

Effect of Different Dosages of Oral Vitamin D Supplementation on Vitamin D Status in Healthy, Breastfed Infants

A Randomized Trial

Sina Gallo, RD, MSc

Kathryn Comeau, RD, MSc

Catherine Vanstone, RN, MSc

Sherry Agellon, MSc

Atul Sharma, MD, MSc

Glenville Jones, PhD

Mary L'Abbé, PhD

Ali Khamessan, PhD

Celia Rodd, MD, MSc

Hope Weiler, RD, PhD

VITAMIN D IS IMPORTANT DURING periods of rapid bone mineral accrual. Nursing infants are susceptible to vitamin D deficiency because vitamin D in breast milk is limited.¹ Pediatric societies in the United States² and Canada³ recommend 400 IU (10 µg) per day based on maintenance of 25-hydroxyvitamin D (25[OH]D) concentrations in the range of 75 to 150 nmol/L (30-60 ng/mL). Similarly, the Institute of Medicine's health policy recommendation for infants in North America is 400 IU/d but targets lower 25(OH)D concentrations, between 40 and 50 nmol/L (16-20 ng/mL).⁴ This policy also established a healthy range of 50 to 125 nmol/L for 25(OH)D concentrations.⁴

Several functional outcomes have been used to systematically assess the

Importance Vitamin D supplementation in infancy is required to support healthy bone mineral accretion. A supplement of 400 IU of vitamin D per day is thought to support plasma 25-hydroxyvitamin D (25[OH]D) concentrations between 40 and 50 nmol/L; some advocate 75 to 150 nmol/L for bone health.

Objective To investigate the efficacy of different dosages of vitamin D in supporting 25(OH)D concentrations in infants.

Design, Setting, and Participants Double-blind randomized clinical trial conducted among 132 one-month-old healthy, term, breastfed infants from Montréal, Québec, Canada, between March 2007 and August 2010. Infants were followed up for 11 months ending August 2011 (74% completed study).

Intervention Participants were randomly assigned to receive oral cholecalciferol (vitamin D₃) supplements of 400 IU/d (n=39), 800 IU/d (n=39), 1200 IU/d (n=38), or 1600 IU/d (n=16).

Main Outcomes and Measures The primary outcome was a plasma 25(OH)D concentration of 75 nmol/L or greater in 97.5% of infants at 3 months. Secondary outcomes included 25(OH)D concentrations of 75 nmol/L or greater in 97.5% of infants at 6, 9, and 12 months; 25(OH)D concentrations of 50 nmol/L or greater across all times; growth; and whole body and regional bone mineral content. Data were analyzed by intention to treat using available data, logistic regression, and mixed-model analysis of variance.

Results By 3 months, 55% (95% CI, 38%-72%) of infants in the 400-IU/d group achieved a 25(OH)D concentration of 75 nmol/L or greater vs 81% (95% CI, 65%-91%) in the 800-IU/d group, 92% (95% CI, 77%-98%) in the 1200-IU/d group, and 100% in the 1600-IU/d group. This concentration was not sustained in 97.5% of infants at 12 months in any of the groups. The 1600-IU/d dosage was discontinued prematurely because of elevated plasma 25(OH)D concentrations. All dosages established 25(OH)D concentrations of 50 nmol/L or greater in 97% (95% CI, 94%-100%) of infants at 3 months and sustained this in 98% (95% CI, 94%-100%) to 12 months. Growth and bone mineral content did not differ by dosage.

Conclusions and Relevance Among healthy, term, breastfed infants, only a vitamin D supplement dosage of 1600 IU/d (but not dosages of 400, 800, or 1200 IU/d) increased plasma 25(OH)D concentration to 75 nmol/L or greater in 97.5% of infants at 3 months. However, this dosage increased 25(OH)D concentrations to levels that have been associated with hypercalcemia.

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Author Affiliations: School of Dietetics and Human Nutrition, McGill University (Mss Gallo, Comeau, Vanstone, and Agellon and Drs Rodd and Weiler), Montreal Children's Hospital, McGill University Health Centre (Drs Sharma and Rodd), and Euro-pharm International Canada Inc (Dr Khamessan), Montréal, Québec, Canada; Departments of Biomedical and Molecular Sciences and Medicine,

Queen's University, Kingston, Ontario, Canada (Dr Jones); and Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada (Dr L'Abbé).

