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KEY WORDS

vitamin D, 25-hydroxyvitamin D, pregnancy, infancy, supplementation

ABBREVIATIONS

25(OH)D—25-hydroxyvitamin D
NZ—New Zealand
RDI—recommended dietary intake

Dr Grant conceived and designed the study, developed the data collection instruments, analyzed and interpreted the data, and completed the first and final drafts of the manuscript; Mr Stewart conceived and designed the study, analyzed and interpreted the data, reviewed and revised the manuscript, and approved the final manuscript as submitted; Dr Robert Scragg conceived and designed the study, developed the data collection instruments, critically reviewed the manuscript, and approved the final manuscript as submitted; Ms Milne and Ms Rowden developed the recruitment and retention strategy for the study, designed the data collection instruments, coordinated the collection of the data, and approved the final manuscript as submitted; Dr Ekeroma conceived and designed the study, developed the data collection instruments, and approved the final manuscript as submitted; Drs Wall and Crengle developed the data collection instruments, critically reviewed the manuscript, and approved the final manuscript as submitted; Dr Mitchell conceived and designed the study, developed the data collection instruments, reviewed and revised the manuscript, and approved the final manuscript as submitted; Dr Trenholme supervised the collection of safety data, critically reviewed the manuscript, and approved the final manuscript as submitted; Dr Crane developed the data collection instruments, reviewed and revised the manuscript, and approved the final manuscript as submitted; and Dr Camargo conceived and designed the study, developed the data collection instruments, analyzed and interpreted the data, reviewed and revised the manuscript, and approved the final manuscript as submitted.

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WHAT'S KNOWN ON THIS SUBJECT: A serum 25-hydroxyvitamin D (25(OH)D) concentration of 20 ng/mL meets the requirements of at least 97.5% of the population older than 1 year. A recommended dietary intake to achieve this serum 25(OH)D concentration has not been established during infancy.

WHAT THIS STUDY ADDS: Daily maternal (during pregnancy) and then infant vitamin supplementation with 1000/400 IU or 2000/800 IU increases the proportion of infants with 25(OH)D ≥20 ng/mL during infancy with the higher dose sustaining this increase for longer.

abstract

FREE

OBJECTIVE: To determine the vitamin D dose necessary to achieve serum 25-hydroxyvitamin D (25(OH)D) concentration ≥20 ng/mL during infancy.

METHODS: A randomized, double-blind, placebo-controlled trial in New Zealand. Pregnant mothers, from 27 weeks' gestation to birth, and then their infants, from birth to age 6 months, were randomly assigned to 1 of 3 mother/infant groups: placebo/placebo, vitamin D_3 1000/400 IU, or vitamin D_3 2000/800 IU. Serum 25(OH)D and calcium concentrations were measured at enrollment, 36 weeks' gestation, in cord blood, and in infants at 2, 4, and 6 months of age.

RESULTS: Two-hundred-and-sixty pregnant women were randomized. At enrollment, the proportions with serum 25(OH)D ≥20 ng/mL for placebo, lower-dose, and higher-dose groups were 54%, 64%, and 55%, respectively. The proportion with 25(OH)D ≥20 ng/mL was larger in both intervention groups at 36 weeks' gestation (50%, 91%, 89%, $P < .001$). In comparison with placebo, the proportion of infants with 25(OH)D ≥20 ng/mL was larger in both intervention groups to age 4 months: cord blood (22%, 72%, 71%, $P < .001$), 2 months (50%, 82%, 92%, $P < .001$), and 4 months (66%, 87%, 87%, $P = .004$), but only in the higher-dose group at age 6 months (74%, 82%, 89%, $P = .07$; higher dose versus placebo $P = .03$, lower dose versus placebo $P = .21$).

CONCLUSIONS: Daily vitamin D supplementation during pregnancy and then infancy with 1000/400 IU or 2000/800 IU increases the proportion of infants with 25(OH)D ≥20 ng/mL, with the higher dose sustaining this increase for longer. *Pediatrics* 2014;133:e143–e153

The Institute of Medicine, in 2011, determined that serum 25-hydroxyvitamin D (25(OH)D) concentrations ≥20 ng/mL meet the requirements of at least 97.5% of the population >1 year old.[1] The recommended dietary intake (RDI) during pregnancy was defined as 600 IU per day, but data were insufficient to allow an RDI to be defined for infants. Instead, an adequate intake of 400 IU per day was established, with this considered sufficient to maintain serum 25(OH)D in the range of 16 to 20 ng/mL.[1]

Vitamin D status at birth and during early infancy, when breast milk is the predominant source of nutrition, is determined by maternal vitamin D status.[2,3] Contemporary population- and primary care–based studies of pregnant women have shown a high prevalence of serum 25(OH)D <20 ng/mL in Asia (70%–96%),[4–6] Australia (10%–47%),[7–10] Europe (15%–44%),[11,12] the United Kingdom (49%–75%),[13–15] India (74%),[16] and the United States (37%).[17] Although less completely studied at the population level, in primary care–based studies from the United States, serum 25(OH)D <20 ng/mL is present in 11% to 12% of infants.[18,19]

It is difficult to meet the vitamin D RDI from dietary sources alone. For this reason, in some countries, including the United States, vitamin D is added to an increasing range of food products.[1,20] Determining the vitamin D intake that achieves a desired serum 25(OH)D concentration in this setting is difficult. In many countries though, vitamin D fortification of foods is not mandated, dietary sources of vitamin D are few, and routine vitamin D supplementation is not recommended.[21] Such countries, for example New Zealand (NZ), provide an opportunity to determine the relationship between vitamin D intake and serum 25(OH)D concentration.[22,23]

In 2010, we commenced enrollment of pregnant women in NZ into a randomized trial of vitamin D supplementation during pregnancy and infancy. We aimed to determine the vitamin D dose during late pregnancy and early infancy that safely and effectively increases serum 25(OH)D concentrations to ≥20 ng/mL in the first 6 months of infancy.

