational age (Table 1). Of the 19 women, 10 completed the study, four have completed through visit 5, and five stopped participation between visits 1 and 4 because of the cessation of breastfeeding.

The women who completed the study were either exclusively or fully breastfeeding, with confirmation of infant dietary intake by a detailed dietary log and monthly interview. There were no differences between the groups in terms of dietary profiles, outdoor activities, and changes in skin pigmentation from baseline. There was an increase in degree of skin pigmentation during warmer months that was

TABLE 1. MATERNAL INFANT CHARACTERISTICS

Characteristic	400 IU Vitamin D_3/d group (n = 10)	6400 IU Vitamin D_3/d group (n = 9)
Maternal age (yr)		
Mean ± SD	30.3 ± 3.3	28.3 ± 5.9
Maternal ethnicity		
African American [n (%)]	1 (11.1%)	1 (11.1%)
White [n (%)]	6 (66.7%)	8 (88.9%)
Hispanic [n (%)]	2 (22.2%)	0
Median number of pregnancies	2.0	2.0
Range	(1–6)	(1–4)
Mean interpregnancy interval		
Months ± SD	24.9 ± 21.2	29.2 ± 24.9
Median interpregnancy interval	24.0	27.5
Infant gender (female/male)	5/4	4/6
Birth weight (grams)		
Months ± SD	3,435.6 ± 440.0	3,614.2 ± 349.8
Gestational age		
Weeks ± SD	38.8 ± 1.2	39.2 ± 0.7

TABLE 2.   SERUM AND URINE CHEMISTRIES OF MOTHER AND INFANTS AS A FUNCTION OF VITAMIN D DOSE[a]

	400 IU Vitamin D$_3$/d			6400 IU Vitamin D$_3$/d		
	Visit 1	Visit 4	Visit 7	Visit 1	Visit 4	Visit 7
Serum calcium						
Maternal	9.1 ± 1.3	9.5 ± 0.4	9.5 ± 0.1	9.4 ± 0.4	9.3 ± 0.3	9.5 ± 0.3
Infant	10.0 ± 0.3	10.0 ± 0.2	9.8 ± 0.3	10.2 ± 0.2	10.3 ± 0.2	10.2 ± 0.3
Urinary Ca/Cr ratio						
Maternal	0.07 ± 0.05	0.10 ± 0.06	0.16 ± 0.18	0.07 ± 0.04	0.16 ± 0.11	0.08 ± 0.02
Infant	0.5 ± 0.4	0.5 ± 0.3	0.3 ± 0.2	0.4 ± 0.2	0.9 ± 0.4	0.6 ± 0.5

[a]$p < 0.05$; no statistically significant differences between groups at all visits.

similar in both groups. Based on pill counts and measurement of multivitamin liquid remaining, there were no differences in adherence to the prescribed regimen between the two study groups. Both groups had ≥80% overall maternal compliance with vitamin D tablet intake; however, both groups had lower compliance with infant vitamins with rates as low as 61%.

As shown in Table 2, maternal and infant serum calcium and phosphorus levels and urinary calcium to creatinine ratios remained in the normal range for both groups. There were no adverse events or serious adverse events related to vitamin D supplementation.

Growth patterns of the infants as measured by infant weight, head circumference, length and BMI were similar between the groups throughout the study period (Table 3). The health characteristics of the two groups also were similar.

As shown in Table 4, the maternal groups did not differ as a function of dietary components. Both groups met the dietary recommended intake for lactating women with respect to total protein, fat, and carbohydrate intakes, and in fact, exceeded the recommended intake for both fat and carbohydrates. Each group met the DRI for calcium, phosphorus, and magnesium, and the current AI for vitamin D of 200 IU/day.[12]

As shown in Figure 1, the total circulating 25(OH)D levels of mothers in Group 1 decreased through visit 5 reaching a nadir of 25.9 ± 9.1; there was slight improvement at visits 6 and 7 that corresponded to increased outdoor activities and sun exposure. Mothers in Group 2 had an immediate increase in 25(OH)D levels that was sustained throughout the study period. Despite the increased outdoor activities and an increase in sunlight ex-