Corresponding Author: Hope Weiler, RD, PhD, School of Dietetics and Human Nutrition, McGill University, 21111 Lakeshore Rd, Ste-Anne-de-Bellevue, Québec H9X 3V9, Canada (hope.weiler@mcgill.ca).

adequacy of vitamin D, including 25(OH)D at various target concentrations (50 and 75 nmol/L), parathyroid hormone concentrations, and bone health.⁴ However, there is a paucity of such data in infants. In one study,⁵ bone mineral content (BMC) was lower in infants receiving a supplement of 400 IU/d compared with placebo. The effects of doses higher than 400 IU on bone health are unknown. While France⁶ and Finland⁷ recommend vitamin D intakes greater than 1000 IU/d for infants, the lack of well-defined recommendations supports the need for dose-response studies.

The objective of this study was to investigate the efficacy of different dosages of oral vitamin D in supporting 25(OH)D concentrations in infants.

METHODS

Overview

The study was a single-center, double-blind, randomized dose-response trial conducted among 132 infants who were randomly assigned at 1 month of age to receive 400, 800, 1200, or 1600 IU/d of oral cholecalciferol (vitamin D₃) for 11 months. The primary objective was to establish a dosage of oral vitamin D supplementation that would support 25(OH)D concentrations of 75 nmol/L or greater in 97.5% of breastfed infants by 3 months of age. Secondary objectives were to evaluate the dosages that would support plasma concentrations of 25(OH)D of 50 nmol/L or greater in 97.5% of infants at 3 months, as well as to evaluate whether concentrations were sustained at 12 months with any dosage, to evaluate growth, and to measure bone mineral accrual from 1 to 12 months of age. The primary outcome was assessed at 3 months of age because most infants are still predominantly breastfed at this age,⁸ other sources of vitamin D are limited, and bone modeling is rapid.⁹ The objectives were designed to provide data to help establish a Recommended Dietary Allowance value, which is defined as the intake level that meets the nutrient requirements of nearly all in-

dividuals (97%-98%) in a life-stage group.⁴ For vitamin D, this recommendation considers 25(OH)D concentration and bone health outcomes. The secondary outcomes, including 25(OH)D concentration, growth, and BMC, were monitored at 5 study visits conducted at 1 (baseline), 3, 6, 9, and 12 months of age. Growth and safety end points were evaluated at each visit and at an additional visit at 2 months. The study was carried out between March 2007 and August 2011 at the Mary Emily Clinical Nutrition Research Unit of McGill University, Montréal, Québec, Canada, and was approved by the institutional review board of McGill University and Health Canada. Parents provided written informed consent and received compensation for travel.

Sample

Newborns (≤ 1 month of age) were referred from Lakeshore General Hospital and 5 pediatric clinics located in greater Montréal between March 2007 and August 2010 (FIGURE 1). The final study measures were obtained in August 2011, when the last infant reached 12 months of age. Infants who were healthy, term, singleton, appropriate size for gestational age, and breastfeeding (consuming $\geq 80\%$ of total milk volume) were eligible to participate. Exclusion criteria included infants of mothers with gestational diabetes, hypertension in pregnancy, chronic alcohol use, or malabsorption syndromes. Parents self-identified race/ethnicity, using Canadian Census criteria, to assist in interpretation of vitamin D status¹⁰ and bone health assessments.¹¹ Other demographic information including education and income were reported by the mothers.

Supplements, Masking, and Adherence

Supplements containing 400, 800, 1200, or 1600 IU of vitamin D₃ were formulated by Europharm International Canada Inc and administered in 2-mL/d volume using a standardized dropper; all had similar taste, smell, and appearance. Supple-

ments were provided in precoded bottles of 60-mL volume. Parents and researchers were blinded to treatment dosage. All dosages were proven to be within 10% of target dosage, with an 18-month shelf life. Adherence evaluation included weighing bottles before and after use and recording number of missed doses reported by mothers.

Randomization and Stopping Rules

Following enrollment into the study and baseline measurements, the infants were randomly assigned to 1 of the 4 groups in a 1:1:1:1 allocation ratio. Randomization was stratified by sex in equal blocks of 4. The randomization list was generated using <http://www.randomization.com> and blinded supplement codes. The codes were revealed only after the statistical analysis was complete.

An independent safety monitoring officer reviewed unblinded data. Stopping rules included hypercalcemia or evidence of plasma 25(OH)D concentrations of 250 nmol/L or greater because such values may be associated with hypercalcemia.³ In July 2008, the 1600-IU/d group was discontinued because 93% of infants ($n=15/16$) developed plasma 25(OH)D concentrations of 250 nmol/L or greater by 3 months of age based on an enzyme immunoassay. The protocol was amended and those receiving 1600 IU/d ($n=10$; 6-9 months of age) were subsequently switched to the standard-of-care dosage (400 IU/d) until 12 months of age; their data were analyzed by intention to treat (ie, 1600-IU/d group). Those recruited at 1 month of age after July 2008 ($n=17$) were randomized in a 1:1:1 ratio to the remaining 3 groups¹² and analyzed in their respective group (Figure 1).