METHODS

Trial Design

We performed a randomized, double-blind, placebo-controlled multiarm parallel study. Pregnant mothers, from enrollment at 27 weeks' gestation to birth, and then their infants, from birth to age 6 months, were randomly and equally assigned, to 1 of 3 groups: placebo, or lower-dose or higher-dose vitamin D_3. Woman/infant pairs received a once-daily oral dose of placebo/placebo, vitamin D_3 1000 IU/400 IU, or vitamin D_3 2000 IU/800 IU.

Ethical approval was obtained from the regional NZ Ministry of Health ethics committee and written informed consent from all participating women. Registration was with the Australian NZ Clinical Trials Registry (ACTRN12610000483055).

Participants

Women were recruited from a community-based primary care maternity clinic in Auckland (latitude 36°S) from April 2010 to July 2011. Women were eligible if their estimated gestation was 26 to 30 weeks and they had a singleton pregnancy. We excluded women taking vitamin D supplementation >200 IU per day, those with a history of renal stones or hypercalcemia, or any serious pregnancy complication at enrollment.

Interventions

Each participant was instructed to take 1 drop per day of study medicine. For pregnant women, 1 drop contained placebo or 1000 IU or 2000 IU of vitamin D_3; for infants, 1 drop contained placebo or 400 IU or 800 IU of vitamin D_3.

Pregnant women took 1 drop per day of study medicine from enrollment until childbirth. They were instructed to then stop taking their study medicine and to start giving their infant 1 drop per day of the infant study medicine until the infant was 6 months old.

Infants admitted to the NICU did not start study medicine until they had discontinued prescribed vitamin D supplements. For those born prematurely, vitamin D supplement was prescribed during their NICU stay and for 3 months after hospital discharge. Infants in the NICU received 160 IU per day while receiving parenteral nutrition and 464 IU per day once orally fed.

Outcomes

The primary end points at study initiation were the proportion of infants achieving a serum 25(OH)D concentration ≥30 ng/mL during the first 6 months of infancy and the number of mothers and infants with hypercalcemia at any measurement point. The 25(OH)D cutoff of 30 ng/mL was chosen because at the time of study initiation this was considered to represent optimal vitamin D status.[24,25] Urinary calcium was not used as a safety measure because physiologic hypercalciuria occurs normally during pregnancy,[26] and urinary calcium excretion during infancy is widely variable.[27]

Sample Size

Sample size calculations were based on the primary study protocol objective of achieving a serum 25(OH)D concentration of 30 ng/mL. In healthy adults, serum 25(OH)D increases by approximately 0.7 ng/mL for every 100 IU per day of vitamin D_3 ingested.[28]

In NZ, the mean 25(OH)D concentration in women of childbearing age and in newborns is approximately 20 ng/mL (interquartile range: women 14–27 ng/mL; newborns 12–31 ng/mL).[23,29] Vitamin D 1000 IU per day was expected to increase average maternal 25(OH)D from 20 to 27 ng/mL and 2000 IU per day to increase 25(OH)D from 20 to

33 ng/mL. We anticipated that the actual increase could be smaller because of the vitamin D demands of the fetus.[25] We estimated that a difference in 25(OH)D concentration of 3 ng/mL between placebo and lower-dose vitamin D and 6 ng/mL between placebo and higher-dose vitamin D could be detected (80% power, α = 0.05) with 70 in each group. We aimed to enroll 260 to have 210 infants complete the study.

Randomization and Blinding

Allocation to the 3 study arms was by restricted randomization within blocks of variable size using a computer-generated randomization list. The allocation sequence was concealed from research staff involved in recruitment. The study statistician randomly allocated a treatment to each participant and labeled identical study medicine bottles such that study staff and participants were unaware of the treatment status.

Study medicine bottles were sequentially numbered with an identical numbering code used for each mother-infant pair. Bottles of study medicine were prepared by the Ddrops Company (Woodbridge, Ontario, Canada) with the study medicine bottles for the 3 groups being identical in color, shape, and volume and the study medicine identical in color, consistency, and taste.

Data Collection

Face-to-face interviews were completed with women at enrollment; at 36 weeks' gestation; and when their infant was 2, 4, and 6 months old. Data collected described demographics, adherence, supplement use, and infant feeding. Mothers were phoned at 2-weekly intervals to check adherence.

