

High-Dose Monthly Maternal Cholecalciferol Supplementation during Breastfeeding Affects Maternal and Infant Vitamin D Status at 5 Months Postpartum: A Randomized Controlled Trial^{1–3}

Benjamin J Wheeler,^{4,7*} Barry J Taylor,⁴ Peter Herbison,⁵ Jillian J Haszard,⁴ Adel Mikhail,⁴ Shirley Jones,⁴ Michelle J Harper,⁶ and Lisa A Houghton⁶

Departments of ⁴Women's and Children's Health and ⁵Preventive and Social Medicine, Dunedin School of Medicine, and ⁶Department of Human Nutrition, University of Otago, Dunedin, New Zealand; and ⁷Pediatric Endocrinology, Southern District Health Board, Dunedin, New Zealand

Abstract

Background: Many countries recommend daily infant vitamin D supplementation during breastfeeding, but compliance is often poor. A monthly, high-dose maternal regimen may offer an alternative strategy, but its efficacy is unknown.

Objective: The objective of the study was to determine the effect of 2 different monthly maternal doses of cholecalciferol on maternal and infant 25-hydroxyvitamin D [25(OH)D] status during the first 5 mo of breastfeeding.

Methods: With the use of a randomized, double-blind, placebo-controlled design, women who were planning to exclusively breastfeed for 6 mo ($n = 90$; mean age: 32.1 y; 71% exclusively breastfeeding at week 20) were randomly assigned to receive either cholecalciferol (50,000 or 100,000 IU) or a placebo monthly from week 4 to week 20 postpartum. The treatment effects relative to placebo were estimated as changes in maternal and infant serum 25(OH)D from baseline to week 20 postpartum by using a linear fixed-effects regression model. Additional secondary analyses, adjusted for potential confounders such as season of birth, vitamin D–fortified formula intake, and infant or maternal skin color, were also conducted.

Results: After 16 wk of supplementation, changes in maternal serum 25(OH)D were significantly higher in the 50,000-IU/mo (12.8 nmol/L; 95% CI: 0.4, 25.2 nmol/L) and 100,000-IU/mo (21.5 nmol/L; 95% CI: 9.2, 33.8 nmol/L) groups than in the placebo group ($P = 0.43$ and $P < 0.001$, respectively). For infants, the unadjusted mean changes in serum 25(OH)D were 4.5 nmol/L (95% CI: –16.2, 25.0 nmol/L) for the 50,000-IU/mo group and 15.8 nmol/L (95% CI: –4.7, 36.4 nmol/L) for the 100,000-IU/mo group, but the changes did not differ from the placebo reference group. However, after adjustment for season of birth, vitamin D–fortified formula intake, and infant skin color, the mean change effect size for the 100,000-IU/mo group was 19.1 nmol/L (95% CI: 2.5, 35.6 nmol/L; $P = 0.025$) higher than that in the placebo group.

Conclusions: Maternal cholecalciferol supplementation at a dose of 100,000 IU/mo during the first 5 mo of breastfeeding potentially benefits infant vitamin D status. Further studies are required to determine optimum dose and dosing frequency. This trial was registered at www.anzctr.org.au as ACTRN12611000108910. *J Nutr* doi: 10.3945/jn.116.236679.

Keywords: vitamin D, cholecalciferol, 25-hydroxyvitamin D, breastfeeding infant, lactation

Introduction

Vitamin D is essential for calcium and bone metabolism. It is unique among vitamins in that it is mainly derived from synthesis in the skin after exposure to UV-B radiation. In the absence of fortifica-

tion, few foods are rich in vitamin D, including human milk, which contains very low amounts (1). Subsequently, breastfeeding infants in higher latitude countries such as New Zealand are at risk of vitamin D deficiency due to suboptimal vitamin D intake, which is further compounded by seasonal changes in UV-B intensity (2–6) and recommendations to avoid direct sunlight exposure in the first 6 mo of life (7).

The most profound manifestation of vitamin D deficiency in growing children is rickets, a mineralization defect of the growth plate, characterized by bone deformities, impaired growth, biochemical abnormalities, and depending on the severity of deficiency, seizures (8–11). Current prospective surveillance studies in New

¹ Supported by Healthcare Otago Charitable Trust and the Dunedin School of Medicine, University of Otago, New Zealand. Breast pumps were donated by Fisher and Paykel and HEHA (Healthy Eating, Healthy Action).

² Author disclosures: BJ Wheeler, BJ Taylor, P Herbison, JJ Haszard, A Mikhail, S Jones, MJ Harper, and LA Houghton, no conflicts of interest.

³ Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

*To whom correspondence should be addressed. E-mail: ben.wheeler@otago.ac.nz.

Zealand, Canada, and Australia have raised considerable concern with regard to an increase in the incidence of rickets, especially among young children (9–11). These studies also identified a number of common factors that potentially affect the risk of rickets, including darker pigmented skin, maternal vitamin D deficiency during pregnancy, season of birth, age <3 y, and exclusive breastfeeding (9–11).

Given the higher risk of rickets in breastfed infants, several countries have recommended universal vitamin D supplementation for all breastfed infants (12). However, compliance is variable (13–16), with recent data published from US NHANES suggesting that 80% of US breastfed infants failed to achieve the recommended intake of 400 IU/d (17). In addition, cases of rickets continue to be reported in countries that use this preventive approach (11, 18).

A potential alternative strategy to improve the vitamin D status of breastfed infants is high-dose vitamin D supplementation to pregnant and lactating women. This maternal supplementation strategy was previously shown to increase both mother and infant serum 25-hydroxyvitamin D [25(OH)D]⁸ concentrations (19–23); however, results from these studies must be interpreted with caution due to the lack of a control group, unblinded treatment, and/or concurrent vitamin D supplementation to infant study participants.

In New Zealand, vitamin D fortification in the food supply is negligible, and at the time of this study guidelines for infant vitamin D supplementation of breastfed infants were recommended only for those considered “at risk.” Thus, the primary aim of this randomized, placebo-controlled study was to determine the effect of 2 different intermittent monthly doses of maternal cholecalciferol supplementation (weeks 4–20 postpartum) on serum 25(OH)D status of non-vitamin D-supplemented breastfed infants. A monthly dose was chosen to improve compliance.