Endogenous and Dietary Vitamin D Sources

Potential for endogenous synthesis of vitamin D through exposure to sunlight was assessed by questionnaire and sun index (hours per week of sun exposure multiplied by percentage body surface area exposed).¹³ Vitamin D in breast milk was estimated from the

volume of breast milk consumed over a 24-hour period.¹⁴ Vitamin D from other foods was assessed using 3-day dietary records completed by parents after each visit. Nutrient intake was generated using Nutritionist Pro software version 4.7.0 (Axxya Systems LLC) and the 2010b Canadian Nutrient File database (Health Canada).

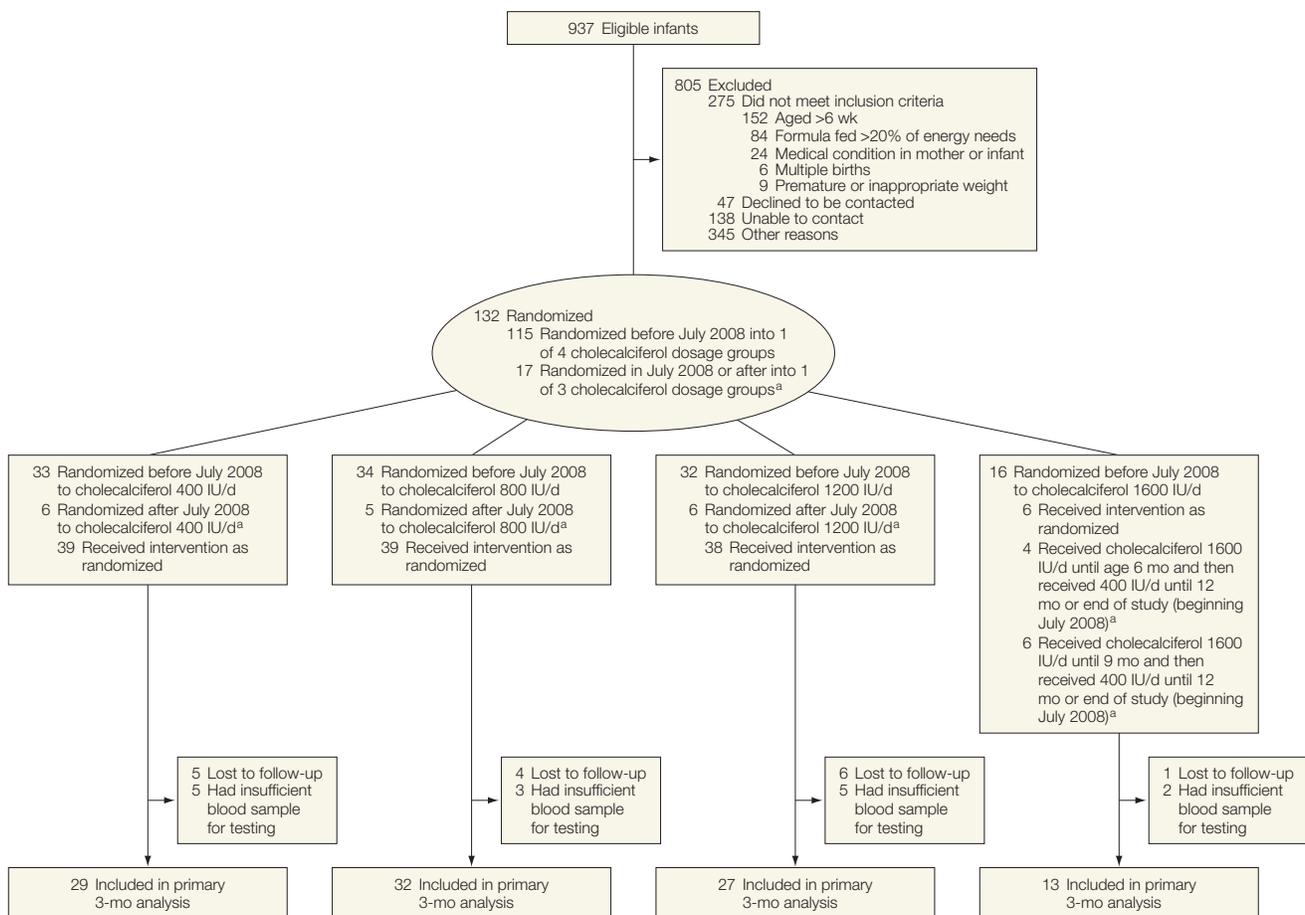
Plasma 25(OH)D and Other Vitamin D Metabolites