TABLE 3.   ANTHROPOMORPHIC MEASUREMENTS OF INFANTS DURING
6-MONTH STUDY PERIOD BY MATERNAL VITAMIN D$_3$ DOSE

Anthropomorphic measures	400 IU Vitamin D$_3$/d	6400 IU Vitamin D$_3$/d	p-Value
Infant BMI			
Visit 1	15.9 ± 0.8	14.9 ± 2.2	0.30
Visit 4	17.0 ± 0.7	16.8 ± 2.3	0.87
Visit 7	16.9 ± 0.5	18.5 ± 0.1	0.22
Infant weight (kg)			
Visit 1	4.7 ± 0.4	4.6 ± 0.7	0.80
Visit 4	6.7 ± 0.5	6.6 ± 0.8	0.91
Visit 7	7.6 ± 0.8	8.4 ± 1.1	0.30
Infant head circumference (cm)			
Visit 1	37.9 ± 1.0	37.6 ± 1.7	0.68
Visit 4	41.7 ± 0.8	41.2 ± 1.6	0.53
Visit 7	44.3 ± 0.9	43.6 ± 0.9	0.27
Infant length (cm)			
Visit 1	54.6 ± 1.1	55.9 ± 2.9	0.29
Visit 4	62.4 ± 1.7	62.8 ± 1.9	0.75
Visit 7	65.5 ± 1.8	69.3 ± 2.9	0.06

BMI, weight(kg)/height^2 [m^2].

posure that paralleled group 1, after achieving steady-state by month 3, there was very little change in maternal 25(OH)D from months 3 to 7. There were no differences between the two groups in terms of exercise, outdoor activities, or skin pigmentation changes throughout the study period to account for the differences in 25(OH)D.

Serum maternal vitamin D_3 levels showed a pattern similar to circulating 25(OH)D. As shown in Figure 2, maternal serum vitamin D_3 levels of mothers in Group 1 were minimal, with a slight increase from 2.1 at baseline to 5.3 and 4.0 at visits 6 and 7, again corresponding to increased outdoor activities and sunlight exposure. In comparison, there was a dramatic and sustained increase in maternal serum vitamin D levels in Group 2, increasing from a mean of 4.1 to 39 by visit 4.

Compared to Group 1, the mean milk antirachitic activity in Group 2 significantly increased to 873 IU/L ($p < 0.0003$; Fig. 3), which resulted in a dramatic rise in infant circulating 25(OH) levels. In fact, as shown in Figure 4, this rise in infant 25(OH)D was almost identical to that in the infants receiving 300 IU/d vitamin D_3 directly via oral supplementation.

DISCUSSION

In this pilot study of lactating women randomized to either 400 or 6400 IU vitamin D_3/day, there were significant differences in the vitamin D status of the women. Compared to a maternal intake of 400 IU vitamin D_3/day, a maternal intake of 6400 IU vitamin D_3/day was associated with a dramatic increase in both circulating maternal vitamin D_3 and 25(OH)D; however, this increase appeared to be limited and controlled with steady-state achieved by visit 3. The current standard dose of 400 IU/day vitamin D, the concentration in prenatal vitamins prescribed to lactating women con-

TABLE 4. MATERNAL DIETARY INTAKE BY FOOD FREQUENCY QUESTIONNAIRE

Dietary component	400 IU Vitamin D_3/d group	6400 IU Vitamin D_3/d group	p-Value
Total protein (g/day)			0.76
Mean ± SD	88.0 ± 46.0	93.7 ± 33.0	
DRI: 71 g/day			
Dietary calories as protein			0.61
Mean % ± SD	15.4 ± 3.1	16.4 ± 3.4	
Total fat (g/day)			0.51
Mean ± SD	89.6 ± 33.0	100.1 ± 24.7	
DRI: 20–35 g/day			
Dietary calories as fat			0.55
Mean % ± SD	36.9 ± 4.3	38.9 ± 7.3	
Total carbohydrates (g/day)			0.91
Mean ± SD	267.8 ± 94.4	273.7 ± 101.0	
DRI: 210 g/day			
Dietary calories as carbohydrates			0.51
Mean % ± SD	48.6 ± 3.9	46.9 ± 8.5	
Total calcium (mg; mean ± SD)	1,116.8 ± 587.3	1,133.2 ± 286.7	0.95
DRI (1): 1300 mg ≤ 18 years old			
1000 mg > 18 years old			
Total vitamin D (IU; mean ± SD)	273.6 ± 274.5	272.6 ± 114.5	0.99
AI (1): 200 IU/day			
Total phosphorus (mg; mean ± SD)	1,536.9 ± 718.2	1,642.6 ± 344.9	0.72
DRI (1): 1250 mg ≤ 18 years old			
700 mg > 18 years old			
Total magnesium (mg; mean ± SD)	328.4 ± 140.7	341.9 ± 100.4	0.84
DRI (1): 360 mg ≤ 18 years old			
310 mg > 18 years old			
Dairy servings/day (mean ± SD)	2.7 ± 1.9	2.4 ± 1.2	0.76
DRI: 3–4 servings/day			
Or 24 oz/24 h			
Total Kcal/day (mean ± SD)	2,213 ± 809	2,341 ± 555	0.73