Methods

Study population and design. Healthy pregnant women planning to exclusively breastfeed for ≥ 5 mo after delivery were recruited from July 2012 to June 2013 through the Queen Mary Maternity Centre, Dunedin Hospital, Dunedin, New Zealand (45°S latitude). The Queen Mary Maternity Centre provides publicly funded, tertiary-level maternity and newborn care to the Otago and Southland region of New Zealand (population ~310,000) and is the only birthing unit in Dunedin, with ~97% of all city births (the remaining 3% being home births). Exclusion criteria included the following: 1) preterm delivery <37 wk of gestation; 2) intent to use vitamin D supplements (either mother or infant) in the postnatal period; 3) a history of disorders known to affect calcium and/or vitamin D metabolism, including abnormal calcium concentrations or urinary calcium-to-creatinine (Ca-Cr) ratio at study baseline; 4) planned travel outside of New Zealand over the study period; and 5) living outside of Dunedin. The study was approved by the New Zealand Lower South Regional Ethics Committee (LRS/11/02/007), and written informed consent was obtained from each mother. The study was registered before study commencement with the Australian New Zealand Clinical Trials Registry at www.anzctr.org.au as ACTRN12611000108910.

The study was a 16-wk randomized, double-blind, placebo-controlled trial in 90 mother and infant pairs, beginning at 4 wk postpartum. Women were enrolled from 20 wk of gestation until delivery. After delivery, lactation support was provided, along with breast pumps as needed. At 4 wk postpartum, breastfeeding mothers were randomly assigned in blocks of 15 to 1 of 3 treatment arms: placebo, 50,000 IU cholecalciferol, or

100,000 IU cholecalciferol to be administered every month, with the final dose given at 16 wk postpartum. Randomization was not stratified by season. These intervention doses were selected because they conformed to commonly available cholecalciferol preparations, and the administration of 50,000 IU cholecalciferol/mo is a commonly prescribed and funded regimen for the treatment of vitamin D insufficiency in New Zealand (with no daily dosing options being publicly funded); and, as previously discussed, daily maternal and infant dosing have been subject to issues of noncompliance. All tablets were supplied to the participants and self-administered, with written instructions and monthly reminder phone calls to take 1 tablet at baseline (4 wk postpartum) and every month thereafter, with the last dose administered at 16 wk postpartum.

Sociodemographic data were obtained from interviews at enrollment (maternal only) and at 4 and 20 wk postpartum (mother and infant). Information was obtained on maternal age, education, ethnicity, smoking status, vitamin and mineral supplement use, breastfeeding, exposure to infant formula (frequency and duration), sun exposure, and sunscreen use. Maternal height and weight measurements were also taken at enrollment and at 4 wk postpartum by using standardized techniques. Infant length and weight measurements were taken at birth and at 4 and 20 wk of age. BMI (in kg/m²) was determined, and infant z scores were calculated by using the WHO Child Growth Standards (24). Blood was drawn at birth (infant cord) and at 4 wk (mother) and 20 wk (infant and mother) postpartum. Last, maternal and infant skin reflectance was measured at each visit by spectrophotometer (CM2006d; Minolta Co. Ltd.) and converted to a final skin color assessment of “very light,” “light,” “intermediate,” “dark,” or “very dark.” Measurement sites included the medial aspect of the upper arm (natural skin color) and dorsal aspect of the forearm (sun-exposed surface).

Supplements. Study supplements were manufactured by OPTIMUS Healthcare Ltd. as identical tablets, each containing lactose powder (placebo) and a target of either 50,000 or 100,000 IU cholecalciferol. In addition, vitamin D content was independently measured by Eurofins NZ Laboratory Services Ltd., which confirmed that the placebo contained no detectable cholecalciferol and the mean cholecalciferol content of tablets used for the 50,000-IU/mo and 100,000-IU/mo interventions were 51,139 and 93,630 IU/tablet, respectively. Supplements were coded by a third party (Dunedin Public Hospital Pharmacy), and participants, investigators, and biostatisticians were blinded to treatment. The randomization list was kept strictly confidential in a sealed envelope until all aspects of the study had been performed. Participants were instructed to take 1 tablet/mo and to return all unused pills. They were also advised to avoid any additional supplemental vitamin D ingestion and overseas travel and to use sunscreen for the duration of the study. Mothers were contacted every month during the study to assess their progress and to remind them to take their monthly dose, with compliance assessed by counting returned pills at the end of the study.

Dietary vitamin D intake. Although all of the mothers planned to exclusively breastfeed from birth to postpartum week 20, they were asked to record the intake of any infant formula and the daily amount consumed. To estimate the daily intake of vitamin D as IU/d, the total IU of vitamin D consumed from infant formula (as per the manufacturer's label, IU/100 g) over the 20-wk study period was divided by the number of study days.

Blood and urine sampling and laboratory analysis. Fasting, second-void morning urine for maternal Ca-Cr ratio was also measured at 4 and 20 wk postpartum. Nonfasting venous maternal, cord, and infant blood samples were collected. Serum calcium, phosphate, albumin, and alkaline phosphatase were measured immediately; and remaining serum was separated into aliquots and stored at -80°C until study completion. The serum total calcium concentration was corrected for serum albumin by using a standard approach because calcium in serum is mainly bound to albumin and thus abnormal albumin may not accurately reflect the physiologically important ionized or free calcium concentrations (25). Urinary creatinine was measured by using photometric analysis via a fully automated Cobas 8000 c502 analyzer (Roche Diagnostics), with the remaining assays analyzed by using a Cobas 8000 c702 analyzer (Roche Diagnostics). The clinical

⁸ Abbreviations used: Ca-Cr, calcium-to-creatinine ratio; DBP, vitamin D binding protein; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D₂, 25-hydroxyvitamin D₂; 25(OH)D₃, 25-hydroxyvitamin D₃.

cutoffs used to define maternal serum hypercalcemia and hypercalciuria at any time point were serum corrected calcium >2.6 mmol/L (26) and Ca-Cr ratio >0.6 mol/100 mol (27), respectively. For infants at 20 wk, serum hypercalcemia was defined as serum corrected calcium >2.8 mmol/L (26).

