Efficacy of daily and monthly high-dose calciferol in vitamin D–deficient nulliparous and lactating women[1–3]

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ABSTRACT

Background: We previously found a high prevalence of vitamin D deficiency and low medication regimen compliance in Arab and East Indian women residing in the United Arab Emirates (UAE). The appropriate dosing regimen for improving vitamin D status in this population is not known.

Objective: We aimed to determine the efficacy of daily and monthly supplementation with vitamin D_2, the only high-dose calciferol available in the UAE, in lactating and nulliparous women.

Design: Healthy lactating ($n = 90$) and nulliparous ($n = 88$) women were randomly assigned to consume 2000 IU vitamin D_2/d or 60 000 IU vitamin D_2/mo for 3 mo. Serum 25-hydroxyvitamin D [25(OH)D] concentrations were measured by radioimmunoassay at baseline and every month.

Results: Most women had vitamin D deficiency [ie, 25(OH)D $<$ 50 nmol/L] at study entry. Mean $\pm$ SD 25(OH)D concentrations at 3 mo were significantly higher than baseline in both lactating (39.8 $\pm$ 12.4 and 25.2 $\pm$ 10.7 nmol/L, respectively) and nulliparous (40.4 $\pm$ 23.4 and 19.3 $\pm$ 12.2 nmol/L, respectively) women ($P < 0.001$ for both). In total, vitamin D supplementation was effective in achieving serum 25(OH)D concentrations of $\geq$50 nmol/L in 21 (30%) of 71 women at endpoint.

Conclusions: Oral vitamin D_2 supplementation with 2000 IU/d or 60 000 IU/mo for 3 mo was safe, and it increased serum 25(OH)D concentrations significantly; however, only a small proportion of the women studied achieved concentrations of $\geq$50 nmol/L. This suggests that, when sunlight exposure is limited, doses of vitamin D_2 higher than those currently studied may be needed. Monthly dosing appears to be a safe and effective alternative to daily dosing. *Am J Clin Nutr* 2007;85:1565–71.

KEY WORDS Vitamin D deficiency, 25-hydroxyvitamin D, 25(OH)D, lactating women, nulliparous women, Arab women

INTRODUCTION

Several studies have shown that women from the Middle East and women from the Indian subcontinent generally have low serum concentrations of 25-hydroxyvitamin D [25(OH)D], mostly as a result of low vitamin D intake and inadequate sunlight exposure (1–8). Infantile vitamin D deficiency rickets also is common in many Middle Eastern and Asian countries (9, 10). Exclusively breastfed infants who lack adequate sunshine exposure and whose mothers have low vitamin D stores are frequently vitamin D deficient and thus at high risk of nutritional rickets (11, 12). Measures to prevent vitamin D deficiency include greater exposure of skin to sunlight, greater fortification of food items with vitamin D, and vitamin D supplementation. Many Middle Eastern and East Indian women residing in the United Arab Emirates (UAE) maintain a very conservative style of dress that covers most of the body when they are outside, which limits sunlight exposure. In addition, vitamin D fortification of food is not mandatory in the UAE and many other Middle Eastern countries, and the current dietary intake of vitamin D is relatively low (8). Vitamin D supplementation currently remains the most appropriate mode for improving the vitamin D status of this high-risk population.

There is mounting evidence that, in the absence of adequate exposure to sunlight, $\geq$1000 IU dietary or supplemental vitamin D/d is required in adults to prevent vitamin D deficiency (13–17). However, a critical factor affecting the outcome of such treatment is adherence to medication. Our clinical experience indicates a low compliance with vitamin D supplement use in women in the UAE. In a survey of prenatal multivitamin supplement use in the UAE, only 40% of Middle Eastern women who delivered at term in a maternity hospital reported using their prescribed prenatal vitamins in the last trimester of pregnancy (18). Intermittent high-dose regimens could overcome low compliance. A dose of 50 000 IU vitamin D_2/d has been suggested as effective in maintaining acceptable vitamin D status (19). Because the biological half-life of 25(OH)D is effectively 1–2 mo (15), dosing less than once every 2 mo may generate large fluctuations in 25(OH)D concentrations that may not be desirable or effective (20). Our objective was to determine the effectiveness and safety of 2 vitamin D supplementation regimens (a daily dose of 2000 IU or a monthly dose of 60 000 IU oral vitamin D_2) in improving the vitamin D status of a convenience sample of lactating and