From: 1997 Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride. National Academy Press, Washington, D.C.
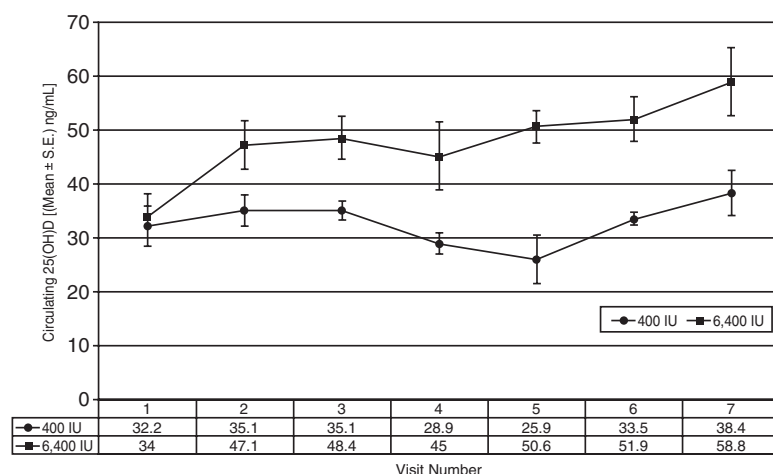
FIG. 1. Maternal 25(OH) status: 400 IU versus 6400 IU vitamin D_3/day supplementation regimen.

|	1	2	3	4	5	6	7
400 IU	32.2	35.1	35.1	28.9	25.9	33.5	38.4
6,400 IU	34	47.1	48.4	45	50.6	51.9	58.8

Visit Number

tributed little to the vitamin D nutritional status of mother and her nursing infant, with 25(OH)D levels reflecting seasonal variation. In comparison, maternal supplementation of 6400 IU vitamin D_3/day appeared to be safe and ensured adequate nutritional vitamin D status of both the mother and her nursing infant independent of season.

In both groups, the vitamin D content of human milk was directly related to the lactating mother's vitamin D status. Vitamin D status in this case refers to both circulating vitamin D and 25(OH)D. In lactating mothers taking 400 IU/day vitamin D, it was previously found that human milk contains 33 to 68 IU/L antirachitic activity.[36] In the authors' more recent supplementation study, in women at baseline taking 400 IU/day vitamin D ($n = 35$), the mean antirachitic activity of the milk was 37.9 ± 10.7 IU/L.[7] There was a dramatic increase in mean antirachitic activity of milk from mothers sup-

plemented with 6400 IU vitamin D_3/day, confirming the hypothesis that maternal vitamin D supply to the infant is directly related to maternal vitamin D status. It is estimated from these data that daily maternal intakes of 6400 IU/day of vitamin D will result in raising the antirachitic activity of their milk to 500 to 800 IU/L. As shown in this study, this level of antirachitic activity in human milk was sufficient for the nursing infant to maintain adequate circulating levels of 25(OH)D, and in fact, was comparable to infants receiving oral vitamin D supplementation. Additional, larger scale studies with more diversified ethnic/racial groups must be conducted to confirm these preliminary findings.