Venous (women and umbilical cord) and capillary (infant) blood samples were collected. Serum calcium concentration was measured and then samples were stored at –80°C until study completion. Serum 25(OH)D concentration was measured using isotope-dilution liquid chromatography–tandem mass spectrometry in a Vitamin D External Quality Assurance Scheme–certified laboratory.[30,31] Total serum calcium was measured using a colorimetric assay on an Abbott Diagnostic Architect instrument (Abbott Park, IL). Hypercalcemia was defined as an adjusted serum calcium concentration >10.4 mg/dL (women), >11.6 mg/dL (cord blood), and >11.2 mg/dL (infants).[32,33]

Statistical Methods

Analyses were performed on an intention-to-treat basis. The χ^2 test, *t* test, and analysis of variance were used for between-group comparisons. The treatment effect at 36 weeks in the mothers was assessed using linear regression in a model that included enrollment 25(OH)D concentration. The treatment effect in the infants was assessed using a linear mixed model with stage (cord [birth], and 2, 4, and 6 months of age) as a repeated measure using an unstructured covariance matrix. Interaction between stage and treatment was assessed first and on finding an interaction, each stage was analyzed separately using linear regression. The 3 treatment groups were compared using the 2 hypothesized, orthogonal contrasts: placebo versus vitamin D supplementation and 400 IU versus 800 IU supplementation.

All comparisons used 2-sided tests at a .05 level of significance. The null hypothesis for all analyses was that there is no difference between the study groups.

RESULTS

Of 404 pregnant women assessed, 260 were randomized to placebo (n = 87), lower-dose vitamin D_3 (n = 87), or higher-dose vitamin D_3 (n = 86) (Fig 1). Serum 25(OH)D concentration was measured on 259 women at enrollment; 228 (88%) women at 36 weeks' gestation; 200 (77%) cord blood samples; and 198 (76%), 189 (73%), and 221 (85%) infants at 2, 4, and 6 months of age, respectively.

Table 1 shows the characteristics of enrolled women. The proportions enrolled during summer, fall, winter, and spring were 0.23, 0.27, 0.26, and 0.24 respectively. At enrollment, 57% of the women were obese (BMI ≥30 kg/m^2).[34] At 36 weeks' gestation, 11 (5%) of the women were taking vitamin D supplements containing between 100 and 500 IU per dose.

Table 2 shows the characteristics of enrolled infants. Ninety-five percent were breastfed, with 26 (12%) exclusively breastfed at age 6 months. The proportion of infants receiving milk formula increased from age 2 to 6 months (P = .002), as did the median daily volume consumed (600 mL to 750 mL, P = .001). Formula milk volume consumed did not differ between study groups at age 2 (P = .25), 4 (P = .30), or 6 months (P = .34). Six infants, 5 of whom were born prematurely, received supplementary vitamin D.

The hours per day each infant spent outdoors increased from age 2 to 6 months (median 0.23 vs 0.40 hours, P = .001) but did not differ between study groups at age 2 (P = .18), 4 (P = .39), or 6 months (P = .55).

Reported compliance did not differ between groups (Table 3). The proportion of infants given 1 drop of study medicine each day decreased during infancy (2 months 90%, 4 months 90%, 6 months 78%, 2 vs 6 months, P < .001).

Maternal serum 25(OH)D concentrations increased from enrollment to 36 weeks' gestation to a similar extent in the 1000 IU and 2000 IU groups while remaining unchanged in the placebo group (Fig 2). In a regression of cord blood 25(OH)D concentration on maternal 36-week gestation 25(OH)D concentration, adjusted for treatment group, the model R^2 was 0.79 (P < .001) (Supplementary Fig 4).

