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Maternal vitamin D_3 supplementation at 50 μg/d protects against low serum 25-hydroxyvitamin D in infants at 8 wk of age: a randomized controlled trial of 3 doses of vitamin D beginning in gestation and continued in lactation[1]

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ABSTRACT

Background: Vitamin D supplementation is recommended for breastfed infants. Maternal supplementation beginning in gestation is a potential alternative, but its efficacy in maintaining infant 25-hydroxyvitamin D [25(OH)D] concentration after birth is unknown.

Objectives: We determined the effect of 3 doses of maternal vitamin D supplementation beginning in gestation and continued in lactation on infant serum 25(OH)D and compared the prevalence of infant serum 25(OH)D cutoffs (>30, >40, >50, and >75 nmol/L) by dose at 8 wk of age.

Design: Pregnant women (n = 226) were randomly allocated to receive 10, 25, or 50 μg vitamin D_3/d from 13 to 24 wk of gestation until 8 wk postpartum, with no infant supplementation. Mother and infant blood was collected at 8 wk postpartum.

Results: At 8 wk postpartum, mean [nmol/L (95% CI)] infant 25(OH)D at 8 wk was higher in the 50-μg/d [75 (67, 83)] than in the 25-μg/d [52 (45, 58)] or 10-μg/d [45 (38, 52)] vitamin D groups ($P < 0.05$). Fewer infants born to mothers in the 50-μg/d group had a 25(OH)D concentration <30 nmol/L (indicative of deficiency) than infants in the 25- and 10-μg/d groups, respectively (2% vs. 16% and 43%; $P < 0.05$). Fewer than 15% of infants in the 10- or 25-μg/d groups achieved a 25(OH)D concentration >75 nmol/L compared with 44% in the 50-μg/d group ($P < 0.05$). Almost all infants (~98%, n = 44) born to mothers in the 50-μg/d group achieved a 25(OH)D concentration >30 nmol/L. At 8 wk postpartum, mean [nmol/L (95% CI)] maternal 25(OH)D concentration was higher in the 50-μg/d [88 (84, 91)] than in the 25-μg/d [78 (74, 81)] or 10-μg/d [69 (66, 73)] groups ($P < 0.05$).

Conclusions: Maternal supplementation beginning in gestation with 50 μg vitamin D_3/d protects 98% of unsupplemented breastfed infants against 25(OH)D deficiency (<30 nmol/L) to at least 8 wk, whereas 10 or 25 μg vitamin D/d protects only 57% and 84% of infants, respectively. This trial was registered at clinicaltrials.gov as NCT01112891. *Am J Clin Nutr* doi: 10.3945/ajcn.114.106385.

Keywords: vitamin D, 25(OH)D, supplement, pregnancy, lactation, postpartum, infant

INTRODUCTION

Vitamin D deficiency during infancy leads to rickets and has been associated with altered calcium metabolism, poor growth, early childhood tooth decay, asthma, and an increased risk of diabetes (1–4). Breast milk is internationally recognized as the best source of nutrition for optimal infant growth and development (5), but it does not normally provide sufficient vitamin D. Numerous organizations, including Health Canada and the American Academy of Pediatrics, recommend direct infant supplementation with ~10 μg vitamin D/d (6–8) rather than sun exposure (9). Rates of vitamin D supplementation in breast milk–fed infants have been reported as variable from 80% (10, 11) to 10% (12). Although infant supplementation is effective, there are several concerns. Infant vitamin D overdose has been reported in the United States, with issuance of warning of the potential risks of liquid vitamin D by the US Food and Drug Administration (13). Furthermore, some breastfeeding advocates, health care providers, and mothers are reluctant to supplement breastfed infants, because it implies that breast milk is an incomplete food for infants.

Most pregnant women in Canada and the United States consume a prenatal multivitamin supplement during pregnancy and lactation, which usually contains ~10–15 μg vitamin D. Vitamin D transport occurs through the placenta in the form of 25-hydroxyvitamin D [25(OH)D],[9] and at birth, serum 25(OH)D in the neonate correlates with that of the mother (14). Therefore,

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[9] Abbreviations used: AT, as treated; IOM, Institute of Medicine; ITT, intention to treat; 25(OH)D, 25-hydroxyvitamin D.

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an alternative approach to improve infant serum 25(OH)D could be to supplement the mother with higher doses of vitamin D throughout pregnancy and lactation to increase in utero transfer and breast milk vitamin D concentration. Breast milk of mothers consuming 10 μg vitamin D/d has been found to contain higher vitamin D concentrations (0.8–1.7 μg/L) compared with unsupplemented women (15, 16). Studies in Finland, the United States, and New Zealand have reported higher serum 25(OH)D concentrations in infants of mothers supplemented with vitamin D (17–19), although the studies in Finland and the United States did not start maternal supplementation until after the infant was born (17, 18), and in the New Zealand study, maternal supplementation began in late pregnancy ($\sim$27 wk of gestation) with infant supplementation from birth onward (19). To our knowledge, no study to date has examined the effect of only maternal vitamin D supplementation starting in pregnancy on maternal and infant serum 25(OH)D concentrations. Thus, the question of whether maternal supplementation alone protects against low infant serum 25(OH)D in breastfed infants remains unanswered.

The primary aim of this trial was to determine and compare the effect of 3 doses of maternal vitamin D_3 (10, 25, and 50 μg/d) starting in midpregnancy (13–24 wk) on infant serum 25(OH)D concentration at 8 wk and to assess the prevalence of infant 25(OH)D deficiency by using cutoffs at 8 wk of age: 30 nmol/L (below which individuals are classified as deficient), 40 nmol/L (the estimated average requirement), 50 nmol/L (suggested to meet or exceed the needs of most individuals) (20), and 75 nmol/L (as recommended by the Canadian Paediatric Society) (21).