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nulliparous women residing in the UAE. We chose to study this relatively high dose of vitamin D because our previous studies showed a high prevalence of severe vitamin D deficiency in this population (8, 11). Furthermore, daily dietary intake of 2000 IU of vitamin D_3 or of a combination of vitamin D_2 and vitamin D_3 was previously reported to be safe and effective in improving vitamin D status in lactating women (21, 22). In the present study, we used vitamin D_2, rather than the more potent vitamin D_3, because the former is the only high-dose calciferol available in the UAE. We are not aware of any studies of this dose in nonlactating women of childbearing age. For ethical reasons, we did not want to use vitamin D doses > 2000 IU/d so as not to exceed the current upper tolerable safe intake level for healthy adults recommended by the Institute of Medicine's Food and Nutrition Board (23), especially when baseline serum 25(OH)D concentration results would not be available until the supplementation dose had been administered. Our hypothesis was that the monthly regimen of vitamin D supplementation would be as effective as the daily regimen in improving vitamin D status and that it would therefore be a reasonable alternative strategy, especially for patients from whom poor compliance is anticipated.

SUBJECTS AND METHODS

Subjects

We recruited 88 generally healthy, nulliparous Emirati women of reproductive age (many of whom were medical students and interns working at Tawam Hospital in Al Ain, UAE) and 90 lactating women (15 Emirati, 61 other Middle Eastern, and 14 East Indian) at the time of their first postnatal visit to the Maternal and Child Health Center in Al Ain (latitude 24°N and longitude 55°E). Lactating women were eligible if they planned to continue breastfeeding for the next 3 mo. Exclusion criteria included current pregnancy, history of metabolic bone disease or calcium disorder, and treatment with vitamin D (other than multivitamins) within the past 1 y. Enrollment began in September 2005 and ended in February 2006.

Subjects received both oral and written information and gave written informed consent. The study protocol was approved by the Human Research Ethics Committee of the Al Ain Medical District.

Study design

The study was an open-label, randomized, parallel-group clinical trial of lactating and nulliparous women. Subjects in each group were randomly allocated to a dose of either 2000 IU vitamin D_2/d (daily regimen) or 60 000 IU vitamin D_2/mo (monthly regimen) in a 1:1 ratio within permuted blocks of size 10. A sample size of 40 subjects per regimen (daily or monthly) was estimated to provide sufficient power (80%) to detect a 10-nmol/L difference in the increase in 25(OH)D concentrations between the 2 regimens. This estimated sample size allowed for a maximum attrition rate of 40%, and Bonferroni's correction for multiple comparisons was applied to the 2-tailed nominal α level of 0.05 when appropriate. The 60 000-IU vitamin D_2 dose was ingested at the time of each visit under direct observation to ensure compliance and prevent accidental ingestion by small children at home. The 2000-IU vitamin D_2 dose was provided in a tamper-resistant container of 90 capsules containing 2000 IU vitamin D_2 each. At 0, 1, 2, and 3 mo of daily vitamin D_2 supplementation and just before the administration of the next monthly vitamin D_2 dose, a nonfasting random urine sample was collected; 10 mL of blood also was collected at the visit, and the serum was separated and frozen at −80 °C until biochemical testing was performed. At the time of each subsequent visit, the vitamin D_2 capsules that remained in the container were counted to monitor compliance. Compliance was based on data collected at the time of last follow-up. All subjects also received supplemental calcium (600 mg elemental Ca/d). Subjects were free to withdraw from the study at any time. A few subjects returned for follow-up visits but declined laboratory tests; their data were not used in the analysis.