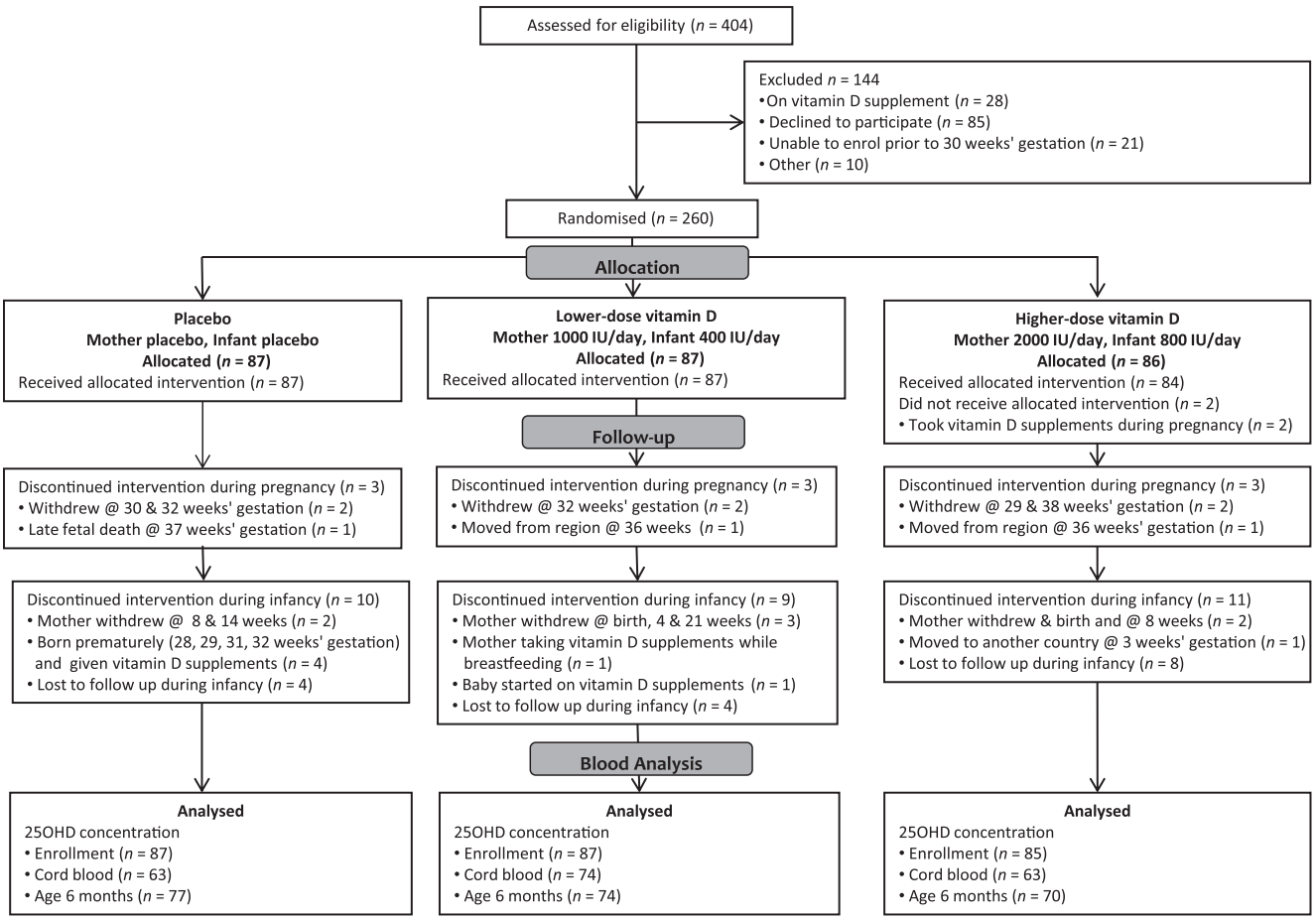

FIGURE 1
Flow diagram of enrollment, randomization, attrition, and data collection.

Median 25(OH)D concentrations at age 2 months in all 3 groups were higher than the cord blood 25(OH)D concentrations. The 25(OH)D concentrations in the 3 groups converged with increasing infant age with 25(OH)D concentrations increasing in the placebo group and decreasing in the higher-dose intervention group (Fig 2).

Maternal 25(OH)D concentrations at 36 weeks in the 3 treatment groups differed ($P < .001$, Table 4). Using the linear mixed model, there was an interaction between age (cord, 2, 4, and 6 months) and treatment group ($P < .001$). At all ages, the order of the estimates was the same, with the higher-dose group having the highest levels and the placebo group the lowest levels. Each stage showed a significant difference across treatment groups ($P < .01$). Table 4 shows estimated differences between groups and their 95% confidence intervals for the 2 hypothesized contrasts.

The proportion of women with 25(OH)D ≥30 ng/mL or ≥20 ng/mL differed among the 3 groups at all postenrollment measurement points (Table 5). In the placebo group, the proportion of infants with 25(OH)D ≥30 ng/mL increased from 33% at age 2 months to 57% at age 6 months ($P = .006$). The proportion of infants with 25(OH)D ≥30 ng/mL did not differ from age 2 to 6 months in the 400 IU (64%–74%, $P = .19$) or the 800 IU group (79%–73%, $P = .44$). In the placebo group, the proportion of infants with 25(OH)D ≥20 ng/mL increased from 50% at age 2 months to 74% at age 6 months ($P = .003$). The proportion of infants with 25(OH)D ≥20 ng/mL did not differ from age 2 to 6 months in the 400 IU (82%–82%, $P = .96$) or the 800 IU group (92%–89%, $P = .54$). Exclusion of women with gestational diabetes ($n = 13$), who took vitamin D supplements ($n = 11$) or whose infants took vitamin D supplements ($n = 7$) made no difference to these comparisons with the exception that the proportion with 25(OH)D ≥20 ng/mL did not differ between groups at age 6 months (Supplementary Table 6).

Based upon the 25(OH)D concentrations at 6 months of age, seasonal variation in vitamin D status was evident with seasonal variation being comparable in all 3 groups (Fig 3). After adjustment for season, the 25(OH)D concentration in