METHODS

Participants

A convenience sample of healthy pregnant women from Greater Vancouver, British Columbia, Canada, was recruited through advertisement in newspapers, electronic media, word of mouth, and the ultrasound clinic of BC Women's Hospital. Eligibility included age of 18–45 y and 13–24 wk of gestation (based on last menstrual period) with exclusion of women taking vitamin D supplements $>$10 μg/d; with metabolic, inflammatory, or genetic problems (e.g., diabetes, tuberculosis, cardiac or renal disease, HIV/AIDS, chronic hypertension, inflammatory bowel disease, autoimmune disease, liver disease, or epilepsy); digestive and intestinal problems that may affect vitamin D absorption (e.g., celiac disease or gastric bypass); or a history of adverse pregnancy outcome (e.g., preterm delivery $<$37 wk of gestation; stillbirth; hemolytic anemia, elevated liver enzymes, and low platelet count syndrome; severe preeclampsia; or eclampsia). The University of British Columbia and the Children's and Women's Clinical Research Ethics Boards (H09-01261) approved the study protocol. The trial was registered at clinicaltrials.gov as NCT01112891. Informed written consent was obtained from women before commencing and participating in the trial.

Study design

This double-blind randomized controlled trial was carried out between June 2010 and March 2013. The recruitment period included 3 calendar years with 4 full seasons each year: summer, fall, winter, and spring. Women attended the study clinics at BC Women's Hospital between 13 and 24 wk of gestation (baseline), 36 $\pm$ 1 wk of gestation, and 8 $\pm$ 1 wk postpartum. Women were blocked by ethnicity as either European or non-European; then, within each block, they were randomly allocated to 1 of the 3 vitamin D doses (10, 25, or 50 μg/d). On study entry, women were asked to discontinue taking all supplements being consumed before enrollment.

At baseline, women completed a self-administered questionnaire on their country and date of birth, ethnicity and ethnicity of their infant's father, their education, occupation, total household income, prepregnancy weight, estimated due date, parity, gravidity, smoking status, alcohol consumption, and use of supplements and prescription and nonprescription medications. Maternal calcium and vitamin D intakes from food over the prior month were estimated by using a semiquantitative food-frequency questionnaire that was validated among a variety of ethnic groups residing in Canada (22). Reported dose and duration of vitamin D supplements were also recorded; however, women consuming $>$10 μg/d were ineligible to participate. Maternal height and weight were measured at each visit according to standardized procedures with a calibrated standing weight scale and a stadiometer after enrollment, at 36 wk of gestation, and 8 wk postpartum. Maternal nonfasting venous blood and urine were collected at each time point, and infant venous blood was collected at 8 wk of age. Mothers were asked not to supplement their infant with vitamin D until after this blood draw, after which all women were provided with vitamin D drops for their infant and advised to give 10 μg/d, as recommended by Health Canada (7). Maternal supplements were dispensed at the first ($\sim$13–24 wk of gestation) and second ($\sim$36 wk of gestation) clinic visits. Women were defined as being "compliant" if they consumed $\geq$80% of the vitamin D tablets, as assessed during the second and final visits (asking women how many pills were consumed over the prior 1 wk and prior 8 wk) and confirmed by pill counts at the final visit (8 wk postpartum).

Supplements

The supplements were manufactured by Natural Factors as tablets all identical in size and color but containing 10, 25, or 50 μg vitamin D_3/d and also including 250 mg calcium, 1000 μg folic acid, 27 mg iron, 1500 IU β-carotene, 1500 IU vitamin A, 3 mg thiamin, 3.4 mg riboflavin, 20 mg niacinamide, 10 mg vitamin B-6, 12 μg vitamin B-12, 10 mg pantothenic acid, 30 μg biotin, 100 mg vitamin C, 30 IU vitamin E, 50 mg magnesium, 5 mg potassium, 25 mg zinc, 1 mg manganese, 0.15 mg iodine, 2 mg copper, 25 μg chromium, 25 μg molybdenum, and 25 μg selenium. All tablets included a blend of vegetable-grade magnesium stearate as a lubricant and microcrystalline cellulose and dicalcium phosphate dehydrate as fillers. The supplements were coded by the manufacturer to ensure blinding of all study staff and participants. The manufacturer analyzed the vitamin D content of supplements at various time points throughout the study to ensure proper doses, with external validation by Heartland Assays LLC, which found vitamin D supplement contents of 12, 29, and 58 μg/tablet for the 3 doses. A placebo was not included because Canadian women are recommended to

take a prenatal supplement containing folic acid and iron (23), most of which contain ~10–15 μg vitamin D/d. The authors thought it would be difficult to enroll pregnant women into a study that required them to discontinue their vitamin D supplements or vitamin D–containing prenatal supplements. This was the main reason for not including a placebo in this study. The highest vitamin D dose of 50 μg/d was chosen based on the current understanding that this intake is below the accepted tolerable upper intake level, which is the maximum usual daily intake level at which no risk of adverse effects is expected, as set by the US Institute of Medicine (IOM) (20).