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.[31,37,38] Further, almost all of the activity was attributable to vitamin D and 25(OH)D.[37] These studies also demon-

FIG. 2. Maternal serum vitamin D status: 400 IU versus 6400 IU vitamin D_3/day supplementation regimen.
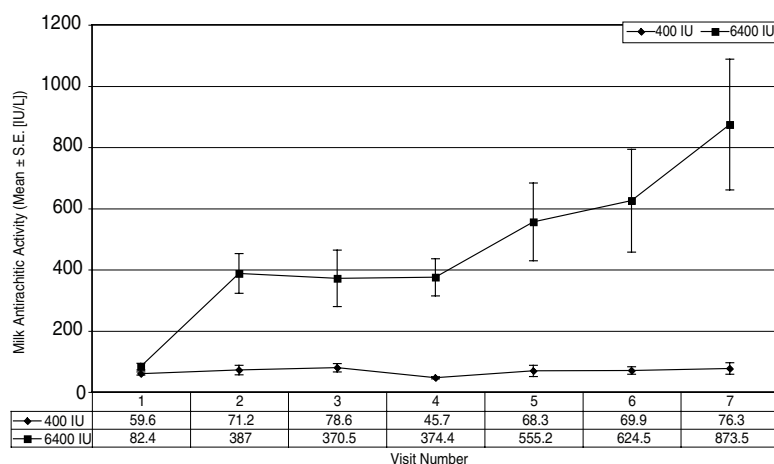

	1	2	3	4	5	6	7
400 IU	2.2	2.8	3.5	2.8	3.7	5.3	4
6,400 IU	4.1	30.3	32.4	39.2	49.6	43.9	49.7

Visit Number
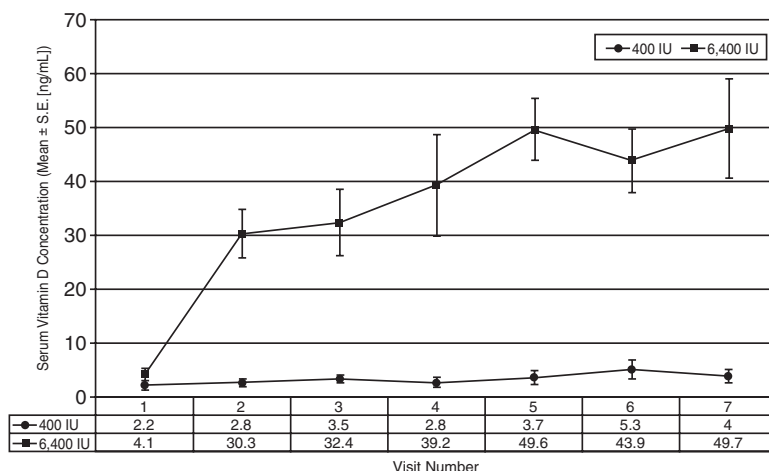
FIG. 3. Milk antirachitic activity as a function of maternal vitamin D_3 dose: 400 vs. 6400 IU/day.

	1	2	3	4	5	6	7
400 IU	59.6	71.2	78.6	45.7	68.3	69.9	76.3
6400 IU	82.4	387	370.5	374.4	555.2	624.5	873.5

strated that dietary maternal vitamin D supplementation and ultraviolet (UV) light exposure increased the vitamin D content of human milk.[31,39,40] Specker et al.[41] determined that the antirachitic content of human milk was lower in black than white mothers. This difference was attributed to variation in dietary intake of vitamin D and UV exposure. An interesting study involving a woman with hypoparathyroidism who was treated with 100,000 IU/day vitamin D for the maintenance of her plasma calcium throughout pregnancy delivered a normal child at term and then breastfed her infant.[30] Analysis of breast milk from this mother showed it to contain over 7000 IU/L of antirachitic activity. In a recent study by the authors' group involving lactating mothers receiving up to 4000 IU vitamin D_2/day, the antirachitic activity of their milk did not rise $\geq$200 IU/L.[7] This was disappointing and largely resulted from the relative inability of vitamin D_2 to raise circulating 25(OH)D in humans.[6,17] However, in the current

study the efficacy of daily doses of 6400 IU vitamin D_3 maternal oral supplementation in achieving antirachitic activity in milk $\geq$500 IU/L supports the premise that the vitamin D content of human milk can be influenced by maternal diet and/or UV exposure. Conversely, if a lactating mother has limited exposure and/or limited vitamin D intake (such as occurs with the current 400 IU/day AI), the vitamin D content of her milk will be poor, especially if she has darker pigmentation. Although supplementation of the infant with vitamin D *may* ameliorate the problem in that age group, it does not address the needs of the mother. By treating the mother with a sufficient dose of vitamin D, both the mother and her recipient infant will achieve normal vitamin D status. The authors strongly believe that the AI of 200 IU vitamin D_3/day and the current practice of prescribing prenatal vitamins containing 400 IU vitamin D_3 per tablet is woefully inadequate, especially in darkly pigmented individuals.