Capillary plasma samples from heel or finger were stored frozen at -80°C for batch analysis. Samples were screened for safety assessments for plasma 25(OH)D at McGill University using an enzyme immunoassay (Octeia, Immu-

nodiagnostic Systems Ltd). A commonly used¹⁵ radioimmunoassay for measurement of 25(OH)D (DiaSorin Inc) and liquid chromatography tandem mass spectrometry (LC-MS/MS)¹⁶ were used to measure the primary outcome. The immunoassays quantify total 25(OH)D, whereas the LC-MS/MS assay separately quantifies 25(OH)D isomers (25[OH]D₂ + 25[OH]D₃), as well as the 3-epimer-25(OH)D¹⁷ and 24,25-dihydroxyvitamin D (24,25[OH]₂D) which may interfere in immunoassays.¹⁸ Plasma 25(OH)D, 3-epimer-25(OH)D, and 24,25(OH)₂D were quantified (Warnex Bio-analytical Services) based on internal standards: 25(OH)D₃-d(6) (Chemaphor Inc), 25(OH)D₂-d(3) (Isosci-

ences), and 3-epi-25(OH)D₃-d(6) and 24,25(OH)₂D₃-d(6) (Toronto Research Chemicals). Briefly, following liquid-liquid extraction, vitamin D metabolites were derivatized using a substituted triazolinedione in a Diels-Alder addition,^{19,20} separated on a high-performance liquid chromatograph system, and detected using an API-4000 (ABSciex ON) or a TSQ-Vantage LC-MS/MS instrument (Thermo Scientific). All infants had vitamin D₂ metabolites below the limit of quantification. The intra-assay coefficient of variation was less than 15% for all vitamin D metabolites across all assays; the laboratories were certified by the Vitamin D External Quality Assessment Scheme to facilitate comparison with other laboratories.

Figure 1. Participant Flow



^aIn July 2008, the 1600-IU/d group was discontinued because 93% of infants developed plasma 25-hydroxyvitamin D concentrations of 250 nmol/L or greater by age 3 months.

Safety Outcomes

The safety end points were selected to comprehensively examine calcium homeostasis and risk of soft tissue calcification.^{3,4} Blood-ionized calcium (ABL 725 series blood gas analyzer; Radiometer America) and plasma total calcium, phosphorus, alkaline phosphatase, and urinary calcium:creatinine were measured (Beckman Coulter DxC600) within 4 hours of collection. Parathyroid hormone was measured using an enzyme-linked immunosorbent assay (Immutopics International). Intra-assay coefficient of variation was less than 5%. Without normative reference limits for ionized calcium and urinary calcium:creatinine in healthy infants, these were extrapolated from hospitalized older children. Any safety measures with results outside the normal range were repeated; if confirmed as such, the infant was switched to the standard of care (400 IU/d vitamin D₃) but analyzed per intention to treat.

Secondary Efficacy Outcomes

The proportion of infants who maintained plasma 25(OH)D concentrations of 75 nmol/L or greater at 6, 9, and 12 months of age were explored as secondary outcomes, as was the proportion who maintained 25(OH)D concentrations of 50 nmol/L or greater. At each visit, nude weight (infant scale model SB 32000, Mettler-Toledo Inc), length (O'Learly Length Board, Ellard Instrumentation Ltd), and head circumference (nonstretchable tape) were measured and reported as absolute units, and z scores were calculated using World Health Organization growth standards.²¹ Bone mineral content and bone mineral density (BMD) were measured at each of the 5 study visits using dual-energy x-ray absorptiometry (Hologic 4500A Discovery, APEX software version 13.2:1, Hologic Inc). Infants were scanned in array mode to obtain BMC of the whole body, lumbar spine vertebrae 1 to 4, and whole femur while sleeping (nonsedated) as previously described.^{10,22} Change in BMC was calculated between visits. The co-

efficient of variation was 1% for BMC and 0.3% for BMD using a spine phantom (Hologic phantom No. 14774).

Sample Size

The primary objective was to determine which dosage met the plasma 25(OH)D threshold of 75 nmol/L in at least 97.5% of infants at 3 months of age. The sample estimate assumed that 50% of the infants in the 400-IU/d group would be able to achieve a 25(OH)D concentration of 75 nmol/L or greater by 3 months. To achieve our target, this would require a change of 47.5% from the 400-IU/d group. Using a χ^2 statistic to compare proportions, 23 infants per group gave the study greater than 90% power at the .05 significance level. Assuming 20% dropout and an additional 20% to compensate for early weaning from breastfeeding, 32 participants per group would be necessary at 3 months.