Questionnaire

After providing written informed consent, eligible women were interviewed in person at each of the 2 study sites (Tawam Hospital or the Maternal and Child Health Center) by 1 observer who used a questionnaire regarding medical history and reproductive, nutritional, and lifestyle factors, including the average amount (in time) of sunlight exposure per day during the 6 wk preceding the visit and the body parts usually directly exposed to the sun while outdoors. A dietary recall using a food-frequency questionnaire estimated daily dietary calcium and vitamin D intake in each subject as described previously (8). Clinical symptoms that have been associated with vitamin D deficiency—muscle pain, muscle cramps, difficulty in ascending a staircase or rising from a chair, gait disturbance, and paresthesia of the hands and feet—were evaluated at the beginning and end of the study.

Materials

Capsules containing 2000 IU vitamin D_2 each were purchased from Tishcon Corp (Westbury, NY). Vitamin D_2 tablets containing 10 000 IU vitamin D_2 each were purchased from Celltech Manufacturing Services Ltd (Ashton-under-Lyne, United Kingdom), and vitamin D_2 gelatin capsules containing 50 000 IU vitamin D_2 each were purchased from Pliva, Inc (East Hanover, NJ). Because the 2000-IU vitamin D_2 preparation was not a marketed product, we asked the supplier to provide a certificate of analysis. The product was assayed on 1 April 2005 and found to contain 2367.6 IU vitamin D_2/capsule.

Tests

Serum calcium and urinary calcium and creatinine were measured with the use of an autoanalyzer (Beckman Coulter DXC800; Beckman Instruments, Inc, Fullerton, CA) immediately after sample collection at the laboratories of the 2 study sites. Urinary calcium excretion was assessed as the ratio of urinary calcium to creatinine concentrations (urinary Ca:Cr, in mmol/mmol); a ratio of >1.0 was considered to represent hypercalciuria (24). Serum 25(OH)D concentrations were measured by radioimmunoassay (DiaSorin, Stillwater, MN). The intrassay and interassay CVs were 8.3% and 3.2%, respectively. Serum 25(OH)D concentrations <50 nmol/L (20 ng/mL) were considered to indicate vitamin D deficiency according to published reports in the literature (19, 25). For quality control, 10 baseline serum samples were shipped on dry ice to the General Clinical Research Center at the Medical University of South Carolina, where 25(OH)D concentrations were measured with the use of HPLC. Serum intact parathyroid hormone (PTH) was

TABLE 1
Characteristics of lactating women by treatment regimen[1]

Characteristic	Daily regimen ($n = 45$)	Monthly regimen ($n = 45$)	P^2
Ethnicity			
UAE (%)	8.9 [4][3]	24.4 [11]	0.09
Other Arab (%)	71.1 [32]	64.4 [29]	0.7
East Indian (%)	20.0 [9]	11.1 [5]	0.4
Age (y)	29.2 ± 5.5[4]	29.9 ± 6.7	0.6
Weight (kg)	72.6 ± 11.6	70.1 ± 16.1	0.4
BMI (kg/m^2)	29.0 ± 4.8	27.9 ± 6.1	0.4
Parity (median)	3.0	3.0	0.9
Sunlight exposure (min/d)	1.0 ± 3.8	0.4 ± 0.6	0.3
Proportion with face and hands exposed to sunlight (%)	51.2	40.9	0.4
Proportion reporting use of multivitamins (%)	41.9	50.0	0.5
Vitamin D intake (μ/d)	4.8 ± 2.8	4.8 ± 3.9	0.9
Calcium intake (mg/d)	652 ± 394	601 ± 397	0.5
Basal 25(OH)D concentration (nmol/L)	27.3 ± 10.4	23.2 ± 10.7	0.07

[1] UAE, United Arab Emirates; 25(OH)D, 25-hydroxyvitamin D.
[2] P value by tailed t tests for continuous variables and by chi-square tests for categorical variables.
[3] n in brackets (all such values).
[4] $\bar{x} \pm$ SD (all such values).

measured by using an immunoradiometric assay (Diagnostic Products Corporation, Los Angeles, CA). The intra- and interassay CVs were 6% and 5.1%, respectively.