Serum samples for 25(OH)D and parathyroid hormone (PTH) concentrations from each participant pair were analyzed in the same assay to avoid interassay variation. Serum total concentrations of 25(OH)D (sum of 25-hydroxyvitamin D₃ [25(OH)D₃] and 25-hydroxyvitamin D₂ [25(OH)D₂]) were measured by isotope-dilution LC–tandem MS (28) by using an API 3200 instrument (Applied Biosystems) connected to a Dionex Ultimate 3000 HPLC system. The limit of quantification for the assay was <5 nmol/L for both metabolites. To assess accuracy and interassay variability, external-quality placebo serum material (UTAK Laboratories) containing low and medium concentrations of both metabolites were analyzed with every assay. Measurements fell within the expected range with mean \pm SD values of 29.0 ± 1.2 and 67.3 ± 2.1 nmol/L for 25(OH)D₃ (UTAK verified values of 25.0 and 69.9 nmol/L, respectively) and 26.2 ± 1.4 and 67.0 ± 2.9 nmol/L for 25(OH)D₂ (UTAK verified values of 29.1 and 67.9 nmol/L, respectively). Internal-quality placebo pooled serum samples were also analyzed, with an interassay CV for 25(OH)D₃ of 3.7% at 56.9 nmol/L; 25(OH)D₂ was below the limit of quantification.

Serum PTH was measured by using an automated electrochemiluminescence immunoassay (Elecsys 2010; Roche Diagnostics). The PTH assay showed a detection sensitivity of 1.2 pg/mL. The placebo samples provided by the manufacturer were within the recommended target value and the interassay CV based on a pooled serum was 4.4% ($n = 22$).

Statistical analyses. The sample size of 84 (28 infants/group) was calculated to detect a minimum difference of 15 nmol/L in infant serum 25(OH)D at 80% statistical power, assuming an SD of 20 nmol/L. To allow for an estimated 10% loss to follow-up, a total of 90 mother-infant pairs were recruited.

Baseline characteristics were summarized as means \pm SDs for continuous variables and as percentage distributions for categorical variables. The primary study outcomes were as follows: 1) change in infant 25(OH)D from baseline to week 20 and 2) proportion of infants defined with deficiency at week 20 [as per Australian and New Zealand guidelines (29)]. Secondary outcome measures included change in maternal 25(OH)D from baseline to week 20 and proportion of maternal deficiency at 20 wk. For the change in infant and maternal 25(OH)D, the data were analyzed by using linear regression with serum 25(OH)D concentration at week 20 as the outcome, treatment as a fixed effect with the placebo group as reference, and baseline serum 25(OH)D concentration as a covariate. Because season of birth, infant underarm skin color, and vitamin D–supplemented infant formula intake are likely prognostic variables for breastfed infant serum 25(OH)D concentrations, a secondary analysis for infants was carried out with adjustment for these variables. A secondary adjusted analysis was also performed to assess change in maternal serum 25(OH)D concentration that included season of birth and maternal underarm skin color as potentially confounding effects. A Fisher's exact test was used to determine if the proportion of infants who were classified as deficient [serum 25(OH)D <50 nmol/L] or moderately or severely deficient [serum 25(OH)D <30 nmol/L] at 20 wk was different between intervention groups (28).

To examine whether supplementation affected other biochemical or safety indexes, such as serum PTH, serum corrected calcium, serum phosphate, and urinary Ca-Cr ratio, changes in these variables for both mother and infant (as appropriate) were assessed by using linear regression, with treatment as a fixed effect with the placebo group as the reference, and the respective baseline concentration as a covariate. Clinically meaningful abnormalities were defined as above, and proportions were compared between groups.

To show changes in 25(OH)D over time, mean serum 25(OH)D and SE bars were plotted with a line graph for both mothers and infants. Pearson's correlation coefficient and *P* values were calculated between baseline maternal 25(OH)D and infant cord 25(OH)D concentrations. Changes in forearm skin color over the trial period were also investigated, as a proxy to sun exposure. Forearm skin color was categorized as "very light," "light," "intermediate," "dark," or "very dark" (as per the spectrophotometric method described above) at baseline and at week 20.

Mothers and infants were then further categorized into 3 categories: "became lighter" (if their skin color changed to a lighter category), "unchanged," or "became darker" (if their skin color changed to a darker category). A chi-square test was used to determine if skin color change categories differed between treatment groups.

All of the statistical analyses were performed by using Stata (version 13.1; StataCorp). Residuals were calculated and plotted to visually assess whether the assumptions of the statistical models had been met (no transformations were made). A 2-sided *P* value <0.05 was considered significant.

Results

Of the 90 women and infant pairs who were randomly assigned, 87 completed the trial (Figure 1), resulting in a 97% retention rate. Across the treatment groups, the loss to follow-up was similar: 3% ($n = 1$) in the placebo group, 6% ($n = 2$) in the 50,000-IU/mo group, and 0% ($n = 0$) in the 100,000-IU/mo group. Baseline participant characteristics of mothers and infants are shown in Table 1. Median weight, length, and BMI for female infants at birth were 3.5 kg (weight-for-age *z* score: 0.5), 51 cm (length-for-age *z* score: 1.1), and 13.0 (BMI-for-age *z* score: -0.2) and for male infants at birth were 3.7 kg (weight-for-age *z* score: 0.6), 53 cm (length-for-age *z* score: 1.0), and 13.5 (BMI-for-age *z* score: -0.2).

Overall, compliance with study supplementation at week 20 was high (96%) and similar for the 3 groups: 94% for placebo, 94% for the 50,000-IU/mo group, and 100% for the 100,000-IU/mo group. As a surrogate for sun exposure, forearm skin color at week 20 was compared with baseline, with 70% of infants and 8% of mothers having a lighter forearm skin color, 27% of infants and 55% of mothers having an unchanged skin color, and 3% of infants and 30% of mothers having a darker skin color at week 20. There were no significant differences in infant and maternal skin color change between baseline to week 20 between study groups.

In terms of baseline vitamin D status, the mean \pm SD maternal serum 25(OH)D concentration at 4 wk postpartum was 51.3 ± 23.6 nmol/L, with 55% (48 of 87) and 14% (12 of 87) of women having serum 25(OH)D concentrations <50 and <30 nmol/L, respectively (Table 1 depicts data by treatment group). Mean infant cord 25(OH)D concentrations of 37.3 ± 19.5 nmol/L were substantially lower than maternal concentrations, and more than three-quarters (77%; 66 of 86) of the infant participants had a cord 25(OH)D concentration <50 nmol/L. A significant positive correlation was observed between cord 25(OH)D and maternal 25(OH)D concentrations ($r^2 = 0.81$, $P < 0.001$).

Over the 20-wk study period, mean maternal serum 25(OH)D concentrations increased in all 3 groups (Supplemental Figure 1), although the change in mean serum 25(OH)D at the study end was significantly greater among mothers randomly assigned to monthly vitamin D supplementation than in the placebo group (Table 2). After adjustment for potential confounding variables (season of delivery and maternal skin color), the change in serum 25(OH)D among vitamin D–supplemented mothers remained similar; however, the precision of the estimates was slightly improved, resulting in an effect size of 10.8 nmol/L (95% CI: 0.5, 21.1 nmol/L; $P = 0.041$) for the 50,000-IU/mo group and 20.7 nmol/L (95% CI: 10.6, 30.9 nmol/L; $P < 0.001$) for the 100,000-IU/mo group compared with the placebo group.