Statistical Analysis

Baseline differences among groups were tested using analysis of variance (ANOVA) for continuous variables and χ^2 (with Fisher exact tests for small sample sizes) for categorical variables (including recruitment site); characteristics with differences among groups were included as covariates in regression modeling. Group differences in breastfeeding status and adherence (proportion of treatments taken) were analyzed using χ^2 at each time point. Infant anthropometry, dietary intake, and sun exposure variables were tested across treatments and time (repeated-measures mixed-model analysis of variance). In evaluating 25(OH)D concentrations at each time, differences among treatments were tested using the existing data and logistic regression without imputing the small number of missing observations. Careful comparisons of participants with missing and fully observed data were consistent with data missing at random. The effects of vitamin D dosage on plasma 25(OH)D concentration, safety biochemistry, growth, and bone mineral accretion were also explored and tested using repeated-

measures mixed-model analysis of variance with time-treatment interactions. The mixed-model analysis of variance estimates the effect size based on available data (Figure 1 and FIGURE 2), and participants with missing data are not dropped, mitigating the need for imputation.²³ Post hoc tests (estimate statements) were used to test for differences between the 400-IU/d and higher dosage groups (800 and 1200 IU/d). The 1600-IU/d group was not included in the statistical models because of its discontinuation; data are presented descriptively. Statistical significance was set at $P \leq .05$ with 2-tailed testing. Data were analyzed using SAS version 9.2 statistical software (SAS Institute Inc).

RESULTS

Of 937 referred infants, 275 were excluded, 530 did not consent, and 132 were enrolled (Figure 1), of whom 84% were taking a vitamin D supplement (400 IU/d). Maternal and infant baseline characteristics were similar among groups except for mother's race ($P = .03$); thus, race was included as a covariate in all analyses (TABLE). There were no differences in attrition rates (Figure 1), referring center, or reported adherence (eTable 1; available at <http://www.jama.com>) across treatment groups. Overall, 88% of infants received breast milk up to 6 months of age and 35% up to 12 months. Time, but not group, differences were observed in nutrient intakes, including dietary vitamin D and sun exposure (eTable 1). Maternal education and infant baseline 25(OH)D concentrations were lower in dropouts vs completers, and more dropouts were nonwhite (eTable 2).

The percentage of infants achieving the primary outcome of 75 nmol/L of 25(OH)D differed at 3 months by group (for 400 IU/d, 55% [95% CI, 38%-72%]; for 800 IU/d, 81% [95% CI, 65%-91%]; for 1200 IU/d, 92% [95% CI, 77%-98%]; and for 1600 IU/d, 100%) (Figure 2A). The percentage of infants at 6, 9, and 12 months who met or exceeded the target cutoff of 75 nmol/L of 25(OH)D declined with time. The crude

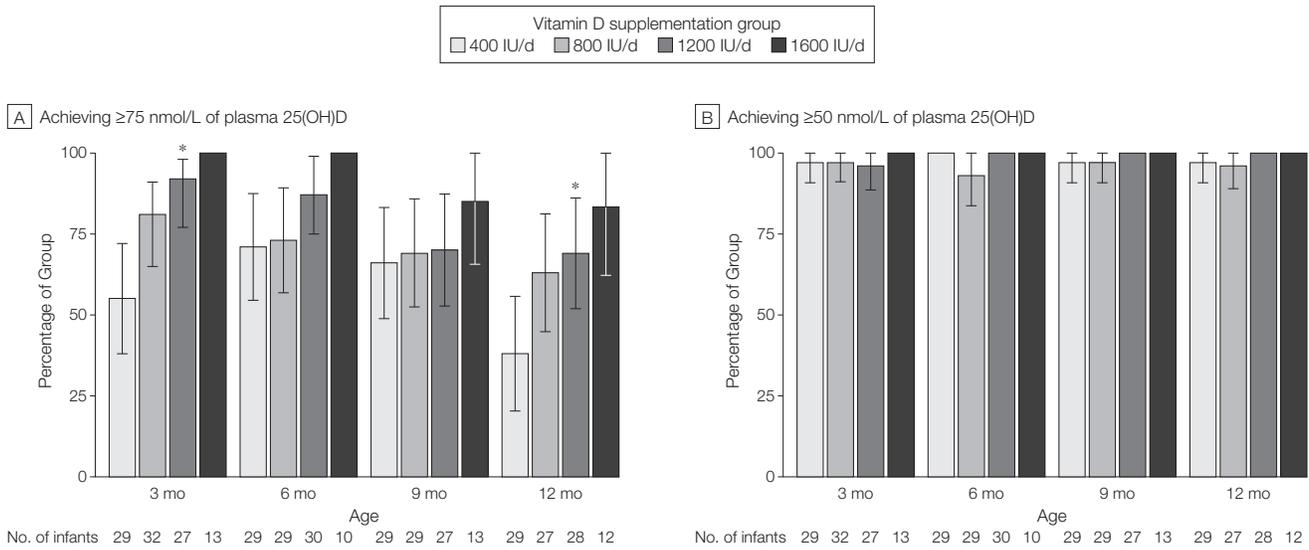
and adjusted logistic regression models (eTable 3) indicate that at 3 months and after adjusting for race only (model 1), the 800-IU/d had an odds ratio of 3.5 (95% CI, 1.1-11.0) vs the 400-IU/d group for achieving a 25(OH)D concentration of 75 nmol/L or greater, whereas the odds ratio for achieving this in the 1200-IU/d vs 400-IU/d group was 9.7 (95% CI,