Data collection procedures

Nonfasting venous blood was collected at the clinic and allowed to clot at room temperature for 30 min. Serum was separated from whole blood by centrifugation (2000 × *g* for 10 min at 4°C) and stored at −80°C. Serum 25(OH)D concentrations were determined at the School of Dietetics and Human Nutrition, McGill University, by using a LIAISON 25-OH Vitamin D TOTAL Assay (DiaSorin). This competitive chemiluminescence immunoassay equally detects 25(OH)D_2 and 25(OH)D metabolites (24). The McGill laboratory participates in the Vitamin D External Quality Assessment Scheme, an external quality control program for 25(OH)D measurement (24, 25), and obtained a Certificate of Proficiency for 2011–2012 and 2012–2013 as relevant to this report. The certificate requires that each laboratory's 25(OH)D values fall within 25% of the All-Laboratory Trimmed Mean for ≥80% of the yearly samples. Maternal serum total calcium and urine creatinine, phosphate, and calcium were measured by using the Ortho-Clinical Diagnostics VITROS 5600 System at BC Women's Hospital. The clinical cutoffs used for elevated serum hypercalcemia and the urine calcium to creatinine ratios were >2.7 nmol/L and >0.7 mg/mg, respectively (26).

Data analyses

The number of participants enrolled in the study was based on detecting a minimum 10-nmol/L difference in maternal 25(OH)D between any 2 doses of vitamin D and 20% attrition. Using an α of 0.05 and a β of 0.8, with baseline measurements, we estimated a sample size of 51 per group needed to detect a 10-nmol/L difference in serum vitamin D between 2 groups based on a mean ± SD serum 25(OH)D concentration of 58 ± 24 nmol/L (27) and thus planned to recruit a total of 210 women (70 women for each of the 3 vitamin D groups). Baseline characteristics were summarized as means ± SDs for numerical variables and as the percentage distribution for categorical variables. Data were analyzed based on intention-to-treat (ITT) for all outcomes, with missing data replaced by carrying forward the most recent available data. A second analysis was also done for as-treated (AT) analysis for subjects with complete data and who were ≥80% compliant in supplement use. Analysis of the data for the 8-wk-old infants was done, excluding infants who had been formula fed for >1 wk or had been given >3 doses of oral vitamin D. Differences in mean 25(OH)D between groups by treatment were assessed by using a general linear model with adjustment for baseline serum 25(OH)D concentration in the mother only. The proportion of subjects achieving serum 25(OH)D cutoffs of 30, 40, 50, and 75 nmol/L was assessed by using a χ^2 test. Statistical analyses were performed with SPSS Statistics 18.0 for Macintosh (SPSS Inc.) with a significance level of 0.05 used for all analyses.

RESULTS

Of the 249 women who consented to participate in the study, 14 subsequently declined participation, 7 miscarried, and 2 terminated their pregnancy before the baseline visit (**Figure 1**). In total, 226 women were randomly allocated to 10, 25, or 50 μg vitamin D/d (n = 76, n = 76, and n = 74, respectively). The overall study retention rate from beginning to end was 76% (n = 172). Across the treatment groups, the dropout rate was similar: 7.5% (n = 17) in the 10-μg/d group, 8.4% (n = 19) in the 25-μg/d group, and 8.0% (n = 18) in the 50-μg/d group. In the 10-μg/d group, 13 women were lost to follow-up, and 4 withdrew from the study for personal reasons. In the 25-μg/d group, 17 women were lost to follow-up, and 2 withdrew from the study for personal reasons. In the 50-μg/d group, 16 women were lost to follow-up, and 2 withdrew from the study for personal reasons. Blood was successfully obtained from 62% of the 8-wk-old infants, with missing samples due to clinical difficulty or the mother's refusal of this test on her infant.

Participant characteristics are shown in **Table 1**. Overall, 72% (n = 163) of women were of Caucasian ethnicity (white/European), and 28% (n = 63) were categorized as "nonwhite" and included a total of 13 different ethnicities: Chinese (n = 19), First Nations Indian (n = 8), Filipino (n = 7), East Indian (n = 6), Japanese (n = 5), and 8 other ethnic groups that each comprised ≤3 women. A total of 69% (n = 145) had a self-reported prepregnancy BMI (in kg/m^2) between 18.5 and 24.9. Income and educational attainment were high, with >70% having at least an undergraduate degree and ~50% having a family income of >$100,000 Canadian. At baseline, the mean (95% CI) serum 25(OH)D concentration of all women was 66 (63, 69) nmol/L, with >90% (n = 218) of women taking a vitamin D–containing supplement (usually a prenatal supplement) and, of these women, 59% (n = 128) taking 10 μg vitamin D/d. Only 9% (n = 20) of women had a serum 25(OH)D concentration <40 nmol/L at baseline. Compliance at 36 wk of gestation was 89% overall, with 85%, 84%, and 97% compliance in the 10-μg/d, 25-μg/d, and 50-μg/d groups, respectively. Compliance at 8 wk postpartum was 75% overall, with 68%, 70%, and 86%, in the 10-μg/d, 25-μg/d, and 50-μg/d groups, respectively. No statistically significant differences were found in the subject characteristics, baseline vitamin D status, or study compliance among the groups.

Mean (95% CI) maternal serum 25(OH)D concentrations at 36 wk of gestation and 8 wk postpartum are shown in **Table 2**. At 36 wk, using ITT analysis and after adjustment for baseline 25(OH)D concentration, mean maternal 25(OH)D concentrations at 36 wk were 6 (1, 12) nmol/L (P = 0.03) and 10 (4, 16) nmol/L higher (P < 0.001) in the 25-μg/d and 50-μg/d groups, respectively, than in the 10-μg/d group. At 8 wk postpartum, mean maternal 25(OH)D concentrations were 9 (3, 15) nmol/L (P < 0.001) and 19 (13, 23) nmol/L higher (P < 0.001) in the 25-μg/d and 50-μg/d groups, respectively, than in the 10-μg/d group.