Among the infants, the mean changes in serum 25(OH)D concentrations from cord blood to 20 wk postnatally by treatment group are shown in Table 2. In contrast to maternal vitamin D status, the magnitude of the change in infant serum 25(OH)D concentrations over time did not significantly differ

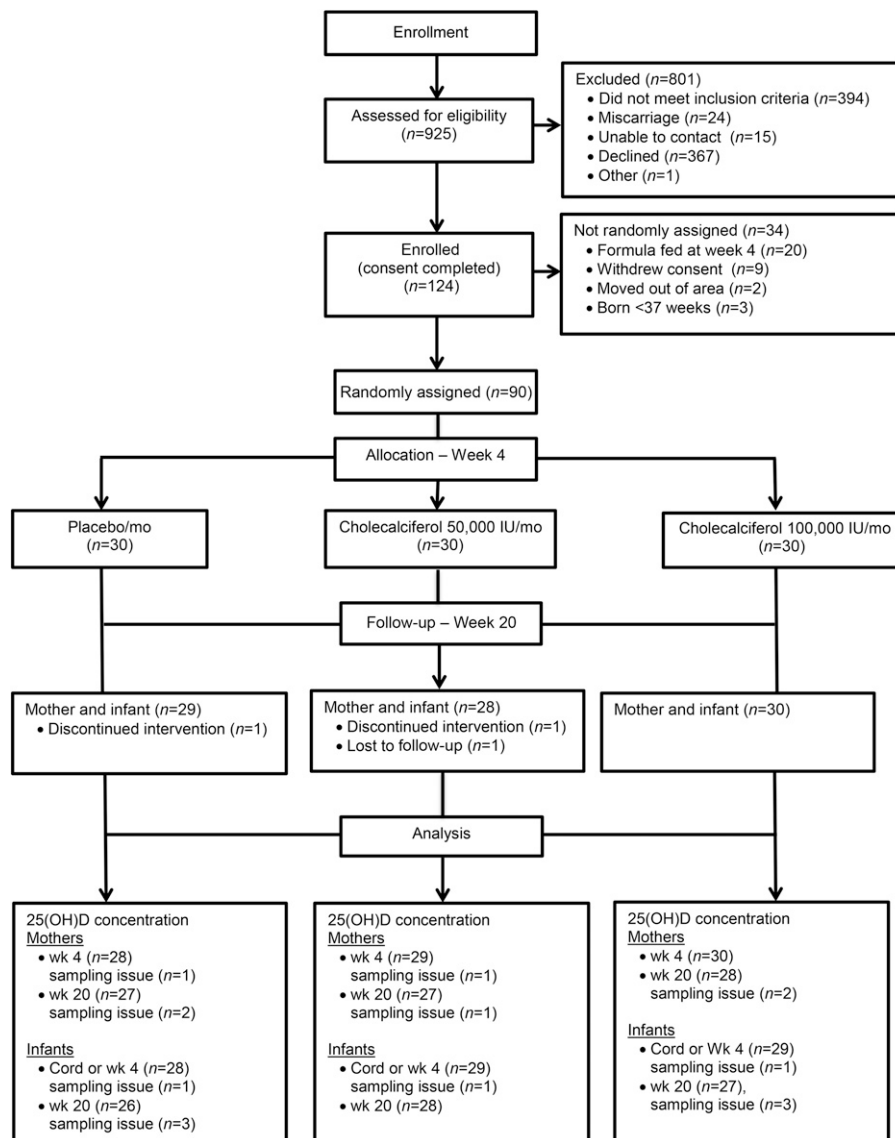


FIGURE 1 CONSORT (Consolidated Standards of Reporting Trials) diagram depicting participant flow and follow-up. 25(OH)D, 25-hydroxyvitamin D

between the 3 groups. After adjustment for known confounding variables, including infant skin color, season of birth, and intake of vitamin D-supplemented infant formula, the effect size estimates for the difference in changes in infant 25(OH)D concentrations were more precise: 5.0 nmol/L (95% CI: -11.3, 21.3 nmol/L; $P = 0.54$) for the 50,000-IU/mo group and 19.1 nmol/L (95% CI: 2.5, 35.6 nmol/L; $P = 0.025$) for the 100,000 IU/mo group compared with the placebo group.

The prevalence of having maternal serum 25(OH)D concentrations <50 nmol/L was 26% (7 of 27) in the placebo group at the end of the study, which was significantly higher than in the 50,000-IU/mo group (4%; 1 of 27) or in the 100,000-IU/mo group (0%) ($P = 0.002$) (Figure 2). Moreover, none of the maternal participants taking vitamin D study supplements had a serum 25(OH)D concentration <30 nmol/L at week 20, whereas 7% (2 of 27) of maternal participants assigned to the placebo group remained at a concentration <30 nmol/L. Among the infant participants, there was no difference in the prevalence of serum 25(OH)D concentration <50 nmol/L among the 3 groups at study completion (27%, 29%, and 19% for the placebo, 50,000-IU/mo, and 100,000-IU/mo groups, respectively; $P = 0.65$) (Figure 2). Likewise, despite a greater number of infants with a 25(OH)D concentration <30 nmol/L in the placebo group (23%;

6 of 26) than in the 50,000-IU/mo (11%; 3 of 28) and 100,000-IU/mo (4%; 1 of 27) groups at the study end, no significant differences were detected between the 3 groups ($P = 0.09$).

In both mothers and infants, there were no significant differences in mean changes in serum PTH, serum corrected calcium, and serum phosphate between groups over the trial period (Table 2). However, 2 infants in the placebo group did have clinically meaningful elevations in serum PTH (above the upper normal limit of 65 pg/mL) of 120 and 281 pg/mL at the end of the intervention. Although normal values for serum PTH concentrations in infants and young children have not been clearly defined, severe vitamin D deficiency [25(OH)D concentrations of 6.4 and 8.6 nmol/L] and elevated alkaline phosphatase values (352 and 507 U/L) were reported for these 2 infants, which suggested possible vitamin D deficiency rickets.