FIG. 4. Infant circulating 25(OH)D as a function of maternal supplementation (400 IU versus 6400 IU vitamin D_3/day) and infant supplementation (300 IU versus 0 IU vitamin D_3/day).

	1	2	7
400 IU	13	33	43
6,400 IU	14	36	46
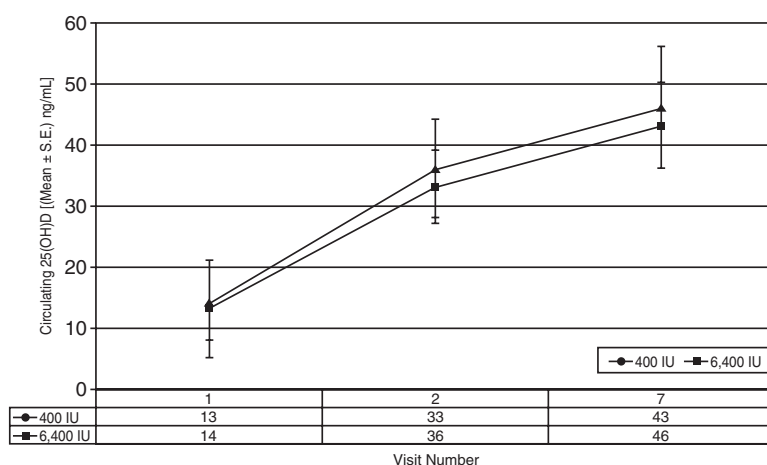
Despite the small sample size of this study, it is clear that a daily dose of 400 IU vitamin D_3 does little to change the nutritional vitamin D status of adults, whether lactating or not. Only with increased sunlight exposure in the 400 IU/day group between visits 5 and 7 did vitamin D status improve. Other studies support this premise.[13,14,20,21,42–45] In order to achieve optimal circulating concentrations of 25(OH)D, what then should the AI for vitamin D be in the adult, especially the adult who is lactating? Before that question can be answered, the optimal concentration of circulating 25(OH)D needs to be determined.

Most studies have concentrated on how much vitamin D is required to avoid deficiency. Available evidence in which circulating intact PTH and 25(OH)D were measured in adult patients indicates that secondary hyperparathyroidism occurs when serum 25(OH)D values fall below the range of 15 to 20 ng/mL.[46–48] A recent report by Vieth et al.[43] demonstrates that maximal suppression of PTH by circulating 25(OH)D occurs at >80 nmol (32 ng/mL) 25(OH)D. Heaney et al. [21] have demonstrated in normal adults that intestinal calcium absorptive performance is reduced in individuals who exhibit circulating 25(OH)D levels of 20 ng/mL compared with subjects with circulating levels >32 ng/mL. They concluded that individuals with circulating 25(OH)D levels at the low end of the current reference range may not be getting the full benefit from their calcium intake. Recent, additional retrospective and interventional studies suggest that circulating 25(OH)D needs to exceed 32 ng/mL (80 nmol/L) to maximize skeletal integrity.[49,50] In lactating women and their infants, it will be necessary to determine optimal 25(OH)D levels in a range of values that ensures both maternal and infant optimization as defined by various biomarkers. It is only with larger, well-designed studies that such data will be generated.

Health professionals need to think of vitamin D in more global terms. For instance, increased circulating 25(OH)D has been linked to improved glucose handling and β-cell function.[51] More importantly, the role of vitamin D and the innate immune system has now been elegantly described with profound implications.[52] Some of this data, as well as additional studies, have been summarized in a recent review regarding the optimization of circulating 25(OH)D levels.[53] The long-term consequences of chronic vitamin D deprivation are just beginning to be understood. Although the effects of acute vitamin D deprivation have been well described and result in rickets in the rapidly growing child and osteopenia and osteoporosis in the mother, the link with disease states that may take years to manifest has been established only recently. Beyond bone, there is increasing evidence of the serious consequences of chronic vitamin D deprivation, including decreased bone mass in later life, as well as increased risks of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, periodontal disease, infections, type I diabetes, neoplasia, myopathy, and depression.[54–68]

Based on the authors' pilot data, it has been shown that 6400 IU/day vitamin D_3 eliminates hypovitaminosis D in both mother and infant. Infants receiving milk from mothers replete in vitamin D have circulating 25(OH)D levels comparable to infants receiving oral vitamin D supplementation. Larger, multisite studies are needed to confirm these findings across a more diverse population of lactating women and their infants.