Using ITT analysis at 36 wk of gestation, no woman had a 25(OH)D concentration <30 nmol/L, and <5% (n = 9) of women in all 3 treatment groups had a 25(OH)D concentration <40 nmol/L (**Table 3**). Only 5% (n = 4) of women had a 25(OH)D

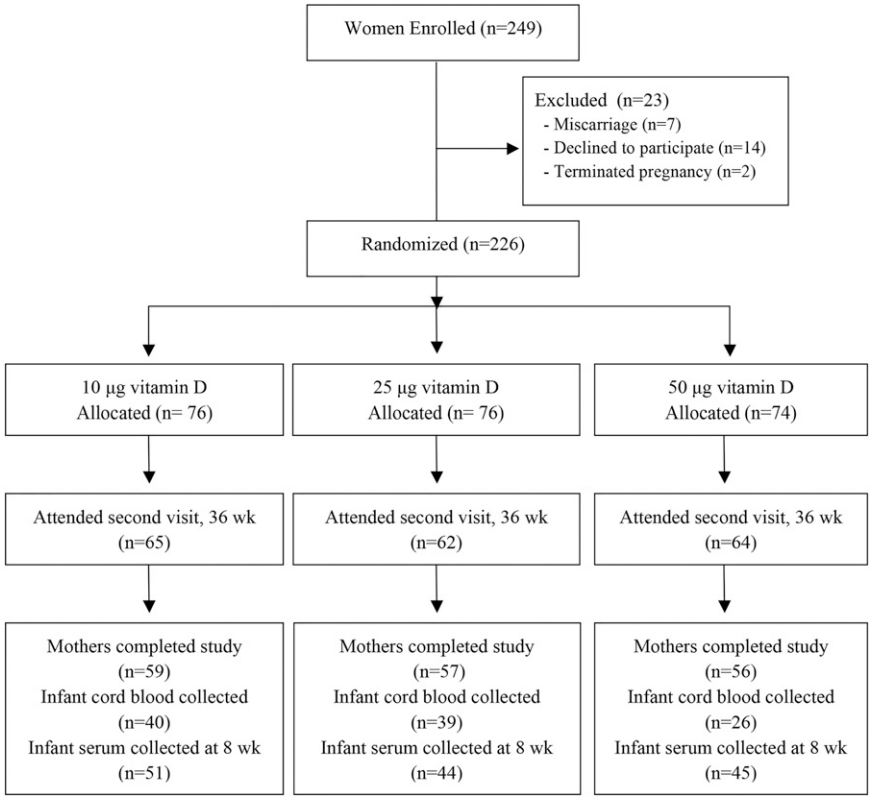

FIGURE 1 Participant flow and follow-up.

concentration <50 nmol/L in the 50-μg/d group compared with 16% (n = 12) and 11% (n = 8) in the 25-μg/d and 10-μg/d groups, respectively, but this was not statistically significant (P = 0.1). A statistically significantly lower proportion of women (38%, n = 28) had a 25(OH)D concentration <75 nmol/L in the 50-μg/d group compared with 60% (n = 45) of women in the 25-μg/d group or 60% (n = 45) of women in the 10-μg/d group (P = 0.01). Using ITT analysis at 8 wk postpartum, the results were similar. Findings were similar with AT analysis for women ≥80% compliant.

We explored whether the magnitude of effect of treatment dose (10, 25, or 50 μg vitamin D/d) on maternal 25(OH)D concentration at 8 wk postpartum was modified by ethnicity (white vs. nonwhite). Using linear regression, with baseline maternal 25(OH)D concentration and treatment group (10, 25, or 50 μg/d) controlled for, ethnicity was not statistically significant in the model (P = 0.6), with no statistically significant interaction between ethnicity and treatment group (P = 0.5). However, we acknowledge that ethnicity was self-reported by women and is only a proxy indicator of skin color. We also explored whether the magnitude of effect of treatment dose (10, 25, or 50 μg vitamin D/d) on maternal 25(OH)D concentration at 8 wk postpartum was modified by season on enrollment (fall/winter vs. spring/summer). Using linear regression, with baseline maternal 25(OH)D concentration and treatment group (10, 25, or 50 μg/d) controlled for, season on enrollment was statistically significant in the model (P = 0.03). Fall/winter enrollment was associated with higher maternal 25(OH)D concentration at 8 wk postpartum. However, there was no statistically significant interaction between season and treatment group (P = 0.2). Although enrollment may occur in winter, the majority of the gestation and postpartum up to the 8-wk period would have occurred in the spring/summer.

We collected cord blood from 105 infants: 40 in the 10-μg/d group, 39 in the 25-μg/d group, and 26 in the 50-μg/d group (**Table 4**). The correlation between infant cord blood 25(OH)D and maternal 25(OH)D concentration at 36 wk of gestation (n = 105) was r = 0.57. Infants whose mothers were in the 50-μg/d vitamin D group had statistically significantly higher mean 25(OH)D concentrations at 8 wk of age than those born to mothers in the 10-μg/d and 25-μg/d groups ($P < 0.0001$). Mean infant 25(OH)D concentration in the 50-μg/d group was 23 (13, 33) nmol/L higher ($P < 0.01$) than in the 25-μg/d group and 30 (20, 40) nmol/L higher ($P < 0.01$) than in the 10-μg/d group. In contrast, the infants whose mothers were receiving 25 μg/d had a 25(OH)D concentration 7 (−2, 16) nmol/L higher than those receiving 10 μg/d (P = 0.2). Findings were similar using AT analysis, excluding infants whose mothers were <80% compliant, infants who had been formula fed for >1 wk, or infants who had received >3 doses of oral vitamin D.