No cases of hyper- or hypocalcemia were found in any mother or infant participant. Mean changes in maternal urinary Ca-Cr ratios over the study intervention were comparable between placebo and treatment groups (50,000-IU/mo: $P = 0.96$; 100,000-IU/mo: $P = 0.22$). Nevertheless, 6 mothers had a higher-than-normal urinary Ca-Cr ratio at week 20 (normal range: <0.6 mol/100 mol): $n = 2$ in the placebo group, $n = 1$ in the 50,000-IU/mo group, and $n = 3$ in the 100,000-IU/mo group. All of these were considered nonfasting

TABLE 1 Baseline characteristics of 90 lactating mother-infant pairs at 4 wk postpartum by maternal treatment dose¹

	Treatment		
	Placebo (<i>n</i> = 30)	50,000 IU/mo (<i>n</i> = 30)	100,000 IU/mo (<i>n</i> = 30)
Maternal			
Age, y	31.2 ± 5.5	32.3 ± 4.0	32.8 ± 5.6
Ethnicity, <i>n</i> (%)			
NZ European	18 (60)	23 (77)	24 (80)
Maori	3 (10)	3 (10)	0
Pacific	0	0	0
Other	4 (13)	6 (20)	6 (20)
Skin color, <i>n</i> (%)			
Very light	15 (50)	18 (60)	16 (53)
Light	11 (37)	11 (37)	11 (37)
Intermediate	1 (3)	1 (3)	2 (7)
Dark	3 (10)	0	1 (3)
Primiparous, <i>n</i> (%)	14 (47)	13 (43)	13 (43)
Tertiary education, ² <i>n</i> (%)	23 (77)	18 (60)	21 (70)
BMI, kg/m ²	27.5 ± 4.4	29.7 ± 4.2	29.6 ± 6.3
Smoker, <i>n</i> (%)	4 (13)	3 (10)	1 (3)
Serum 25(OH)D, nmol/L	51.7 ± 23.4	51.6 ± 24.9	50.6 ± 23.1
Serum PTH, pg/mL	34 ± 14	30 ± 12	38 ± 21
Serum corrected calcium, ³ mmol/L	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1
Serum phosphate, mmol/L	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.2
Urinary Ca-Cr ratio	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.2
Infant			
Gestational age, wk	39.6 ± 1.0	39.7 ± 1.0	39.7 ± 1.1
Male sex, <i>n</i> (%)	16 (53)	12 (40)	15 (50)
Birth weight, g	3613 ± 559	3546 ± 495	3376 ± 435
Birth season, <i>n</i> (%)			
Spring	9 (30)	9 (30)	9 (30)
Summer	4 (13)	3 (10)	5 (17)
Autumn	5 (17)	6 (20)	4 (13)
Winter	12 (40)	12 (40)	12 (40)
Skin color, ⁴ <i>n</i> (%)			
Very light	3 (10)	3 (10)	1 (3)
Light	17 (59)	15 (52)	15 (50)
Intermediate	9 (31)	9 (31)	11 (37)
Dark	0 (0)	2 (7)	3 (10)
Exclusive breastfeeding, ⁵ <i>n</i> (%)	22 (73)	22 (73)	20 (67)
Infant vitamin D intake, ⁶ IU/d			
Total group	14 ± 47	16 ± 42	23 ± 70
Consumers only	49 ± 83	61 ± 66	69 ± 111
Serum total 25(OH)D, nmol/L	37 ± 21	36 ± 18	40 ± 20
Serum PTH, pg/mL	6.1 ± 7.9	4.6 ± 1.7	4.8 ± 3.7
Serum corrected calcium, ³ mmol/L	2.6 ± 0.2	2.6 ± 0.1	2.6 ± 0.2
Serum phosphate, mmol/L	2.0 ± 0.4	1.9 ± 0.4	2.0 ± 0.4

¹ Values are means ± SDs unless otherwise indicated. Ca-Cr, calcium-to-creatinine; NZ, New Zealand; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

² Completed university or other higher education qualification.

³ Serum calcium calculated by using standard corrections for serum albumin (25).

⁴ As determined by skin reflectance with the use of a spectrophotometer.

⁵ Infants received only breast milk; no other liquid or solids were given over 20 wk.

⁶ Birth to postnatal week 20. Number of infants consuming any formula: placebo, *n* = 8; 50,000 IU/mo, *n* = 8; 100,000 IU/mo, *n* = 10.

specimens and a repeat fasting specimen as per study protocol was not undertaken.

Discussion

The results of the present study reveal new insights into the potential effectiveness of a maternal vitamin D dosing strategy for

breastfed infants. To our knowledge, this study is the first to assess the effect of maternal vitamin D supplementation on serum 25(OH)D concentrations in nonsupplemented breastfeeding infants with the use of a double-blind, randomized, placebo-controlled study design. Not surprisingly, we found that mothers who received the moderate (50,000-IU/mo) and high (100,000-IU/mo) doses of cholecalciferol had significantly higher maternal serum 25(OH)D at

TABLE 2 Mean changes in maternal and infant serum 25(OH)D and other biochemical indexes from baseline to week 20 postpartum by maternal cholecalciferol treatment dose¹

	Placebo (n = 28)	Treatment			
		50,000 IU/mo (n = 28)		100,000 IU/mo (n = 29)	
		Value	P ²	Value	P ²
Maternal (week 4 to week 20 postpartum)					
Serum 25(OH)D, nmol/L	17.3 ± 32.7	30.2 ± 25.9		40.0 ± 29.3	
Change in 25(OH)D, ³ nmol/L (×16 wk)	Reference	12.8 (0.4, 25.2)	0.043	21.5 (9.2, 33.8)	0.001
Serum PTH, pg/mL	-1.1 ± 12.8	-0.8 ± 14.2		-5.5 ± 19.6	
Change in PTH, ³ pg/mL (×16 wk)	Reference	-1.6 (-8.7, 5.4)	0.65	-1.9 (-8.8, 5.1)	0.59
Serum corrected calcium, mmol/L	0.07 ± 0.11	0.07 ± 0.13		0.11 ± 0.12	
Change in corrected calcium, ³ mmol/L (×16 wk)	Reference	-0.01 (-0.07, 0.04)	0.63	0.02 (-0.03, 0.07)	0.46
Serum phosphate, mmol/L	0.01 ± 0.20	-0.01 ± 0.18		0.05 ± 0.28	
Change in phosphate, ³ mmol/L (×16 wk)	Reference	-0.05 (-0.14, 0.04)	0.27	0.01 (-0.07, 0.10)	0.75
Infant (birth to 20 postnatal weeks)					
Serum 25(OH)D, nmol/L	37.4 ± 51.9	44.7 ± 44.7		52.8 ± 46.4	
Change in 25(OH)D, ⁴ nmol/L (×20 wk)	Reference	4.5 (-16.1, 25.0)	0.67	15.8 (-4.7, 36.4)	0.13
Serum PTH, pg/mL	31.9 ± 61.4	17.6 ± 10.9		13.6 ± 7.8)	
Change in PTH, ⁴ pg/mL (×20 wk)	Reference	-17.0 (-38.2, 4.2)	0.11	-20.7 (-42.8, 1.4)	0.066
Serum corrected calcium, mmol/L	0.06 ± 0.22	0.04 ± 0.19		0.04 ± 0.21	
Change in corrected calcium, ⁴ mmol/L (×20 wk)	Reference	0.02 (-0.02, 0.07)	0.35	0.01 (-0.03, 0.06)	0.56
Serum phosphate, mmol/L	-0.22 ± 0.43	-0.06 ± 0.43		-0.13 ± 0.48	
Change in phosphate, ⁴ mmol/L (×20 wk)	Reference	0.06 (-0.02, 0.15)	0.15	0.06 (-0.02, 0.15)	0.14