TABLE 1 Demographics and Clinical Characteristics of Enrolled Pregnant Women

Variable	Study Group		
	Placebo (Mother Placebo/ Infant Placebo)	Lower-Dose Vitamin D_3 (Mother 1000 IU Daily/Infant 400 IU Daily)	Higher-Dose Vitamin D (Mother 2000 IU Daily/Infant 800 IU Daily)
	$n_1 = 87$	$n_2 = 87$	$n_3 = 86$
Season of enrollment,[a] *n* (%)			
Summer	20 (23)	21 (24)	18 (21)
Fall	25 (29)	22 (25)	24 (27)
Winter	23 (26)	23 (27)	22 (26)
Spring	19 (22)	21 (24)	22 (26)
Maternal demographics			
Age, y, mean ± SD.	28±6	27±6	26±7
Gestation at enrolment, in weeks (median, 25th, 75th centiles)	27 (26, 29)	28 (26, 29)	27 (26, 29)
BMI at enrolment, in kg/m^2 (mean ± SD)	32 (7)	33 (8)	32 (7)
Ethnic group,[b] *n* (%)			
European	33 (38)	27 (31)	28 (33)
Māori	21 (24)	23 (26)	19 (22)
Pacific	40 (46)	44 (51)	43 (50)
Other	22 (25)	20 (23)	23 (27)
Took vitamin D supplements during pregnancy, *n* (%)			
Yes	2 (2)	6 (7)	3 (4)
No	78 (98)	75 (93)	68 (96)
Cigarette smoker during current pregnancy, *n* (%)			
Yes	18 (21)	17 (20)	14 (16)
No	69 (79)	70 (80)	72 (84)
Education, *n* (%)			
Primary	10 (12)	17 (20)	12 (14)
Secondary	23 (26)	28 (32)	23 (27)
Tertiary	54 (62)	42 (48)	51 (59)
Maternal pregnancy history and health			
Previous pregnancies, *n* (%)			
Yes	67 (77)	66 (76)	58 (67)
No	20 (33)	21 (24)	28 (33)
Gestational diabetes during current pregnancy,[c] *n* (%)			
Yes	4 (5)	7 (9)	2 (3)
No	79 (95)	73 (91)	75 (97)
Maternal sunlight exposure and sunlight-related behavior			
Use sunscreen with an SPF15 or stronger when outside in summer, *n* (%)			
Yes	32 (37)	36 (41)	40 (47)
No	55 (63)	51 (59)	45 (53)
Avoid direct sun exposure between 10 AM and 4 PM,[d] *n* (%)			
Yes	51 (59)	53 (61)	61 (71)
No	35 (41)	34 (39)	25 (29)
Reaction of skin to sun exposure, *n* (%)			
Always burn, never tan	3 (3)	2 (2)	5 (6)
Usually burn, tan with difficulty	10 (12)	4 (5)	15 (17)
Sometimes mild burn	30 (35)	41 (47)	23 (27)
Rarely burn, tan with ease	23 (26)	19 (22)	22 (26)
Not stated	21 (24)	21 (24)	21 (24)
Time per spent outdoors on average during month previous to enrollment, in hours, median (25th, 75th centiles)	0.5 (0.3, 1.6)	1.0 (0.5, 2.0)	1.0 (0.5, 1.8)
Headwear worn when outside, *n* (%)			
Veiled	2 (2)	0 (0)	0 (0)

TABLE 1 Continued

Variable	Study Group		
	Placebo (Mother Placebo/ Infant Placebo)	Lower-Dose Vitamin D_3 (Mother 1000 IU Daily/Infant 400 IU Daily)	Higher-Dose Vitamin D (Mother 2000 IU Daily/Infant 800 IU Daily)
	$n_1 = 87$	$n_2 = 87$	$n_3 = 86$
Hat	9 (11)	12 (14)	13 (15)
No head covering	55 (63)	53 (61)	51 (59)
Not stated	21 (24)	22 (25)	22 (26)

[a] Summer (December to February), Fall (March to May), Winter (June to August), Spring (September to November)
[b] Ethnic groups are those used for the national census. Māori is New Zealand's indigenous population. Ethnicity was defined by the participants. More than 1 ethnic group could be identified; therefore, percentages do not add to 100.
[c] At either enrollment or 36-week gestation interview $n_1 = 83$, $n_2 = 80$, $n_3 = 77$.
[d] Includes using protective clothing and/or remaining under a shade cover (not stated by 1 mother).

the placebo group at age 6 months was lower than that in the intervention groups ($P = .025$) but did not differ between intervention groups ($P = .40$). Serum calcium was not elevated in any participant, nor did mean serum calcium concentration differ between study groups at any measurement point (Supplementary Table 7). At age 2 months, 1 infant in the 400-IU group (130 ng/mL) and 4 infants in the 800-IU group (104, 128, 130, 134 ng/mL)

TABLE 2 Demographics and Clinical Characteristics of Enrolled Infants

Variable	Study Group		
	Placebo (Mother Placebo/ Infant Placebo)	Lower-Dose Vitamin D_3 (Mother 1000 IU Daily/Infant 400 IU Daily)	Higher-Dose Vitamin D (Mother 2000 IU Daily/Infant 800 IU Daily)
	$n_1 = 85$	$n_2 = 83$	$n_3 = 81$
Male gender, *n* (%)	44 (52)	41 (49)	36 (44)
Gestation, wk, median (25th, 75th centile)	39 (38, 40)	40 (39, 40)	40 (39, 40)
Birth weight, g, median (25th, 75th centile)	3465 (3160, 3780)	3480 (3140, 3870)	3540 (3230, 3900)
Breastfed, *n* (%)			
Any			
Yes	74 (94)	70 (92)	69 (99)
No	5 (6)	6 (8)	1 (1)
Exclusively at age 6 mo			
Yes	10 (13)	5 (7)	11 (16)
No	68 (87)	71 (93)	59 (84)
Received infant formula, n (%)			
At age 2 mo			
Yes	42 (57)	45 (63)	38 (58)
No	32 (43)	27 (37)	28 (42)
At age 4 mo			
Yes	44 (62)	44 (66)	42 (68)
No	27 (38)	23 (34)	20 (32)
At age 6 mo			
Yes	56 (71)	55 (74)	50 (74)
No	28 (29)	19 (26)	18 (26)
Average daily volume of infant milk formula consumed, mL, median (25th, 75th centile)			
At age 2 mo	400 (150, 800)	550 (170, 750)	662 (200, 900)
At age 4 mo	500 (150, 1000)	890 (310, 1000)	805 (300, 1000)
At age 6 mo	720 (120, 1000)	900 (250, 1000)	750 (300, 1000)
Used multivitamin that contains vitamin D,[a] *n* (%)			
Yes	3 (4)	1 (1)	2 (3)
No	77 (96)	76 (99)	68 (97)
Time per day spent outdoors on average over past 2 mo, h, median (25th, 75th centiles)			
At age 2 mo	0.21 (0.08, 0.65)	0.17 (0.06, 0.48)	0.27 (0.08, 0.65)
At age 4 mo	0.25 (0.08, 0.62)	0.33 (0.08, 0.52)	0.26 (0.08, 0.65)
At age 6 mo	0.40 (0.12, 0.90)	0.33 (0.13, 0.75)	0.50 (0.17, 0.68)

[a] Vitadol-C (Nutricia; NZ Limited, Auckland, NZ), which contains 466 IU of vitamin D per daily recommended dose.