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REFERENCES

1. Bachrach S, Fisher J, Parks JS. An outbreak of vitamin D deficiency rickets in a susceptible population. *Pediatrics* 1979;64:871–877.
2. Taha SA, Dost SM, Sedrani SH. 25(OH)D and total calcium: Extraordinarily low plasma concentrations in Saudi mothers and their neonates. *Pediatric Res* 1984;18:739–741.
3. Elidrissy ATH, Sedrani SH, Lawson DEM. Vitamin D deficiency in mothers of rachitic infants. *Calcif Tissue Int* 1984;36:266–268.
4. Sills I, Skuza K, Horlick M, et al. Vitamin D deficiency rickets. Reports of its demise are exaggerated. *Clin Pediatr* 1994;33:491–493.

5. Eugster EA, Sane KS, Brown DM. Need for a policy change to support vitamin D supplementation. *Minnesota Med* 1996;79:29–32.

6. Hollis BW, Wagner CL. Assessment of dietary vitamin D requirements during pregnancy and lactation. *Am J Clin Nutr* 2004;79:717–726.

7. Hollis BW, Wagner CL. Vitamin D requirements during lactation: High-dose maternal supplementation as therapy to prevent hypovitaminosis D in both mother and nursing infant. *Am J Clin Nutr* 2004;80S:1752S–1758S.

8. Gartner L, Greer F, Breastfeeding So, Nutrition Co. Prevention of rickets and vitamin D deficiency: New guidelines for vitamin D intake. *Pediatrics* 2003;111(4):908–910.

9. Kreiter S. The reemergence of vitamin D deficiency rickets: The need for vitamin D supplementation. *AMB News* 2001;7:1,5.

10. Breastfeeding. So. Breastfeeding and the use of human milk. *Pediatrics* 2005;115:496–506.

11. Shaikh U, Alpert P. Practices of vitamin D recommendation in Las Vegas, Nevada. *J Human Lact* 2004;20(1):56–61.

12. Committee S. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride*. National Academy Press, Washington, DC, 1997.

13. Heaney RP, Davies KM, Chen TC, et al. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr* 2003;77:204–210.

14. Vieth R, Chan PCR, MacFarlane GD. Efficacy and safety of vitamin D_3 intake exceeding the lowest observed adverse effect level (LOAEL). *Am J Clin Nutr* 2001;73(2):288–294.

15. Ala-Houhala M. 25(OH)D levels during breast-feeding with or without maternal or infantile supplementation of vitamin D. *J Pediatr Gastroenterology Nutr* 1985;4:220–226.

16. Ala-Houhala M, Koskinen T, Terho A, et al. Maternal compared with infant vitamin D supplementation. *Arch Dis Child* 1986;61:1159–1163.

17. Armas L, Hollis BW, Heaney RP. Vitamin D_2 is much less effective than vitamin D_3 in humans. *J Clin Endocrinal Metab* 2004;89:5387–5391.

18. Cancela L, LeBoulch N, Miravet L. Relationship between the vitamin D content of maternal milk and the vitamin D status of nursing women and breastfed infants. *J Endocrinol* 1986;110:43–50.

19. Greer FR, Marshall S. Bone mineral content, serum vitamin D metabolite concentrations and ultraviolet B light exposure in infants fed human milk with and without vitamin D_2 supplements. *J Pediatr* 1989;114:204–212.

20. Vieth R. Vitamin D supplementation, 25-hydroxy-vitamin D concentrations, and safety. *Am J Clin Nutr* 1999;69:842–856.

21. Heaney R, Dowell M, Hale C, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J Am College Nutr* 2003;22(2):142–146.

22. Coffin CF, Labbok MH, Belsey M. Breastfeeding definitions. *Contraception* 1997;55:323–325.

23. Block G, DiSogra C. *Validation, Self-Administered, in Low-Income White, African-American and Hispanic Women*. Food and Nutrition Service, U.S. Department of Agriculture, Alexandria, VA, 2001.