¹ Values are means ± SDs or means (95% CIs) unless otherwise indicated. PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

² Placebo compared with cholecalciferol interventions with the use of linear regression.

³ Change over 16 wk compared with placebo.

⁴ Change over 20 wk compared with placebo.

20 wk postpartum than those mothers who received the placebo. Moreover, the pharmacologic cholecalciferol doses given once monthly over a period of 4 mo appeared to be safe and well tolerated. However, the effects of maternal vitamin D supplementation on infant serum 25(OH)D were more complex. For the primary unadjusted analysis, there was no significant differential effect of maternal supplementation on infant vitamin D status or a decreased prevalence of having a serum 25(OH)D concentration <50 nmol/L at the end of the study period. However, after adjustment for potentially important confounders, including season of birth, infant formula exposure, and infant skin color, we found that high-dose maternal monthly cholecalciferol supplementation (100,000 IU/mo) resulted in a significant and clinically meaningful increase in infant serum 25(OH)D of 19 nmol/L compared with that in the placebo group. Moreover, the higher dose of 100,000 IU/mo may also offer some protection against moderate to severe deficiency in infants, with only 1 infant in this group (compared with 6 infants in the placebo group) presenting with a serum 25(OH)D concentration of <30 nmol/L.

Importantly, infants in the present study were not administered any vitamin D supplements. Thus, the observed changes in infant vitamin D status can therefore be attributed to the effect of maternal vitamin D supplementation, particularly given that the adjusted analysis for the intake of vitamin D-containing infant formula showed significantly higher infant 25(OH)D concentrations in the 100,000-IU/mo group. These findings align with those of previous maternal supplementation studies in lactating women (19, 21–23, 30). These previous studies trialed both daily and monthly supplementation; however, all lacked controls and often had inadequate blinding. One of the first maternal vitamin D supplementation trials conducted in lactating women (19) was designed to provide either 1600 or 3600 IU ergocalciferol/d for a period of 3 mo beginning at 1 mo postpartum together with an

infant supplement of 400 IU cholecalciferol/d. At 4 mo of age, infants had attained mean 25(OH)D concentrations of 69 and 77 nmol/L, respectively. The authors concluded that maternal vitamin D intakes ≥4000 IU/d appeared to ensure adequate infant vitamin D status. Of importance, we reported a similar mean serum infant 25(OH)D concentration of 76 nmol/L at week 20 in study infants whose mothers were receiving a placebo. This observed increase in 25(OH)D concentrations in placebo-assigned infants over the 20-wk postnatal period highlights one of the most notable findings of our study, and similar increases in serum 25(OH)D have been shown in a number of longitudinal studies in unsupplemented breastfed infants (4, 31–35). The observed increase in serum 25(OH)D over the course of the study may be the result of a seasonal imbalance in recruitment and/or possibly a physiologic increase over time in the circulation of vitamin D binding proteins (DBPs), a key determinant of 25(OH)D concentrations (36). Information on DBP in early infancy is limited, but it is known that adult albumin concentrations and binding affinity are not reached until 10–12 mo of age. Regardless, these results emphasize the importance of a placebo group. In light of our findings, the reported effect size of previous uncontrolled studies may have been potentially confounded by a natural temporal trend toward increased vitamin D status in the first year of life.

It is unknown whether a higher monthly maternal dose would have achieved greater improvements in infant vitamin D status, but the lower 50,000-IU cholecalciferol dose every 4 wk appeared to be ineffective. A recent uncontrolled, blinded study administering a single maternal cholecalciferol dose of 150,000 IU showed a significant increase in infant serum 25(OH)D concentrations from baseline (40.8 nmol/L) to day 28 (96.8 nmol/L) (22). However, the safety of this dosage with regular use (every 4 wk) is unknown and currently exceeds the Tolerable Upper Intake Level of daily intake suggested by the Institute of Medicine by >30% (37). In our

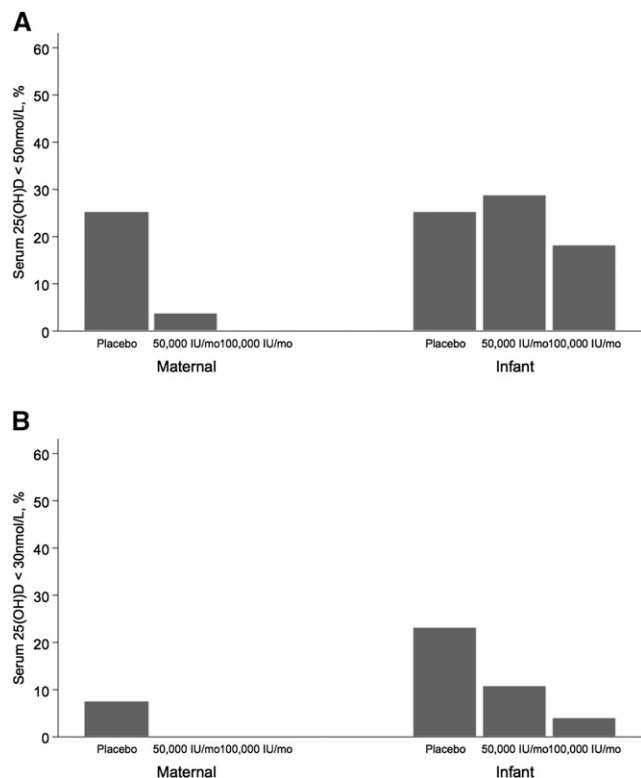


FIGURE 2 Prevalence of maternal and infant serum 25(OH)D concentrations <50 nmol/L (A) or <30 nmol/L (B) at 20 wk postpartum, by maternal monthly treatment dose. Cholecalciferol supplement intake (IU/mo) is shown in 3 categories. For maternal groups: placebo ($n = 27$), 50,000 IU/mo ($n = 27$), and 100,000 IU/mo ($n = 28$); for infant groups: placebo ($n = 26$), 50,000 IU/mo ($n = 28$), and 100,000 IU/mo ($n = 27$). Fisher's exact test was used to test for differences between maternal groups with concentrations <30 nmol/L (not testable) and <50 nmol/L ($P = 0.002$) and infant groups with concentrations <30 nmol/L ($P = 0.09$) and <50 nmol/L ($P = 0.65$). 25(OH)D, 25-hydroxyvitamin D.