TABLE 3 Reported Compliance With Study Medicine[a]

Variable	Study Group			*P* Value
	Placebo (Mother Placebo/Infant Placebo)	Lower-Dose Vitamin D_3 (Mother 1000 IU Daily/ Infant 400 IU Daily)	Higher-Dose Vitamin D (Mother 2000 IU Daily/ Infant 800 IU Daily)	
	$n_1 = 87$	$n_2 = 87$	$n_3 = 86$	
	n (%)	*n* (%)	*n* (%)	
Took 1 drop daily at 36 wk gestation				.66
Yes	77 (96)	77 (95)	66 (93)	
No	3 (4)	4 (5)	5 (7)	
Gave 1 drop daily to infant				
Age 2 mo				
Yes	67 (92)	64 (89)	58 (91)	.84
No	6 (8)	8 (11)	6 (9)	
Age 4 mo				
Yes	67 (94)	58 (87)	54 (87)	.25
No	4 (6)	9 (13)	8 (13)	
Age 6 mo				
Yes	65 (83)	57 (75)	52 (74)	.33
No	13 (17)	19 (25)	18 (26)	

[a] Reported by 220 (95%) of 232 of the women at 36 weeks' gestation and by them for 189 (90%) of 209, 179 (90%) of 200, and 174 (78%) of 224 of the infants at 2, 4, and 6 months of age, respectively.

had a serum 25(OH)D concentration ≥100 ng/mL.

DISCUSSION

Vitamin D supplementation of pregnant women, from 27 weeks' gestation until childbirth, and then their infants, from birth until age 6 months, results in 71% to 79% of women, at 36 weeks' gestation, and 73% to 74% of infants at age 6 months achieving serum 25(OH)D concentrations ≥30 ng/mL when a woman/infant dosing regimen of either vitamin D_3 1000 IU/400 IU, or vitamin D_3 2000 IU/800 IU is used. Ninety percent of women, at 36 weeks' gestation, and 82% to 92% of infants, to age 6 months, achieve serum 25(OH)D concentrations ≥20 ng/mL when either of these dosing regimens are used. In comparison with placebo, the proportion of infants achieving a serum 25(OH)D concentration ≥20 ng/mL was greater for the higher-dose group to age 6 months and for the lower-dose group to age 4 months.

Neither vitamin D dosing regimen caused hypercalcemia. In particular, hypercalcemia did not occur in the 5 infants who at age 2 months had serum 25(OH)D concentrations ≥100 ng/mL. These findings are consistent with data from other contemporary pregnancy and infancy studies of vitamin D supplementation. Separate studies have shown that vitamin D 4000 IU per day during pregnancy and 1200 IU per day during infancy does not cause hypercalcemia.[35,36] Vitamin D 1600 IU per day from age 2 weeks to 3 months does not cause hypercalcemia despite resulting in serum 25(OH)D concentrations up to 92 ng/mL.[37]

Serum 25OHD concentrations at enrollment (4–80 ng/mL) spanned the expected range seen in populations without supplementation.[38] Recruitment was evenly distributed across seasons and the recruited sample included a diversity of skin pigmentation types. Only 5% of the women and 3% of the infants took vitamin D supplements during the study; these few were distributed evenly across the study arms.

Our intervention ceased at age 6 months. Therefore, we cannot comment on the vitamin D dose required during later infancy. With increasing intake of infant formula, dietary intake of vitamin D is likely to increase and, hence, requirement for vitamin D supplementation may be less critical than earlier in infancy.

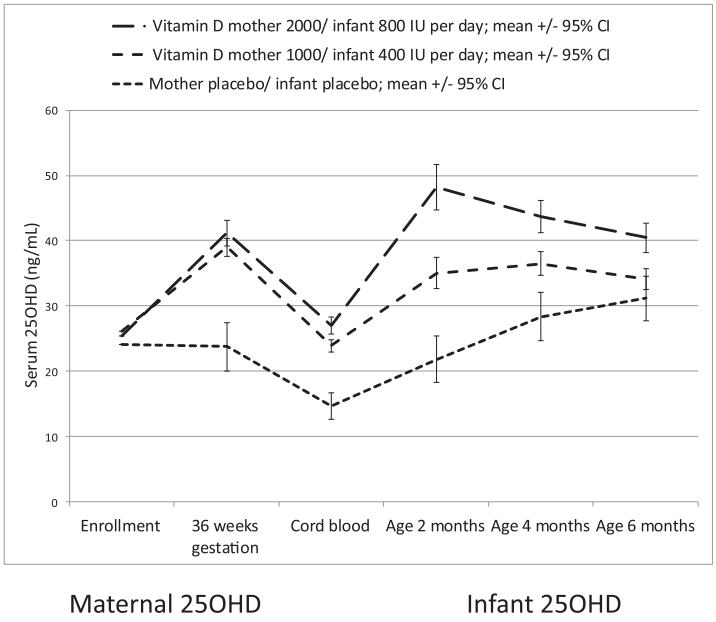

FIGURE 2
Serum 25(OH)D concentration for mother/infant pairs who were randomly assigned to placebo, lower-dose, or higher-dose vitamin D supplementation.