Statistical analysis

Data were analyzed by using both SPSS (version 13; SPSS Inc, Chicago, IL) and SAS (version 8.01; SAS Institute, Cary, NC) statistical software. Methods used were Student's t test, chi-square test, analysis of variance (ANOVA), and analysis of covariance (ANCOVA). Adjustments for multiple comparisons were carried out with Tukey's test for post hoc pairwise comparisons. A 2-tailed $P < 0.05$ was considered significant. Paired binomial observations were analyzed by using McNemar's test. To compare the effects of regimen and reproductive status on follow-up serum 25(OH)D concentrations, a mixed-model ANCOVA was employed by using SAS PROC MIXED, with "individual" specified as a repeated effect and with time, regimen, and reproductive status as categorical (class) covariates; baseline 25(OH)D concentrations and body weight were used as covariates to adjust for baseline differences.

RESULTS

Of the 90 lactating women enrolled initially, 69 completed 1 mo of the study, 58 completed 2 mo of the study, and 48 completed the 3-mo study. Reasons for withdrawal included pregnancy (1 subject), diarrhea in the breastfed infant (1 subject), leaving the country (3 subjects), and no specific reason (the remainder of those who withdrew). Of the 88 nulliparous women enrolled initially, 55 completed 1 mo of the study, 27 completed 2 mo of the study, and 23 completed the 3-mo study. Most subjects who withdrew from the study were contacted, and none gave any specific reason for withdrawal. Comparison of the baseline characteristics of the women who completed the study and those who dropped out found no significant differences in age, ethnicity profile, number of pregnancies, vitamin D intake, and baseline 25(OH)D concentrations between the 2 groups (data not shown). Serum concentrations of 25(OH)D were measured by both radioimmunoassay and HPLC in 10 baseline samples. The measurement by radioimmunoassay (18.7 ± 11.6 nmol/L) did not differ significantly ($P = 0.12$, t test) from that by HPLC (14.8 ± 16.7 nmol/L). The 2 measurements were strongly positively correlated ($r = 0.93$, $P < 0.01$).

Lactating women

Forty-five lactating women were allocated to each of the 2 regimens. No significant differences in baseline characteristics were seen, which indicated effective randomization (**Table 1**). All women who were assigned daily vitamin D took more than two-thirds of the prescribed capsules (median: 100%; range: 67–100%). Overall mean baseline serum 25(OH)D concentrations were 25.2 ± 10.7 nmol/L. All women except one had 25(OH)D concentrations <50 nmol/L, and one-third had concentrations <20 nmol/L. The mean baseline serum 25(OH)D concentration in Emirati women was lower than that in other Middle Eastern and East Indian women, but these differences were not significant (21.7 ± 12.4, 26.0 ± 10.1, and 25.7 ± 11.6 nmol/L, respectively; $P = 0.6$).

Serum 25(OH)D concentrations increased significantly from baseline in the daily and monthly supplementation groups ($P <$ 0.001 by ANOVA; **Table 2**). The highest 25(OH)D concentration achieved was 75.2 nmol/L. The mean increments from baseline in serum 25(OH)D concentrations at 3 mo in the daily (12.4 ± 13.9 nmol/L) and monthly (14.8 ± 10.8 nmol/L) regimens did not differ significantly ($P = 0.5$). The mean observed increment in serum 25(OH)D concentration per 100 IU vitamin D_2 administered was 0.7 nmol/L.

Vitamin D supplementation was effective at ensuring 25(OH)D concentrations of ≥50 nmol/L at 3 mo in 8 (35%) of 23 women on the daily regimen and 5 (20%) of 25 women on the monthly regimen. When lactating women with baseline 25(OH)D concentrations <20 nmol/L were excluded from the efficacy analysis, the efficacy rates increased only marginally—to 40% for the daily regimen and 31% for the monthly regimen. Serum calcium concentrations increased during follow-up but did not exceed the upper limit of normal in any

TABLE 2
Biochemical variables at baseline and follow-up in lactating women according to treatment regimen[1]