current investigation, we found that doses of $\leq 100,000$ IU every 4 wk were well tolerated and the incidence of maternal hypercalcemia and hypercalciuria did not differ between groups, nor was vitamin D treatment associated with any laboratory, clinical, or serious adverse effect. In addition, we did not observe hypervitaminosis D [defined as serum 25(OH)D concentrations >225 nmol/L] in any of our participants [peak maternal and infant serum 25(OH)D of 136 and 140 nmol/L, respectively].

Because cord blood serum concentrations of 25(OH)D in the present study were highly correlated with maternal concentrations, an alternative strategy for improving the vitamin D status of breastfed infants would be to increase fetal vitamin D status via maternal supplementation during pregnancy. A number of studies have examined this outcome (4, 21, 38, 39), with the most recent findings reported by March et al. (21), which showed that cholecalciferol doses between 400 and 2000 IU/d during pregnancy resulted in substantially higher cord 25(OH)D, ranging from 73 to 95 nmol/L. In contrast, the mean cord 25(OH)D in unsupplemented mothers in the present study was 37.3 nmol/L. March et al. also reported that supplementation with 2000 IU/d antenatally appeared to protect 98% of unsupplemented breastfed infants against vitamin D deficiency [serum 25(OH)D <30 nmol/L] until ≥ 8 wk postnatally (21).

The strengths of this present study are its randomized, double-blind, placebo-controlled design conducted in a setting with little interference from additional vitamin D supplements or food fortification. In addition, the longer duration of our

study (encompassing the recommended period for exclusive breastfeeding), high exclusive breastfeeding rates achieved (ranging from 67% to 73%), and close to 100% rate of current breastfeeding at study completion are also strengths in comparison to previous studies (4, 19, 21–23, 30). Study limitations include the convenience sample of healthy women in the South Island of New Zealand who were mainly of European descent (72%). As a result, it is unknown whether these results can be generalized to other ethnic groups or other regions of varying latitude in New Zealand. Another limitation is that breast-milk analysis for vitamin D content was not available, which would have been ideal as a secondary outcome measure, even though the half-life of cholecalciferol in milk is ~ 24 –72 h (22). Last, the lack of effect in the primary analysis may be due to type 2 error and is a limitation that reflects both a potentially small sample size and an imbalance of recruitment by season.

In conclusion, our study shows that maternal vitamin D supplementation in lactating women during the first 5 mo of breastfeeding improved the vitamin D status of the mother and suggests that doses of 100,000 IU cholecalciferol/mo are not only safe but may improve the vitamin D status of the breastfeeding infant, and subsequently prevent deficiency in the first 6 mo of life. In addition, these data show a general increase in serum 25(OH)D concentrations over the first 20 wk of life, which questions the effect size and beneficial effects of previous uncontrolled maternal supplementation studies. Further work is warranted to support our findings and to more fully investigate the efficacy of varying maternal dosage regimens (e.g., daily or weekly) on both maternal and infant vitamin D status.

Acknowledgments

BJW, BJT, and LAH conceived and designed the study and obtained funding; BJW, BJT, PH, SJ, and LAH further refined and finalized the research protocol; BJW, AM, and SJ conducted the research including coordinating data collection and recruitment; BJW, SJ, MJH, and LAH oversaw the laboratory analyses; PH and JJH conducted the statistical analysis; and BJW, JJH, SJ, and LAH drafted the research manuscript. All authors contributed to the review and revision of the manuscript, and read and approved the final manuscript.

References

- Hollis BW, Roos BA, Draper HH, Lambert PW. Vitamin D and its metabolites in human and bovine milk. *J Nutr* 1981;111:1240–8.
- McKenzie RL, Liley JB, Bjorn LO. UV radiation: balancing risks and benefits. *Photochem Photobiol* 2009;85:88–98.
- Rockell JE, Green TJ, Skeaff CM, Whiting SJ, Taylor RW, Williams SM, Parnell WR, Scragg R, Wilson N, Schaaf D, et al. Season and ethnicity are determinants of serum 25-hydroxyvitamin D concentrations in New Zealand children aged 5–14 y. *J Nutr* 2005;135:2602–8.
- Grant CC, Stewart AW, Scragg R, Milne T, Rowden J, Ekeroma A, Wall C, Mitchell EA, Crengle S, Trenholme A, et al. Vitamin D during pregnancy and infancy and infant serum 25-hydroxyvitamin D concentration. *Pediatrics* 2014;133:e143–53.
- Wall CR, Grant CC, Jones I. Vitamin D status of exclusively breastfed infants aged 2–3 months. *Arch Dis Child* 2013;98:176–9.
- Houghton LA, Szymlek-Gay EA, Gray AR, Ferguson EL, Deng X, Heath AL. Predictors of vitamin D status and its association with parathyroid hormone in young New Zealand children. *Am J Clin Nutr* 2010;92:69–76.
- American Academy of Pediatrics Committee on Environmental Health. Ultraviolet light: a hazard to children. *Pediatrics* 1999;104:328–33.
- Pettifor JM, Prentice A. The role of vitamin D in paediatric bone health. *Best Pract Res Clin Endocrinol Metab* 2011;25:573–84.