1.9-49.7). Adjusting for sex and period of birth (model 2) did not change these results.

Overall, 97% (95% CI, 94%-100%) of infants in all treatment groups achieved the secondary outcome of 50 nmol/L or greater of plasma 25(OH)D by 3 months of age, with no differences among groups (for 400 IU/d, 97% [95% CI, 91%-100%];

800 IU/d, 97% [95% CI, 91%-100%]; 1200 IU/d, 96% [95% CI, 89%-100%]; and 1600 IU/d, 100%) (Figure 2B). This concentration was sustained in 98% (95% CI, 94%-100%) of infants at 12 months. The 25(OH)D concentrations in all groups peaked at approximately 3 months with mean concentrations of 78 (95% CI, 71-84) nmol/L in the 400-IU/d group, 102

Figure 2. Percentage of Infants Achieving at Least 75 nmol/L and 50 nmol/L of Plasma 25(OH)D Concentration at 3, 6, 9, and 12 Months Using Liquid Chromatography Tandem Mass Spectrometry



Error bars indicate 95% CIs. 25(OH)D indicates 25-hydroxyvitamin D. *P < .05 vs 400-IU/d group.

Table. Baseline Characteristics of Participating Infants and Their Mothers

| Characteristics | Vitamin D ₃ Supplementation Dosage ^a | | | |
|---|--|------------------------|--------------------------|--------------------|
| | 400 IU/d (n = 39) | 800 IU/d (n = 39) | 1200 IU/d (n = 38) | 1600 IU/d (n = 16) |
| Infants | | | | |
| Sex | | | | |
| Male | 22 (56.4) | 23 (59.0) | 22 (57.9) | 9 (56.2) |
| Female | 17 (43.6) | 16 (41.0) | 16 (42.1) | 7 (43.8) |
| Race | | | | |
| White | 30 (79.0) | 31 (79.5) | 37 (97.4) ^{b,c} | 13 (86.7) |
| Other ^d | 8 (21.0) | 8 (20.5) | 1 (2.6) | 2 (13.3) |
| Age, mean (95% CI), d | 36 (34-38) | 34 (32-36) | 34 (33-36) | 32 (31-34) |
| Birth during vitamin D synthesizing period ^e | 24 (61.5) | 21 (53.9) | 25 (65.8) | 9 (56.3) |
| Receiving vitamin D supplement at baseline | 37 (97.4) | 30 (79.0) ^b | 33 (89.2) | 11 (73.3) |
| Mothers | | | | |
| Age, mean (95% CI), y | 33 (32-34) | 32 (31-34) | 38 (32-34) | 33 (31-36) |
| Primiparous | 18 (46.2) | 15 (38.5) | 12 (31.6) | 6 (37.5) |
| Income ≥Can \$75 000 | 27 (69.2) | 20 (52.6) | 22 (59.5) | 9 (56.3) |
| Completed college/university | 34 (87.2) | 36 (92.3) | 33 (86.8) | 14 (87.5) |

^aData are reported as No. (%) unless otherwise indicated.

^bP = .03 vs 400 IU/d.

^cP = .03 vs 800 IU/d by Fisher exact test.

^dOther race includes black, Hispanic, First Nations, Asian, Hawaiian/Pacific Islander, and nonwhite mixed race.

^eInfants categorized as born during months of the year when cutaneous vitamin D production is possible (April-October), based on latitude.²⁴

(95% CI, 90-114) nmol/L in the 800-IU/d group, 134 (95% CI, 118-150) nmol/L in the 1200-IU/d group, and 180 (95% CI, 154-207) nmol/L in the 1600-IU/d group (FIGURE 3).