A lower proportion of infants had insufficient vitamin D concentrations, irrespective of cutoff, at 8 wk if their mothers were in the 50-μg/d group compared with the 10-μg/d and 25-μg/d treatment groups (**Table 5**). More than 40% (n = 22) of infants had a 25(OH)D concentration <30 nmol/L in the 10-μg/d group compared with 16% (n = 7) in the 25-μg/d group and 2% (n = 1) in the 50-μg/d group. Around 56% (n = 25) of infants in the 50-μg/d group had a 25(OH)D concentration <75 nmol/L, but this was statistically significantly less than the 25-μg/d or the 10-μg/d groups, in which 84–87% of infants were <75 nmol/L. Using AT analysis and excluding infants whose mothers were

TABLE 1
Baseline characteristics of 226 pregnant women (aged 18–45 y) at 13–24 wk of gestation[1]

	Vitamin D_3/d		
Characteristic	10 μg (n = 76)	25 μg (n = 76)	50 μg (n = 74)
Age, y	33.2 ± 4.2[2]	33.4 ± 4.4	34.5 ± 4.6
Gestation, wk	21 ± 2.5	21 ± 2.8	21 ± 3.3
Prepregnancy BMI,[3] kg/m^2	23.7 ± 7.8	23.3 ± 6	22.6 ± 5
18.5–24.9, n (%)	48 (68)	44 (62)	53 (78)
25.0–29.9, n (%)	10 (14)	20 (28)	12 (18)
≥30, n (%)	13 (18)	7 (10)	3 (4)
Ethnicity, n (%)			
White	56 (74)	55 (72)	52 (70)
Nonwhite	20 (26)	21 (28)	22 (30)
Education, n (%)			
High school	3 (4)	3 (4)	6 (8)
College	16 (21)	19 (26)	9 (13)
Undergraduate degree or higher	57 (75)	51 (70)	56 (79)
Annual household income (CDN), n (%)			
<$50,000	12 (16)	16 (22)	15 (22)
$50,000–$100,000	23 (31)	21 (28)	22 (32)
>$100,000	40 (53)	37 (50)	31 (46)
Season at study entry, n (%)			
April–September	51 (67)	47 (62)	49 (66)
October–March	25 (33)	29 (38)	25 (34)
Nutrient intake[4]			
Dietary vitamin D, μg/d	5 (3, 6)	4 (3, 8)	5 (3, 6)
Supplemental vitamin D, μg/d	10 (10, 10)	10 (6, 10)	10 (10, 10)
Dietary calcium, mg	831 (654, 1120)	800 (460, 1100)	832 (654, 1119)
Supplemental calcium, mg	250 (250, 250)	250 (238, 300)	250 (250, 250)
25(OH)D,[5] nmol/L	68 (63, 73)	64 (59, 68)	67 (63, 71)
25(OH)D, nmol/L, n (%)			
<30	0	0	0
<40	5 (7)	6 (8)	4 (5)
<50	18 (24)	21 (28)	9 (12)
<75	47 (62)	58 (76)	52 (70)

[1] t tests, ANOVA, and χ^2 tests were used to compare baseline characteristics by using SPSS Statistics 18.0 for Macintosh (SPSS Inc.) with a significance level of 0.05 for all analyses. CDN, Canadian dollars; 25(OH)D, 25-hydroxyvitamin D.
[2] Mean ± SD (all such values).
[3] Missing data (n = 16) because of unknown weight history of participant.
[4] Values are medians; first, third quartiles in parentheses.
[5] Values are means; 95% CIs in parentheses.

<80% compliant, all 3 groups differed statistically significantly for the proportion of infants with a serum 25(OH)D concentration <40 nmol/L. Findings were similar using AT analysis, excluding infants who had been formula fed for >1 wk or excluding infants who had received >3 doses of oral vitamin D. Overall, a total of 40 infants were excluded for these reasons. In the 10-μg/d group, 12 infants were excluded: 6 because of formula feeding and 6 because of vitamin D supplements. In the 25-μg/d group, 16 infants were excluded: 8 because of formula feeding and 8 because of vitamin D supplements. In the 50-μg/d group, 15 infants were excluded: 6 because of formula feeding, 8 because of vitamin D supplements, and 1 for both reasons.

Hypercalcemia (defined as serum total calcium >2.7 nmol/L) was found in ~10% (n = 18) of women overall; however, proportions did not statistically significantly differ between groups (P = 0.5) (**Table 6**). Symptomatic hypercalcemia, defined as a serum total calcium >11 nmol/L, was not present in any subject. The incidence of maternal urinary calcium to creatinine ratios >0.7 mg/mg were also not statistically significantly different between the treatment groups (P = 0.5), with this occurring in 13, 11, and 16 women in the 10-μg/d, 25-μg/d, and 50-μg/d groups, respectively. During pregnancy, urinary calcium to creatinine ratios >1.0 mg/mg can result from an increased glomerular filtration rate from hemodilution rather than being indicative of hypervitaminosis D or hypercalciuria (28). Although maternal plasma volume has generally decreased to normal concentrations by 8 wk after delivery, glomerular filtration rate may still remain elevated (29).