	Time (mo)				
	0	1	2	3	*P*
Subjects (*n*)					
Daily regimen	45	35	28	23	
Monthly regimen	45	34	30	25	
Serum 25(OH)D (nmol/L)					
Daily	27.3 ± 10.4[2,a]	34.3 ± 12.6[b]	43.0 ± 13.1[c]	42.2 ± 13.9[c]	<0.01
Monthly	23.2 ± 10.7[a]	29.5 ± 10.1[b]	35.4 ± 9.5[c]	37.6 ± 10.5[c]	<0.01
Serum PTH (pmol/L)					
Daily	4.3 ± 2.6[a]	4.2 ± 1.8[a]	4.0 ± 1.6[a]	6.3 ± 3.2[b]	<0.01
Monthly	6.1 ± 4.3[a]	5.1 ± 2.9[a,b]	4.1 ± 2.0[b]	6.1 ± 3.6[a]	0.03
Serum calcium (mmol/L)					
Daily	2.3 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.4 ± 0.1	NS
Monthly	2.2 ± 0.2[a]	2.3 ± 0.1[b]	2.4 ± 0.1[b]	2.3 ± 0.1[b]	<0.01
Urinary calcium (mmol/mmol creatinine)					
Daily	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	NS
Monthly	0.1 ± 0.1[a]	0.2 ± 0.2[a,b]	0.2 ± 0.3[b]	0.1 ± 0.2[a,b]	0.04

[1] 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.
[2] $\bar{x}$ ± SD (all such values). Means in a row with different superscript letters are significantly different, $P < 0.05$ (Tukey test).

subject (normal range: 2.2–2.68 mmol/L; Table 2). The mean Ca:Cr excretion increased slightly in the monthly regimen group, and one subject had a ratio >1.0 mmol/mmol (Table 2). The mean serum PTH concentration showed no significant decrease from baseline except the measurement at 2 mo in the monthly regimen group ($P < 0.05$; Table 2).

Nulliparous women

Forty-three nulliparous women were allocated to the daily regimen, and 45 nulliparous women were allocated to the monthly regimen. The baseline characteristics of nulliparous women by treatment regimen are shown in **Table 3**. No significant differences in baseline characteristics were seen, which indicated effective randomization. All but 1 of the women assigned to the daily vitamin D regimen took more than two-thirds of the prescribed capsules (median: 100%; range: 50–100%). The overall mean baseline serum 25(OH)D concentration was 19.3 ± 12.2 nmol/L. All but 1 of the women had 25(OH)D concentrations <50 nmol/L, and two-thirds had concentrations <20 nmol/L.

Serum 25(OH)D concentrations increased significantly from baseline in the daily and monthly supplementation groups ($P <$ 0.001 by ANOVA; **Table 4**). The highest 25(OH)D concentration achieved was 96.5 nmol/L. The mean increments from baseline in serum 25(OH)D concentration at 3 mo in the daily (23.7 ± 20.9 nmol/L) and monthly (23.1 ± 26.3 nmol/L) regimens did not differ significantly ($P = 0.95$). The mean observed increment in serum 25(OH)D concentration per 100 IU vitamin D_2 administered was 1.2 nmol//L.

Vitamin D supplementation was effective at ensuring 25(OH)D concentrations of ≥50 nmol/L at 3 mo in 4 (36%) of 11 women on the daily regimen and in 4 (33%) of 12 women on the monthly regimen. When nulliparous women with baseline 25(OH)D concentrations <20 nmol/L were excluded from the efficacy analysis, the efficacy rates increased only marginally—to 50% for the daily regimen and 40% for the monthly regimen. Serum calcium concentrations increased during follow-up but did not exceed the upper limit of normal in any subject (Table 4). There was no significant change in the Ca:Cr excretion, but a ratio of >1.0 was seen in 1 subject in each of the

TABLE 3
Characteristics of nulliparous women by treatment regimen[1]

Characteristic	Daily regimen ($n = 43$)	Monthly regimen ($n = 45$)	P^2
Age (y)	23.0 ± 5.2[3]	24.6 ± 5.1	0.2
Weight (kg)	62.9 ± 17.5	59.9 ± 12.9	0.4
BMI (kg/m^2)	24.4 ± 6.9	24.2 ± 5.5	0.8
Sunlight exposure (min/d)	4.0 ± 10.0	6.6 ± 16.2	0.3
Proportion with face and hands exposed to sunlight (%)	73.8	63.0	0.4
Proportion reporting use of multivitamins (%)	7.3	6.5	1.0
Vitamin D intake (μ/d)	3.4 ± 2.3	3.6 ± 2.9	0.8
Calcium intake (mg/d)	416 ± 345	582 ± 496	0.07
Basal 25(OH)D concentration (nmol/L)	19.6 ± 12.4	19.0 ± 12.3	0.8

[1] 25(OH)D, 25-hydroxyvitamin D.
[2] *P* value by tailed *t* tests for continuous variables and by chi-square tests for categorical variables.
[3] $\bar{x}$ ± SD (all such values).