Differences were observed in the values of plasma 25(OH)D obtained using different methods (eFigure 1); the enzyme immunoassay tended to overestimate and the radioimmunoassay to underestimate 25(OH)D concentrations vs LC-MS/MS. The 3-epimer-25(OH)D, measured by LC-MS/MS, was present in 98% of all samples tested. In proportion to plasma 25(OH)D, 3-epimer-

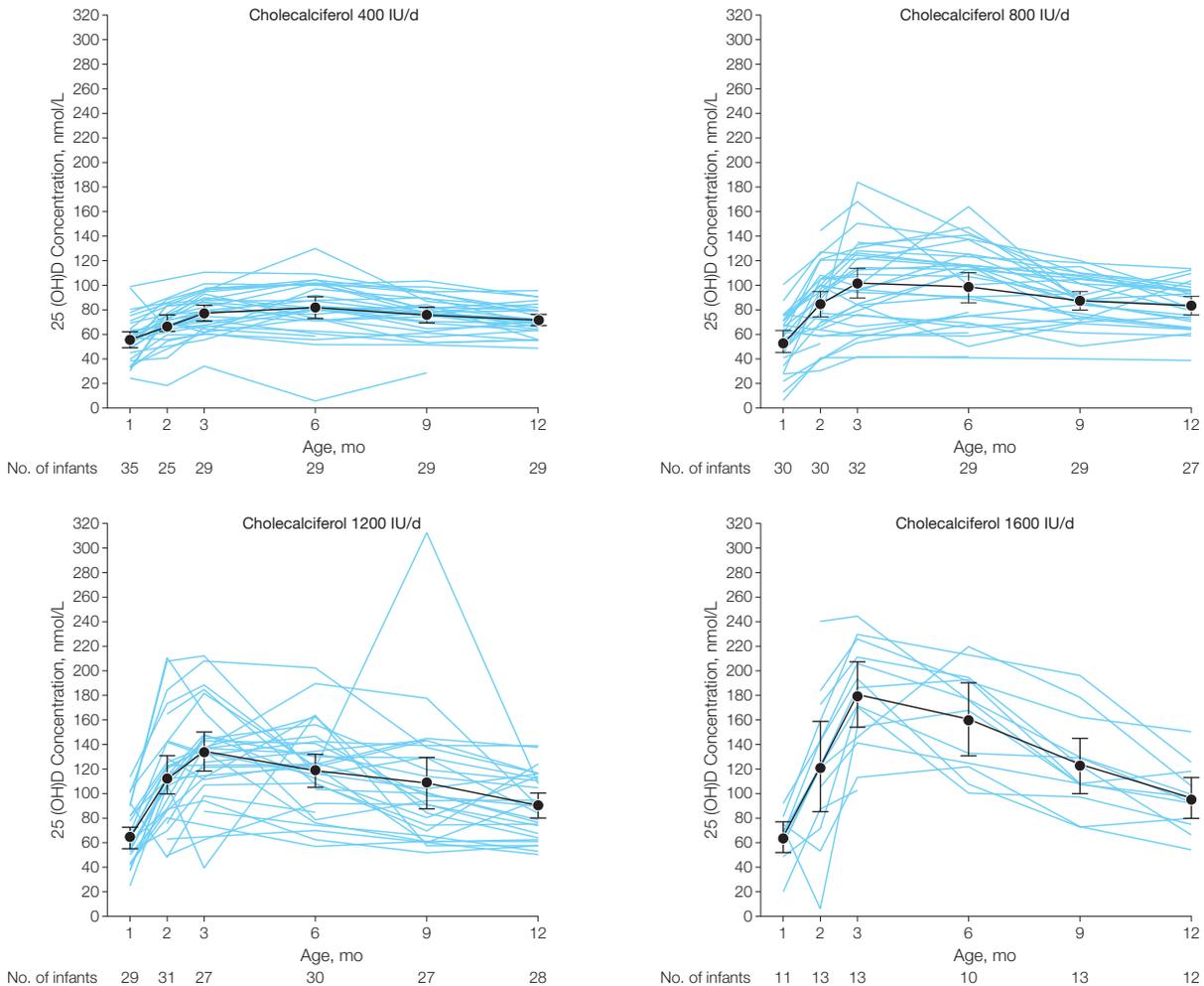
25(OH)D was equivalent to 30% to 40% of plasma 25(OH)D at baseline and declined to approximately 10% by 12 months (eFigure 2A). Plasma 24,25(OH)₂D, measured by LC-MS/MS, was equivalent to approximately 15% of plasma 25(OH)D concentration (eFigure 2B). Concentrations of both 3-epimer-25(OH)D and 24,25(OH)₂D₃ were different only at 2 and 3 months and only in the 400-IU/d vs 1200-IU/d groups.

Bone mineral concentration increased over time for lumbar spine, femur, and whole body (eFigure 3) but

did not differ by group. Similarly, lumbar spine BMD did not differ by group or time. Infants grew in an age-appropriate way over time, with no differences by group (eFigure 4A-C).