DISCUSSION

This clinical trial aimed to examine the effect of maternal vitamin D supplementation during pregnancy and lactation, without direct infant supplementation, on infant 25(OH)D. We found that infants of mothers receiving 50 μg vitamin D_3/d had statistically significantly higher 25(OH)D concentrations at 8 wk than those of mothers receiving lower doses of 10 μg/d or 25 μg/d. Maternal supplementation at 50 μg/d was effective at

TABLE 2
Maternal 25(OH)D at baseline, 36 wk of gestation, and 8 wk postpartum by maternal vitamin D dose[1]

	Maternal 25(OH)D, nmol/L					
	Baseline		36 wk of gestation[2]		8 wk postpartum[2]	
	n	Mean (95% CI)	*n*	Mean (95% CI)	*n*	Mean (95% CI)
Intent-to-treat analysis[3]						
10 μg/d	76	68 (63, 73)	76	69 (66, 73)[a]	76	69 (66, 73)[a]
25 μg/d	76	64 (59, 68)	76	76 (72, 79)[b]	76	78 (74, 81)[b]
50 μg/d	74	67 (63, 69)	74	79 (76, 83)[b]	74	88 (84, 91)[c]
As-treated analysis (≥80% compliant)[4]						
10 μg/d	76	68 (63, 73)	65	70 (66, 73)[a]	59	71 (67, 75)[a]
25 μg/d	76	64 (59, 68)	62	77 (73, 80)[b]	57	79 (75, 83)[b]
50 μg/d	74	67 (63, 69)	64	81 (77, 85)[b]	56	91 (87, 95)[c]

[1]A general linear model with adjustments for baseline maternal 25(OH)D concentration was used to assess differences in mean 25(OH)D concentration between groups by treatment with a significance level of 0.05 for all analyses. Values that do not share a common superscript letter in the column are statistically significantly different, $P < 0.05$. 25(OH)D, 25-hydroxyvitamin D.

[2]Adjusted for baseline maternal 25(OH)D concentration.

[3]Intent-to-treat analysis in which last value carried forward in the case of missing values.

[4]As-treated analysis in which dropouts were excluded and mothers were ≥80% compliant with the supplement regimen.

preventing 98% of infants against low infant 25(OH)D concentrations (<30 nmol/L) without having to supplement infants during the first 8 wk of life. We acknowledge the lack of evidence around optimal 25(OH)D concentrations, especially during infancy. The IOM notes that in infants aged 0–6 mo, maintaining 25(OH)D concentrations >30 nmol/L and more likely closer to 50 nmol/L probably covers the majority of infant needs (20). Still, in our study, only 2% ($n = 1$) and 8% ($n = 4$) of infants born to mothers in the 50-μg/d group had a 25(OH)D concentration <40 and 50 nmol/L, respectively, suggesting that this dose is effective at achieving these higher cutoffs. If 75 nmol/L is needed for optimal health of infants, as suggested by some (21), even 50 μg/d would allow only just over half of infants to achieve this concentration.

Hollis and Wagner (18) supplemented lactating women ($n = 18$) starting at 1 mo postpartum with either 50 μg or 100 μg oral vitamin D_2/d, a form of vitamin D that is considered less effective in raising serum 25(OH)D concentrations than vitamin D_3 (14), which was used in our study. At 4 mo of age infants, had attained a mean 25(OH)D concentration of 69 and 77 nmol/L, respectively. In our study, infants achieved 75 nmol/L with 50 μg/d maternal supplementation, which was similar to the value observed by Hollis and Wagner when mothers received 100 μg/d. Infants in our study likely started at higher 25(OH)D concentrations, because the duration of maternal supplementation was much longer in our study (~28 vs. 12 wk), and infants were smaller during the maternal supplementation period. Hence, the dose per kilogram would have been somewhat higher, given

TABLE 3
Prevalence of maternal 25(OH)D concentration <30, <40, <50, and <75 nmol/L at 36 wk of gestation and 8 wk postpartum by maternal vitamin D dose[1]

	Maternal 25(OH)D									
	36 wk of gestation					8 wk postpartum				
	n	<30 nmol/L, *n* (%)	<40 nmol/L, *n* (%)	<50 nmol/L, *n* (%)	<75 nmol/L, *n* (%)	*n*	<30 nmol/L, *n* (%)	<40 nmol/L, *n* (%)	<50 nmol/L, *n* (%)	<75 nmol/L, *n* (%)
Intent-to-treat[2]										
10 μg/d	76	0	3 (4)[a]	8 (11)[a]	45 (60)[a]	76	0	3 (4)[a]	10 (13)[a]	45 (60)[a]
25 μg/d	76	0	4 (5)[a]	12 (16)[a]	45 (60)[a]	76	0	3 (4)[a]	10 (13)[a]	42 (55)[a]
50 μg/d	74	0	2 (3)[a]	4 (5)[a]	28 (38)[b]	74	0	1 (1)[a]	2 (3)[b]	22 (30)[b]
As-treated (≥80% compliant)[3]										
10 μg/d	55	0	2 (4)[a]	4 (7)[a]	28 (50)[a]	39	0	0[a]	2 (5)[a]	18 (46)[a]
25 μg/d	52	0	1 (2)[a]	6 (12)[a]	30 (58)[a]	40	0	0[a]	3 (8)[a]	18 (45)[a]
50 μg/d	62	0	0[a]	2 (3)[a]	21 (34)[b]	47	0	0[a]	0 (0)[a]	10 (21)[b]

[1]The proportion of women achieving serum 25(OH)D cutoffs was assessed by using a χ^2 test with a significance level of 0.05 for all analyses. Values that do not share a common superscript letter in the column are statistically significantly different, $P < 0.05$. 25(OH)D, 25-hydroxyvitamin D.