24. Block G, Thompson FE, Hartman AM, et al. Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1–year period. *J Am Diet Assoc* 1992;92:686–693.

25. Mares-Perlman JA, Klein BEK, Klein R, et al. A diet history questionnaire ranks nutrient intakes in middle-aged and older men and women similarly to multiple food records. *J Nutr* 1993;123:489–501.

26. Coates RJ, Eley JW, Block G, et al. An evaluation of a food frequency questionnaire for assessing dietary intake of specific carotenoids and vitamin E among low-income black women. *Am J Epidemiol* 1991;134:658–671.

27. Sinha R, Patterson BH, Mangels AR, et al. Determinants of plasma vitamin E in health males. *Cancer Epidemiol Biomarkers Prev* 1993;2:473–479.

28. Sobell J, Block G, Koslowe P, Tobin J, Andres R. Validation of a retrospective questionnaire assessing diet 10–15 years ago. *Am J Epidemiol* 1989;130:173–187.

29. Hollis BW. Comparison of equilibrium and disequilibrium assay conditions for ergocalciferol, cholecalciferol and their major metabolites. *J Steroid Biochem* 1984:81–86.

30. Greer FR, Hollis BW, Napoli JL. High concentrations of vitamin D_2 in human milk associated with pharmacologic doses of vitamin D_2. *J Pediatrics* 1984;105:61–64.

31. Hollis BW. Individual quantitation of vitamin D_2, vitamin D_3, 25(OH)D_2 and 25(OH)D_3 in human milk. *Analytical Biochem* 1983;131:211–219.

32. Hollis BW, Pittard WB. Evaluation of the total fetomaternal vitamin D relationships at term: Evidence for racial differences. *J Clin Endorcrinol Metab* 1984;59:652–657.

33. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD. Determination of vitamin D status by radioimmunoassay with a ^{125}I-labeled tracer. *Clin Chem* 1993;39:529–533.

34. Gillings D, Koch G. The application of the principle of intention-to-treat in the analysis of clinical trials. *Drug Inf J* 1991;25:411–424.

35. *SAS Software* [computer program]. Version 8.2 for Windows 95/98. SAS Institute, Inc., Cary, NC, 1999–2001.

36. Hollis BW, Pittard WB, Reinhardt TA. Relationships among vitamin D, 25(OH)D, and vitamin D-binding protein concentrations in the plasma and milk of human subjects. *J Clin Endocrinol Metab* 1986;62:41–44.

37. Hollis B, Roos B, Lambert P. Vitamin D and its metabolites in human and bovine milk. *J Nutr* 1981;111:1240–1248.

38. Reeve LE, Chesney RW, Deluca HF. Vitamin D of human milk: Identification of biologically active forms. *Am J Clin Nutr* 1982;26:122–126.

39. Takeuchi A, Okano T, Tsugawa H, et al. Effects of er-

gocalciferol supplementation on the concentration of vitamin D and its metabolites in human milk. *J Nutr* 1989;119:1639–1646.

40. Greer FR, Hollis BW, Cripps DJ, Tsang RC. Effects of maternal ultraviolet B irradiation on vitamin D content of human milk. *J Pediatr* 1984;105:431–433.

41. Specker BL, Tsang RC, Hollis BW. Effect of race and diet on human milk vitamin D and 25(OH)D. *Am J Dis Child* 1985;139:1134–1137.

42. Dawson-Hughes B, Heaney RP, Holick MF, et al. Vitamin D Round Table. *Nutritional Aspects of Osteoporosis*, 2nd ed. Elsevier Science, St. Louis, 2004.

43. Vieth R, Ladak Y, Walfish P. Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. *J Clin Endocrinal Metab* 2003;88:185–191.

44. Vieth R, Cole D, Hawker G, Trang H, Rubin L. Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it. *Eur J Clin Nutr* 2001;55:1091–1097.

45. Heaney R, Abrams S, Dawson B, et al. Peak bone mass. *Osteoporos Int* 2001;11:985–1009.

46. Gloth FM, Tobin JD, Sherman SS, Hollis BW. Is the recommended daily allowance for vitamin D too low for the homebound elderly? *J Am Geriatric Soc* 1991;39:137–141.