TABLE 4 Maternal and Infant 25(OH)D Concentrations by Study Group Assignment

Stage	Study Group			Comparisons Between Study Groups		
	Placebo (Mother Placebo/Infant Placebo)[a]	Lower-Dose Vitamin D (Mother 1000 IU Daily/Infant 400 IU Daily)[b]	Higher-Dose Vitamin D (Mother 2000 IU Daily/Infant 800 IU Daily)[c]	Difference Between Vitamin D and Placebo	Difference Between Lower Dose and Higher Dose Vitamin Groups	Overall *P* Value
	Serum 25(OH)D Concentration, ng/mL Median (25th, 75th Centile)			Estimate (95% CI)	Estimate (95% CI)	
Maternal at enrollment	22 (13, 32)	23 (16, 36)	22 (13, 35)			
Maternal at 36 wk gestation	20 (12, 30)	39 (32, 46)	41 (29, 50)	14 (11–17)	2 (−1–6)	<.001
Cord blood	13 (9, 18)	24 (18, 30)	26 (18, 35)	11 (8–14)	3 (0–6)	<.001
Infant at age 2 mo	20 (7, 35)	34 (24, 46)	43 (36, 54)	20 (14–26)	13 (6–21)	<.001
Infant at age 4 mo	30 (14, 41)	38 (29, 45)	45 (35, 54)	12 (7–17)	7 (1–13)	<.001
Infant at age 6 mo	31 (19, 40)	34 (29, 44)	38 (28, 51)	6 (2–10)	6 (0–11)	.004

CI, confidence interval.

[a] For enrollment, 36 weeks, cord, 2, 4, and 6 months $n = 87, 78, 63, 70, 68$, and 77, respectively.

[b] For enrollment, 36 weeks, cord, 2, 4, and 6 months $n = 87, 78, 74, 67, 61$, and 74, respectively.

[c] For enrollment, 36 weeks, cord, 2, 4, and 6 months $n = 85, 72, 63, 61, 60$, and 70, respectively.

Serum 25(OH)D concentration increased during infancy in our placebo group. This was also observed in a study of exclusively breastfed infants born during winter in Ioannina, Greece (39°N).[39] In our sample, the increased serum 25(OH)D concentration during infancy was probably multifactorial, but we suspect was largely due to increased intake of vitamin D from milk formula. In NZ, infant formula is fortified with 360 IU/L of vitamin D.[40] Therefore by age 6 months, infants randomized to the placebo group were, on average, receiving 260 IU per day of vitamin D.

Serum 25(OH)D concentrations decreased during infancy in the 800-IU per day group. Although decreasing compliance may have contributed to this, it is likely also due to the vitamin D dose per kilogram of body weight decreasing.[41] A similar pattern has been observed in other infant supplementation trials and suggests that a vitamin D dose per kilogram may be necessary in this age group.[36,42,43]

Our study is the first randomized controlled trial of vitamin D supplementation during infancy that commenced supplementation before birth. By doing so, ~90% of the pregnant women randomized to vitamin D achieved a serum 25(OH)D concentration ≥20 ng/mL. Consistent with other reports, there was a linear positive association between the cord 25(OH)D and maternal 36-week gestation 25(OH)D concentration.[10,35,44] In our study, the cord 25(OH)D concentration was, on average, 13.8 ng/mL lower than the maternal concentration. Although this also has been observed previously,[44] cord blood 25(OH)D concentrations in other populations have been similar to[45] or higher than maternal late-pregnancy serum 25(OH)D concentrations.[10] For neonates to be born with adequate vitamin D status requires their mothers to be vitamin D sufficient during pregnancy.[46]

TABLE 5 Maternal and Infant 25(OH)D Concentrations by Study Group Assignment and Proportion With 25(OH)D ≥20 ng/mL and ≥30 ng/mL

	Study Group			Comparisons Between Study Groups			
	Placebo (Mother Placebo/Infant Placebo)[a]	Lower-Dose Vitamin D (Mother 1000 IU Daily/ Infant 400 IU Daily)[b]	Higher-Dose Vitamin D (Mother 2000 IU Daily/ Infant 800 IU Daily)[c]	*P* Value (All 3 Groups)	*P* Value (Lower-Dose Vitamin D Versus Placebo)	*P* Value (Higher-Dose Vitamin D Versus Placebo)	*P* Value (Higher-Dose Vitamin D Lower-Dose Vitamin D)
Serum 25(OH)D concentration ≥20 ng/mL, *n* (%)							
Maternal at enrollment	47 (54)	56 (64)	47 (55)				
Maternal at 36 wk gestation	39 (50)	71 (91)	64 (89)	< .001	< .001	< .001	.66
Cord blood	14 (22)	53 (72)	45 (71)	< .001	< .001	< .001	.98
Infant at age 2 mo	35 (50)	55 (82)	56 (92)	< .001	< .001	< .001	.11
Infant at age 4 mo	45 (66)	53 (87)	52 (87)	.004	.006	.007	.97
Infant at age 6 mo	57 (74)	61 (82)	62 (89)	.07	.21	.03	.30
Serum 25(OH)D concentration ≥30 ng/mL, *n* (%)							
Maternal at enrollment	26 (30)	32 (37)	31 (36)				
Maternal at 36 wk gestation	21 (27)	62 (79)	51 (71)	< .001	< .001	< .001	.22
Cord blood	3 (5)	18 (24)	25 (40)	< .001	.002	< .001	.05
Infant at age 2 mo	24 (33)	43 (64)	48 (79)	< .001	< .001	< .001	.07
Infant at age 4 mo	34 (50)	43 (70)	48 (80)	.001	.02	< .001	.23
Infant at age 6 mo	44 (57)	55 (74)	51 (73)	.04	.03	.05	.84