[2]Intent-to-treat analysis in which the last value carried forward in the case of missing values.

[3]As-treated analysis in which dropouts were excluded and mothers were ≥80% compliant to the supplement regimen.

TABLE 4
Infant cord blood 25(OH)D concentration at birth and infant serum 25(OH)D concentration at 8 wk of age by maternal vitamin D dose[1]

	Cord blood 25(OH)D at birth, nmol/L		Infant serum 25(OH)D at 8 wk, nmol/L	
Maternal vitamin D dose	*n*	Mean (95% CI)	*n*	Mean (95% CI)
All infants				
10 μg/d	40	76 (68, 84)[a]	51	45 (38, 52)[a]
25 μg/d	39	73 (65, 81)[a]	44	52 (45, 58)[a]
50 μg/d	26	95 (87, 111)[b]	45	75 (67, 83)[b]
Infants born to mothers ≥80% compliant				
10 μg/d	32	78 (70, 87)[a]	34	45 (37, 53)[a]
25 μg/d	32	74 (65, 83)[a]	33	54 (45, 61)[a]
50 μg/d	22	96 (88, 104)[b]	39	78 (70, 85)[b]
Exclusively breastfed[2]				
10 μg/d		NA	39	45 (37, 53)[a]
25 μg/d		NA	31	51 (43, 59)[a]
50 μg/d		NA	30	75 (64, 85)[b]

[1]A general linear model was used to assess differences in mean 25(OH)D concentration between groups by treatment with a significance level of 0.05 for all analyses. Values that do not share a common superscript letter in the column are statistically significantly different, $P < 0.05$. NA, not applicable; 25(OH)D, 25-hydroxyvitamin D.
[2]Excluding infants directly supplemented with >3 doses of vitamin D (i.e., drops) or formula fed for >1 wk.

equivalent doses. Compliance in the Hollis and Wagner study (~90%) was similar to our study and therefore is not likely a factor contributing to the differences observed.

That direct infant supplementation is an effective way of ensuring adequate infant 25(OH)D concentration is not in doubt. Gallo et al. (11) randomly allocated infants at birth to 10, 20, 30, or 40 μg/d for 1 y. Even 10 μg/d was effective at maintaining a 25(OH)D concentration at >50 nmol/L in nearly all infants over the year. Interestingly, only 40 μg/d allowed nearly all infants to achieve a 25(OH)D concentration >75 nmol/L, but this dose was discontinued because of hypercalcemia in some infants. In the 1980s, researchers in Finland directly compared maternal supplementation with infant supplementation (17). Lactating women (n = 49) were randomly allocated soon after birth of their infant to receive 25 μg/d or 50 μg/d or placebo; infants in the placebo group received 10 μg vitamin D/d (17). At 8 and 15 wk of age, infant 25(OH)D concentrations were similar in infants whose mothers had received 10 μg/d and those whose mothers had received 50 μg/d. However, concentrations were lower in infants whose mothers received only 25 μg/d, suggesting that direct infant supplementation at 10 μg/d and maternal supplementation of 50 μg/d are comparable (17).

A secondary aim was to examine the effect of the 3 doses on maternal serum 25(OH)D at 36 wk of gestation and 8 wk postpartum. We found that maternal 25(OH)D concentrations generally increased in a dose-response manner. Likewise,

TABLE 5
Prevalence of infant 25(OH)D below 30, 40, 50, and 75 nmol/L at 8 wk of age by maternal vitamin D dose[1]

	Infant serum 25(OH)D				
Maternal vitamin D dose	*n*	<30 nmol/L	<40 nmol/L	<50 nmol/L	<75 nmol/L
Infants at 8 wk					
10 μg/d	51	22 (43)[a]	24 (47)[a]	30 (59)[a]	43 (84)[a]
25 μg/d	44	7 (16)[b]	14 (33)[a]	20 (46)[a]	39 (87)[a]
50 μg/d	45	1 (2)[c]	1 (2)[b]	4 (9)[b]	25 (56)[b]
Infants born to mothers ≥80% compliant					
10 μg/d	34	15 (44)[a]	16 (47)[a]	20 (59)[a]	28 (82)[a]
25 μg/d	33	4 (12)[b]	9 (27)[b]	14 (42)[a]	29 (88)[a]
50 μg/d	39	0 (0)[c]	1 (3)[c]	3 (8)[b]	39 (37)[b]
Exclusively breastfed[2]					
10 μg/d	39	17 (44)[a]	18 (46)[a]	23 (59)[a]	34 (87)[a]
25 μg/d	31	4 (13)[b]	10 (32)[a]	15 (48)[a]	28 (90)[a]
50 μg/d	30	1 (3)[c]	2 (7)[b]	4 (13)[b]	17 (57)[b]

[1]The proportion of infants achieving serum 25(OH)D cutoffs was assessed by using a χ^2 test with a significance level of 0.05 for all analyses. Values that do not share a common superscript letter in the column are statistically significantly different, $P < 0.05$. 25(OH)D, 25-hydroxyvitamin D.
[2]Excluding infants directly supplemented with >3 doses of vitamin D (i.e., drops) or formula fed for >1 wk.