TABLE 4
Biochemical variables at baseline and follow-up in nulliparous women according to treatment regimen[1]

	Time (mo)				
	0	1	2	3	*P*
Subjects (n)					
Daily	43	26	13	11	
Monthly	45	29	14	12	
Serum 25(OH)D (nmol/L)					
Daily	19.6 ± 12.2[2,a]	33.6 ± 12.4[b]	56.2 ± 18.2[c]	41.7 ± 26.5[b]	<0.01
Monthly	19.0 ± 12.3[a]	23.5 ± 8.6[a]	45.8 ± 16.2[b]	39.2 ± 21.4[b]	<0.01
Serum PTH (pmol/L)					
Daily	6.7 ± 3.0	6.1 ± 2.4	4.8 ± 1.8	5.5 ± 2.5	NS
Monthly	7.5 ± 3.4	6.5 ± 2.1	6.5 ± 2.5	6.9 ± 1.8	NS
Serum calcium (mmol/L)					
Daily	2.3 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	NS
Monthly	2.3 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	NS
Urinary calcium (mmol/mmol creatinine)					
Daily	0.3 ± 0.2	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	NS
Monthly	0.3 ± 0.2	0.4 ± 0.3	0.3 ± 0.2	0.4 ± 0.3	NS

[1] 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.
[2] $\bar{x}$ ± SD (all such values). Means in a row with different superscript letters are significantly different, $P < 0.05$ (Tukey test).

2 regimens (Table 4). There was no significant change in serum PTH concentration (Table 4).

Comparisons between lactating and nulliparous women

The mean baseline serum 25(OH)D concentration was significantly lower in nulliparous than in lactating women (19.3 ± 12.2 and 25.2 ± 10.7 nmol/L, respectively; $P < 0.001$). Almost one-third of nulliparous women and one-half of lactating women dressed to cover the whole body, including hands and face, while outside of their homes, and only a few exposed more than their face and hands. A small proportion of the nulliparous women reported intake of multivitamin (6.9%) or calcium (4.7%) supplements. In contrast, 46% of lactating women reported multivitamin intake, and 27% reported supplemental calcium intake. Dietary vitamin D and calcium intakes also were significantly ($P < 0.05$, *t* test) higher in lactating women. In nulliparous women, muscle pain was reported in 33%, muscle cramps in 30%, difficulty in ascending a staircase or rising from a chair in 13%, gait disturbance in 1%, and paresthesia of hands and feet in 25% at study entry. In contrast, the reported rates for the above symptoms in lactating women were much lower: 16%, 9%, 13%, 1%, and 8%, respectively. A total of 25 nulliparous and lactating women completed a questionnaire on clinical symptoms at the end of the study. The reported frequency of clinical symptoms decreased significantly only for muscle cramps (66% compared with 4%; $P < 0.05$, McNemar's test). Overall, 50% of women felt better at the end of the study, 50% felt the same, and none felt worse. The improvement in symptoms corresponded with a significant increase in serum 25(OH)D concentrations (from 21.0 ± 12.3 to 40.2 ± 23.0 nmol/L; $P < 0.01$).

To compare the effects of regimen and reproductive status (nulliparous or lactating) on follow-up 25(OH)D concentration, we used SAS PROC MIXED as described in Methods, in which baseline 25(OH)D concentrations and body weight were used as covariates to adjust for baseline differences (**Table 5**). Overall, the daily regimen resulted in higher mean follow-up 25(OH)D concentrations than did the monthly regimen (estimated difference ± SE: 4.8 ± 1.8 nmol/L; $P < 0.01$). Lactating women had slightly lower mean follow-up 25(OH)D concentrations than did nulliparous women (estimated difference ± SE: −3.6 ± 1.9 nmol/L; $P = 0.064$).