[a] For enrollment, 36 weeks, cord, 2, 4, and 6 months *n* = 87, 78, 63, 70, 68, and 77, respectively.
[b] For enrollment, 36 weeks, cord, 2, 4, and 6 months *n* = 87, 78, 74, 67, 61, and 74, respectively.
[c] For enrollment, 36 weeks, cord, 2, 4, and 6 months *n* = 85, 72, 63, 61, 60, and 70, respectively.

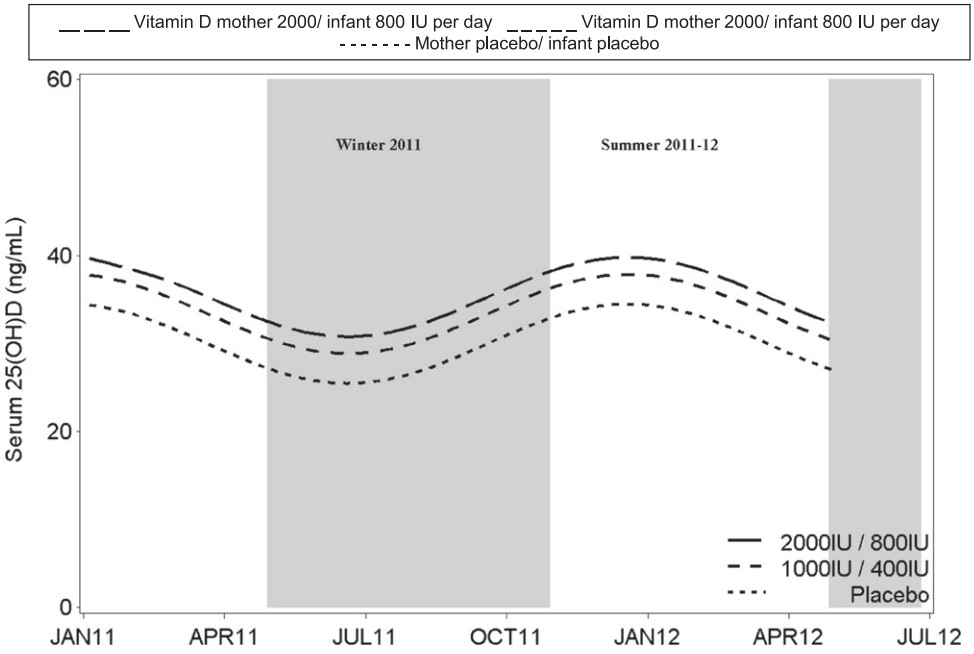

FIGURE 3
Seasonal patterns of 25(OH)D concentration for infants at age 6 months who were randomly assigned to placebo (mother placebo/infant placebo), lower-dose (mother 1000/infant 400 IU per day) or higher-dose (mother 2000/infant 800 IU per day) vitamin D supplementation.

Among infants randomized to 400 IU per day, the median serum 25(OH)D concentration at age 2 (33.5 ng/mL) and 6 months (34.4 ng/mL) approximated that achieved in the 3 other clinical trials reporting comparable data for infants receiving 400 IU per day from the first weeks of life. These trials enrolled infants in Cincinnati, Ohio (39°N) (25[OH]D 2 months 37 ng/mL, 6 months 33 ng/mL),[47] Madison, Wisconsin (43°N) (2 months 30 ng/mL, 6 months 24 ng/mL),[43] and Montreal, Quebec (46°N) (3 months, 31 ng/mL).[36]

At age 2 months, serum 25(OH)D concentrations ≥20 ng/mL were achieved in 82% of infants in the 400-IU per day group and 92% in the 800-IU per day group. In comparison, 97% of breastfed infants living in Montreal and randomized at age 1 month to vitamin D 400, 800, 1200, or 1600 IU per day had a serum 25(OH)D concentration at age 3 months ≥20 ng/mL, as did almost all infants in Finland randomized at age 2 weeks to 400, 1200, or 1600 IU per day.[36,37] The lower percentages in our study are possibly due to differences in the enrolled populations. In the Canadian study >85% of mothers had completed tertiary education. The mothers of the Finnish infants were considered more health-orientated than the general population.[36,37] In comparison, our sample of mothers was less well educated (57% tertiary education) and comparable demographically to the region from which they were recruited.[48]

CONCLUSIONS

If the objective of vitamin D supplementation is to achieve a serum 25(OH)D concentration ≥20 ng/mL in 97.5% of infants, then it seems likely that this requires both maternal vitamin D supplementation during pregnancy and high compliance with daily dosing regimens. For serum 25(OH)D concentration to be maintained throughout infancy, it is likely to also require dose adjustment to meet the demands created by rapid growth during infancy. Given the global variation in serum 25(OH)D concentration during pregnancy, recommended infant vitamin D supplementation in different countries will need to take into account maternal pregnancy vitamin D status in each country.

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(Continued from first page)

Dr Grant and Mr Stewart had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Vitamin D During Pregnancy and Infancy and Infant Serum 25-Hydroxyvitamin D Concentration

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