TABLE 6
Elevated maternal serum total calcium and urine calcium to creatinine ratio at 8 wk postpartum by maternal vitamin D dose[1]

Supplement dose	*n*	Serum total calcium >2.7 nmol/L, *n* (%)	Urine calcium to creatinine ratio >0.70 mg/mg, *n* (%)
10 μg/d	59	5 (9)[a]	13 (22)[a]
25 μg/d	57	5 (9)[a]	11 (19)[a]
50 μg/d	56	8 (14)[a]	16 (29)[a]

[1]The proportion of women achieving serum 25(OH)D cutoffs was assessed by using a χ^2 test with a significance level of 0.05 for all analyses. Values that do not share a common superscript letter in the column are statistically significantly different, $P < 0.05$.

Hollis et al. (30) reported a dose-response increase in 25(OH)D concentration among pregnant women ($n = 350$) randomly allocated at 12–16 wk of gestation to 10, 50, or 100 μg vitamin D/d until delivery. In their study, mean ± SD serum 25(OH)D concentrations were 79 ± 34 nmol/L, 105 ± 35 nmol/L, and 119 ± 35 nmol/L ($P < 0.0001$), respectively, at 1 mo before delivery. Women in their study attained a higher maternal 25(OH)D concentration compared with ours, even at the same dose (105 vs. 79 nmol/L taking 50 μg/d). Women in the Hollis et al. study could have been supplemented up to 8 wk longer in duration compared with our study. At 8 wk postpartum, maternal 25(OH)D concentrations in our study were higher in the 50-μg/d group than at 36 wk of gestation, suggesting that a steady state had not been reached by 36 wk. With respect to cutoffs, no woman in our study was classified as deficient (<30 nmol/L), irrespective of dose. The IOM set an estimated average requirement and a Recommended Dietary Allowance for vitamin D of 10 and 15 μg/d, respectively, during pregnancy and lactation; these correspond to plasma 25(OH)D concentrations of 40 and 50 nmol/L (20). Almost all women achieved these plasma concentrations irrespective of dose. Given that women were attaining ~5 μg/d from dietary sources, supplementation with 10 μg/d resulted in a total intake corresponding to the Recommended Dietary Allowance of 15 μg/d. If a plasma 25(OH)D concentration of ≥75 nmol/L is required for optimal health (20), supplementation with 50 μg/d allowed 60–70% of women to achieve this cutoff. Interestingly, in our study, cord blood 25(OH)D concentration was ~9% higher than maternal 25(OH)D concentration measured at 36 wk, whereas in the study by Hollis and Wagner (18), cord blood 25(OH)D concentrations were ~40% lower than those in mothers measured at a similar time. The reason for this difference is not clear but may relate to different study populations, dose, and 25(OH)D assay used. However, our 9% higher cord blood vs 36 wk in maternal blood is very similar to the 7.5% difference that Zhang et al. (31) found between 36 and 40 wk in a longitudinal study of vitamin D status during pregnancy

Hypercalcemia (defined as serum calcium >2.7 nmol/L) was reported in a small proportion of women ($n = 18$ of 172) but did not differ between treatment groups ($P = 0.523$). No adverse effects of hypervitaminosis D (defined as 25(OH)D concentration >225 nmol/L) were reported. The dose of 50 μg/d is within the safe range of intake defined by the IOM, where 100 μg/d is set as the tolerable upper intake level (20). In previous studies, Hollis et al. (30) and Roth et al. (32) supplemented pregnant women with 100 μg vitamin D/d and 875 μg vitamin D/wk, respectively, and similarly, neither study reported any adverse events with these higher doses of vitamin D.

A strength of this study is that it is the first known to supplement mothers during gestation and continue through 8 wk of lactation, without infant supplementation. Furthermore, we investigated 3 different doses of vitamin D to compare dose-responsiveness and to monitor reports of adverse effects between these 3 groups. Limitations include that we recruited a convenience sample of healthy women in the Greater Vancouver area of British Columbia, Canada, who were mainly European and were generally of high socioeconomic status based on income and education. Most women were taking a vitamin D prenatal supplement at entry of the study, and there were no 25(OH)D concentrations indicative of deficiency. However, our mean 25(OH)D at baseline of 66 nmol/L was similar to the mean of 70 nmol/L reported for nonpregnant women in the Canadian Health Measures Survey, a nationally representative survey (33). We cannot confirm whether infant 25(OH)D concentrations were reflective of in utero vitamin D accretion or the result of higher vitamin D content of breast milk from maternal supplementation. As such, we do not know whether 25(OH)D concentrations would be maintained throughout the exclusive breastfeeding period or during the subsequent period when complementary foods are introduced and breastfeeding continues. We did not analyze breast milk samples for vitamin D, which would have partially addressed this issue. Some evidence that breast milk vitamin D increases with maternal supplementation is available from a small pilot study ($n = 19$) (16). Breast milk vitamin D (as anti–rachitic factor) increased (2.1–21.8 μg/L; $P < 0.0003$) in lactating women supplemented with 160 μg vitamin D/d but not with 10 μg/d (16). Last, perinatal or infant outcomes such as preeclampsia, preterm birth, small for gestational age, and infant bone mass were not determined.

We conclude that maternal supplementation beginning in gestation with 50 μg vitamin D_3/d protects 98% of unsupplemented breastfed infants against 25(OH)D deficiency (<30 nmol/L) to at least 8 wk, whereas 10 or 25 μg vitamin D/d protects only 57% and 84% of infants, respectively. Maternal supplementation has an advantage over infant supplementation because it has potential to improve vitamin D status of both the mother and infant without having to directly supplement the infant. Maternal doses of 50 μg vitamin D/d are recognized as safe by the IOM (20). Future research is warranted on the analysis of breast milk vitamin D among supplemented mothers and on vitamin D status of infants throughout the lactation period.

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