47. Lips P, Wiersinga A, Van Ginkel FC, et al. The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. *J Clin Endocrinol Metab* 1988;67:644–650.

48. Gloth FM, Gundberg CM, Holllis BW, et al. Vitamin D deficiency in homebound elderly persons. *JAMA* 1995;274:1683–1686.

49. Bischoff-Ferrari H, Dietrich T, Orav E, Dawson-Hughes B. Positive association between 25(OH)D levels and bone mineral density: A population-based study of younger and older adults. *Am J Med* 2004;116:634–639.

50. Meier C, Woitge H, Witte K, et al. Supplementation with oral vitamin D_3 and calcium during winter prevents seasonal bone loss: A randomized controlled open-label prospective trial. *J Bone Mineral Res* 2004;19:1221–1230.

51. Chiu K, Chu A, Go V, Soad M. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 2004;79:820–825.

52. Liu P, Stenger S, Li H, et al. Triggering of vitamin D receptor-dependent antimicrobial response by human toll like receptor 2/1. *Science* 2006;311:1770–1772.

53. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin sufficiency: Implications for establishing a new effective DRI for vitamin D. *J Nutr* 2005;135:317–322.

54. Merlino L, Curtis J, Mikuls T, et al. Vitamin D intake is inversely associated with rheumatoid arthritis. *Arthritis Rheum* 2004;50(1):72–77.

55. Holick MF. Vitamin D: Importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* 2004;79:362–371.

56. Fronczak CM, Barón AE, Chase HP, et al. In utero dietary exposures and risk of islet autoimmunity in children. Diabetes Care 2003;26:3237–3242.

57. Hypponen E, Laara E, Reunanen A, et al. Intake of vitamin D and risk of type 1 diabetes: A birth-cohort study. *Lancet* 2001;358:1500–1503.

58. Zamora SA, Rizzoli R, Belli DC, Slosman DO, Bonjour JP. Vitamin D supplementation during infancy is associated with higher bone mineral mass in prepubertal girls. *J Clin Endocrinol Metab* 1999;84:4541–4543.

59. Garland C, Comstock G, Garland F, et al. Serum 25(OH)D and colon cancer: Eight-year prospective study. *Lancet* 1989;2:1176–1178.

60. Garland F, Garland C, Gorham E, Young J. Geographic variation in breast cancer mortality in the United States: A hypothesis involving exposure to solar radiation. *Prev Med* 1990;19:614–622.

61. Lefkowitz E, Garland C. Sunlight, vitamin D, and ovarian cancer mortality rates in US women. *Int J Epidemiol* 1994;23:1133–1136.

62. Platz EA, Hankinson SE, Hollis BW, et al. Plasmas 1,25-dihydroxy-and 25-hydroxyvitamin D and adenomatous polyps of the distal colorectum. *Cancer Epidemiol Biomarkers Prev* 2000;9:1059–1065.

63. Prabhala A, Garg R, Dandona P. Severe myopathy associated with vitamin D deficiency in western New York. *Arch Internal Med* 2000;160:1199–1203.

64. Gloth FM, Alam W, Hollis BW. Vitamin D versus broad-spectrum phototherapy in the treatment of seasonal affective disorder. *J Nutr Hlth Aging* 1999;3:5–7.

65. Krall EA, Wehler C, Garcia RI, et al. Calcium and vitamin D supplements reduce tooth loss in the elderly. *Am J Med* 2001;111(6):452–456.

66. Dietrich T, Joshipura KJ, Dawson-Hughes B, Bischoff-Ferrari HA. Association between serum concentrations of 25-hydroxyvitamin D3 and periodontal disease in the US population. *Am J Clin Nutr* 2004; 80(1):108–113.

67. Munger K, Zhang S, O'Reilly E, et al. Vitamin D intake and incidence of multiple sclerosis. *Neurology* 2004;62:60–65.

68. Hayes CE. Vitamin D: A natural inhibitor of multiple sclerosis. *Proc Nutr Soc* 2000;59:531–535.

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3. L A Basile, S N Taylor, C L Wagner, L Quinones, B W Hollis. 2007. Neonatal vitamin D status at birth at latitude 32°72′: evidence of deficiency. *Journal of Perinatology* **27**:9, 568. [CrossRef]