9. Wheeler BJ, Dickson NP, Houghton LA, Ward LM, Taylor BJ. Incidence and characteristics of vitamin D deficiency rickets in New Zealand children: a New Zealand Paediatric Surveillance Unit study. *Aust N Z J Public Health* 2015;39:380–3.
10. Munns CF, Simm PJ, Rodda CP, Garnett SP, Zacharin MR, Ward LM, Geddes J, Cherian S, Zurynski Y, Cowell CT, et al. Incidence of vitamin D deficiency rickets among Australian children: an Australian Paediatric Surveillance Unit study. *Med J Aust* 2012;196:466–8.
11. Ward LM, Gaboury I, Ladhani M, Zlotkin S. Vitamin D-deficiency rickets among children in Canada. *CMAJ* 2007;177:161–6.
12. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* 2008;122:1142–52.
13. Perrine CG, Sharma AJ, Jefferds ME, Serdula MK, Scanlon KS. Adherence to vitamin D recommendations among US infants. *Pediatrics* 2010;125:627–32.
14. Taylor JA, Geyer LJ, Feldman KW. Use of supplemental vitamin D among infants breastfed for prolonged periods. *Pediatrics* 2010;125:105–11.
15. Gallo S, Jean-Philippe S, Rodd C, Weiler HA. Vitamin D supplementation of Canadian infants: practices of Montreal mothers. *Appl Physiol Nutr Metab* 2010;35:303–9.
16. Crocker B, Green TJ, Barr SI, Beckingham B, Bhagat R, Dabrowska B, Douthwaite R, Evanson C, Friesen R, Hydamaka K, et al. Very high vitamin D supplementation rates among infants aged 2 months in Vancouver and Richmond, British Columbia, Canada. *BMC Public Health* 2011;11:905.
17. Ahrens KA, Rossen LM, Simon AE. Adherence to vitamin D recommendations among US infants aged 0 to 11 months, NHANES, 2009 to 2012. *Clin Pediatr (Phila)* 2016;55:555–6.
18. Weisberg P, Scanlon KS, Li R, Cogswell ME. Nutritional rickets among children in the United States: review of cases reported between 1986 and 2003. *Am J Clin Nutr* 2004;80:1697S–705S.
19. Hollis BW, Wagner CL. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. *Am J Clin Nutr* 2004;80 (Suppl):1752S–8S.
20. Basile LA, Taylor SN, Wagner CL, Horst RL, Hollis BW. The effect of high-dose vitamin D supplementation on serum vitamin D levels and milk calcium concentration in lactating women and their infants. *Breastfeed Med* 2006;1:27–35.
21. March KM, Chen NN, Karakochuk CD, Shand AW, Innis SM, von Dadelszen P, Barr SI, Lyon MR, Whiting SJ, Weiler HA, et al. Maternal vitamin D3 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. *Am J Clin Nutr* 2015;102:402–10.
22. Oberhelman SS, Meekins ME, Fischer PR, Lee BR, Singh RJ, Cha SS, Gardner BM, Pettifor JM, Croghan IT, Thacher TD. Maternal vitamin D supplementation to improve the vitamin D status of breast-fed infants: a randomized controlled trial. *Mayo Clin Proc* 2013;88:1378–87.
23. Saadi HF, Dawodu A, Afandi B, Zayed R, Benedict S, Nagelkerke N, Hollis BW. Effect of combined maternal and infant vitamin D supplementation on vitamin D status of exclusively breastfed infants. *Matern Child Nutr* 2009;5:25–32.
24. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatr Suppl* 2006;450:76–85.
25. Cooper MS, Gittoes NJ. Diagnosis and management of hypocalcaemia. *BMJ* 2008;336:1298–302.
26. Chan MK, Seiden-Long I, Aytekin M, Quinn F, Ravalico T, Ambruster D, Adeli K. Canadian Laboratory Initiative on Pediatric Reference Interval Database (CALIPER): pediatric reference intervals for an integrated clinical chemistry and immunoassay analyzer, Abbott ARCHITECT ci8200. *Clin Biochem* 2009;42:885–91.
27. Metz MP. Determining urinary calcium/creatinine cut-offs for the paediatric population using published data. *Ann Clin Biochem* 2006;43:398–401.
28. Maunsell Z, Wright DJ, Rainbow SJ. Routine isotope-dilution liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of the 25-hydroxy metabolites of vitamins D₂ and D₃. *Clin Chem* 2005;51:1683–90.
29. Paxton GA, Teale GR, Nowson CA, Mason RS, McGrath JJ, Thompson MJ, Siafarikas A, Rodda CP, Munns CF; Australian and New Zealand Bone and Mineral Society; Osteoporosis Australia. Vitamin D and health in pregnancy, infants, children and adolescents in Australia and New Zealand: a position statement. *Med J Aust* 2013;198:142–3.
30. Hollis BW, Wagner CL, Howard CR, Ebeling M, Shary JR, Smith PG, Taylor SN, Morella K, Lawrence RA, Hulsey TC. Maternal versus infant vitamin D supplementation during lactation: a randomized controlled trial. *Pediatrics* 2015;136:625–34.
31. Challa A, Ntourtoui A, Cholevas V, Bitsori M, Galanakis E, Andronikou S. Breastfeeding and vitamin D status in Greece during the first 6 months of life. *Eur J Pediatr* 2005;164:724–9.
32. Kim MJ, Na B, No SJ, Han HS, Jeong EH, Lee W, Han Y, Hyeun T. Nutritional status of vitamin D and the effect of vitamin D supplementation in Korean breast-fed infants. *J Korean Med Sci* 2010;25:83–9.
33. Ziegler EE, Hollis BW, Nelson SE, Jeter JM. Vitamin D deficiency in breastfed infants in Iowa. *Pediatrics* 2006;118:603–10.
34. Narchi H, Kochiyil J, Zayed R, Abdulrazzak W, Agarwal M. Longitudinal study of vitamin D status in the 1st 6 months of life. *Ann Trop Pediatr* 2011;31:225–30.
35. Perumal N, Al Mahmud A, Baqui AH, Roth DE. Prenatal vitamin D supplementation and infant vitamin D status in Bangladesh. *Public Health Nutr* 2015;6:1–9.
36. Carpenter TO, Zhang JH, Parra E, Ellis BK, Simpson C, Lee WM, Balko J, Fu L, Wong BY, Cole DE. Vitamin D binding protein is a key determinant of 25-hydroxyvitamin D levels in infants and toddlers. *J Bone Miner Res* 2013;28:213–21.
37. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, et al. The 2011 report on Dietary Reference Intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2011;96:53–8.
38. Roth DE, Al Mahmud A, Raqib R, Akhtar E, Perumal N, Pezzack B, Baqui AH. Randomized placebo-controlled trial of high-dose prenatal third-trimester vitamin D3 supplementation in Bangladesh: the AViDD trial. *Nutr J* 2013;12:47.
39. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res* 2011;26:2341–57.