DISCUSSION

Our data confirm previous studies showing a high prevalence of severe vitamin D deficiency in Arab and East Indian women residing in the UAE (8, 11). Vitamin D_2 supplementation with 2000 IU/d or 60 000 IU/mo resulted in significant increases in

TABLE 5
Effects of regimen, reproductive status, and selected covariates on follow-up serum 25(OH)D concentration[1]

	Regression coefficient of estimates	SE	*P*
Intercept	43.6	4.6	<0.001
Factors			
Regimen			
Daily regimen	4.83	1.78	0.0075
Monthly regimen	0 (reference)	—	—
Reproductive status			
Lactating status	−3.6	1.93	0.064
Nulliparous status	0 (reference)	—	—
Time			
1 mo	−10.9	1.7	<0.001
2 mo	2.44	1.8	0.18
3 mo	0 (reference)	—	—
Overall trend	—	—	<0.001
Covariate			
Baseline 25(OH)D (nmol/L)	0.46	0.08	<0.001
Weight (kg)	−0.2	0.06	0.0018

[1] 25(OH)D, 25-hydroxyvitamin D. Expected mean values, according to the model, can be calculated by using this table. For example, a nulliparous woman weighing 60 kg and with a baseline 25(OH)D concentration of 25 nmol/L who is treated with a daily vitamin D_2 regimen will be expected to have a mean 25(OH)D concentration at 2 mo of 43.6 + 4.83 + 0 + 2.44 + (0.46 × 25) − (0.2 × 60) = 50.37 nmol/L, where "25" refers to the baseline 25(OH)D concentration, and "60" refers to the person's weight.

serum 25(OH)D concentrations in lactating and nulliparous women at 3 mo. The mean increment observed in 25(OH)D concentration was slightly higher than that reported by Cooper et al (26), who found that the administration of 10 000 IU vitamin D_2/wk for 2 y resulted in an increment of only 5.3 ± 18.1 nmo/L (or 0.4 nmol/L per 100 IU vitamin D_2) in serum 25(OH)D concentrations in the first year and a decline toward baseline concentrations in the second year. The difference between the findings of that study and those of the present study may be partly explained by the fact that subjects in the study by Cooper et al had healthy baseline 25(OH)D concentrations. The response to treatment is inversely related to baseline vitamin D status (27), probably as a result of mechanisms that accelerate metabolic clearance of vitamin D when serum concentrations of 25(OH)D begin to rise (26–28). The increments in serum 25(OH)D concentrations noted in the present study are substantially lower, however, than those reported for equimolar doses of vitamin D_3 (20, 27, 29). This difference was not unexpected, because recent reports showed that vitamin D_3 supplementation is more effective than is vitamin D_2 supplementation in increasing the serum 25(OH)D concentration (30, 31). The dose of vitamin D_2 used in our study was effective at achieving serum 25(OH)D concentrations of ≥50 nmol/L in only 20–36% of subjects, which suggests that, when sunlight exposure is limited and the more potent vitamin D_3 preparation is not available, higher doses of vitamin D_2 than currently studied may be needed. The safety of higher doses of vitamin D_2, however, should be studied further. None of our subjects experienced hypercalcemia or hypervitaminosis D. However, there were 3 episodes of Ca:Cr excretion >1.0 mmol/mmol. Our results are similar to those of other studies showing no or very few episodes of hypercalciuria in response to treatment with 1000–4000 IU oral vitamin D/d (21, 22, 24). Until the implications of these few episodes of hypercalciuria are determined, it is prudent to monitor urinary calcium excretion ratios in subjects treated with such high doses of vitamin D.

Currently, no increase in the vitamin D intake of lactating women is recommended by the Food and Nutrition Board (23) except when milk and other foods fortified with vitamin D are avoided, in which case a daily supplement of 10 μg (400 IU) vitamin D_3 is recommended (32). Our results in lactating women are in agreement with those of others (21, 22, 33), which indicates that a much higher vitamin D intake than currently recommended is needed in lactating women. We are not aware of any studies comparing vitamin D requirements in lactating and nonlactating women. However, the higher incremental change observed in nulliparous women in the present study even after adjustment for body weight and baseline 25(OH)D concentration leads us to suggest that lactating women need a higher dose of vitamin D to obtain a similar increase in serum 25(OH)D concentration.

The increases from baseline serum 25(OH)D concentration at 3 mo in lactating and nulliparous women did not differ significantly between the daily and monthly regimens. However, when lactating and nulliparous women were considered together, the daily regimen resulted in significantly higher serum 25(OH)D concentrations than did the monthly regimen in the multiple regression model after adjustment for baseline 25(OH)D concentration. We are not aware of any previous randomized studies addressing this issue. The observed difference between daily and monthly dosing could be related to different clearance patterns and could be influenced by differences in blood sampling time. Because we drew blood samples just before the next dose, the reported measurements for serum concentrations of 25(OH)D in the monthly regimen group were trough values that may not be truly representative of the average 25(OH)D concentrations typically present during the month after each dose. For example, after the administration of a single 50 000-IU dose of vitamin D_2, Armas et al (31) found that the peak serum 25(OH)D concentration occurred on day 3; the concentration then began to fall until, by day 14, it did not differ significantly from baseline. Thus, the actual concentration achieved by the monthly regimen may be higher than that which we reported, which suggests that the observed differences between daily and monthly dosing regimens may be smaller. Although the median rate of compliance with the daily dose in the present study was high, in our clinical experience, compliance rates are much lower (18). Compliance rates are known to be high (90–100%) in clinical trials because of patient selection and the special attention given to the study patients to encourage their adherence to the medication regimens (34). The special attention to compliance in our study may have encouraged adherence to medication. However, if poor compliance is anticipated, monthly supplementation could be a safe and effective alternative option.

Vitamin D deficiency was significantly more severe in the nulliparous than in the lactating women. This difference could be attributed, in part, to higher intakes of vitamin D and multivitamin supplement in the lactating women. Vitamin D deficiency causes osteomalacia, which is associated with nonspecific bone pain, muscle aches, and weakness. These symptoms were highly prevalent, especially in nulliparous women, in the present study, and they tended to improve with treatment. Similar findings were reported in Danish women of Middle Eastern descent who presented with muscle pains and weakness and who were found to have severe vitamin D deficiency (mean serum 25(OH)D concentration: 6.7 nmol/L) and osteomalacia (13). In addition, all isometric muscle-strength measurements in that group of vitamin D–deficient Danish women of Middle Eastern background were significantly lower than those in healthy Danish control subjects with mean serum 25(OH)D concentrations of 47 nmol/L (35). After 3 mo of vitamin D treatment, all muscle-related variables improved significantly in the Middle Eastern women in that study.

Some limitations of the present study deserve comment. First, the dropout rates were very high. This is unlikely to have affected our results because the baseline characteristics of the subjects who dropped out did not differ significantly from the characteristics of those who completed the study. Second, we used vitamin D_2, which could have contributed to the lower increments in serum 25(OH)D concentrations than are likely to be seen with vitamin D_3 supplementation. In addition, not all of the doses of vitamin D_2 used in the present study were independently tested for their true vitamin D content, and therefore the differences between the responses to the daily and monthly regimens may not be accurate. Third, the study included neither a control group taking the current recommended Dietary Reference Intake of 400 IU vitamin D_3 nor a reference group to account for temporal changes in serum 25(OH)D concentrations related to season. Our previous studies show no significant seasonal variation in 25(OH)D concentrations between September and May in the UAE, where there is abundant sunshine year-round (8). Despite the above limitations, we believe that our results are valid and that they provide important information with which to address

appropriate dosing regimens to improve the vitamin D status of women of childbearing age and lactating mothers.

In conclusion, vitamin D_2 supplementation with 2000 IU/d or 60 000 IU/mo for 3 mo increased serum 25(OH)D concentrations significantly, but these concentrations became acceptable ($\geq$50 nmol/L) in only a small proportion of the women studied. In the absence of adequate sunlight exposure and if the more potent vitamin D_3 preparation is not available, higher doses of vitamin D_2 than currently studied may be needed. Monthly dosing appears to be a safe and an effective alternative to daily dosing, especially in patients from whom poor compliance is anticipated.

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