Ionized calcium and urinary calcium:creatinine values (eFigure 4D-E) declined over time, with no treatment interaction. Plasma parathyroid hormone values increased over time without treatment or interaction effects (eFigure 4F). Over the 11 months, total plasma calcium (median, 2.52 [interquartile range {IQR}, 2.47-2.57] mmol/L), alkaline phosphatase (median, 240 [IQR,

Figure 3. Mean Plasma 25(OH)D Concentrations by Vitamin D₃ Supplementation Dosage Using Liquid Chromatography Tandem Mass Spectrometry



Black data markers indicate means; error bars indicate 95% CIs. 25(OH)D indicates 25-hydroxyvitamin D. Data for each participant are shown as a spaghetti plot underlying the summary estimates.

193-289] U/L), and phosphate (median, 1.89 [IQR, 1.74-2.01] mmol/L) differed over time, but no differences among groups were noted. Forty-two infants were retested because the results of their safety measurements were outside the normal range (n=9 in the 400-IU/d, n=10 in the 800-IU/d, n=17 in 1200-IU/d, and n=6 in the 1600-IU/d groups). Based on these results and suspected hypercalcemia (n=2 in the 800-IU/d, n=2 in the 1200-IU/d, and n=2 in the 1600-IU/d groups) or suspected hypercalciuria (n=1 in the 800-IU/d, n=1 in the 1200-IU/d, and n=1 in the 1600-IU/d groups), additional safety procedures (electrocardiogram or renal ultrasound) were conducted at the Montreal Children's Hospital; all results were normal.

DISCUSSION

Our primary objective was to establish a vitamin D dosage that would support a plasma concentration of 25(OH)D of 75 nmol/L or greater in 97.5% of infants at 3 months of age. Only the 1600-IU/d dosage of vitamin D met this criterion; however, this dosage was discontinued because most infants in that group developed elevated plasma 25(OH)D concentrations that have been associated with hypercalcemia.⁴ Although a higher percentage of infants in the 800-IU/d and 1200-IU/d groups achieved 75 nmol/L of 25(OH)D (81% and 92%, respectively), only 55% of infants did so in the 400-IU/d group at 3 months. Thus, the primary outcome was not achieved at 3 months, when plasma 25(OH)D concentrations were highest; all dosages failed except the highest dosage, which appears to be too high. Furthermore, in none of the groups was this concentration sustained in 97.5% of infants at 12 months. In contrast, regardless of vitamin D dosage, 97% of our infant population achieved the secondary outcome of a 25(OH)D concentration of 50 nmol/L at 3 months and 98% from 6 to 12 months.

A dose-response study of vitamin D intake and bone health has been lacking in infants.⁴ Despite our demonstration of a dose-response relationship in

plasma 25(OH)D concentrations relative to vitamin D dosage, no such response was observed in BMC or accrual. Similarly, older studies did not find improvements in bone mineral accretion⁵ or content²⁵ over 26 weeks with 400 IU/d of vitamin D vs placebo. Bone mineral accretion in breastfed infants is likely optimal and less affected by vitamin D supplementation unless an underlying deficiency exists. Alternatively, the sensitivity of dual-energy x-ray absorptiometry to detect small changes in infants over an 11-month period may not be sufficient. Possible benefits to bone may take longer to present, as shown in prospective studies.²⁶

The safety of vitamin D dosages greater than 400 IU/d has not been tested in infants. The tolerable upper intake levels for infants aged 0 to 6 months of 1000 IU/d and for those aged 6 to 12 months of 1500 IU/d were established based on a no-observed-adverse-effect level of 1800 IU/d.⁴ This level was established from data collected in 1938²⁷ showing that vitamin D greater than 1800 IU/d for 6 months impaired linear growth. Our data did not demonstrate abnormalities in growth or calcium homeostasis. Although the 1600-IU/d group was discontinued, this was based on 25(OH)D concentrations exceeding the normal range.³ The other safety procedures were designed to monitor safety end points at the individual level and were thus not powered to test for safety at the population level. Reassuringly, a cohort of 10 060 Finnish infants followed up prospectively⁷ for 31 years showed that dosages up to 2000 IU/d did not alter linear growth. A randomized clinical trial²⁸ found similar increases in circulating 25(OH)D concentrations with dosages up to 1600 IU/d for 3 months; however, uncertainty still remains about this high dosage.

Concentrations of 25(OH)D declined from 3 to 12 months of age in all groups although dietary vitamin D sources increased. This decline in all groups may be due to decreased adherence, but it is more likely that the changes reflect the relative intake over time. For example, infants in

the 400-IU/d group at 3 months would have received 64 IU/kg, whereas this declined to 42 IU/kg at 12 months. Whether the lower values may reflect increased requirements based on larger size and hepatic maturity is unclear.

During the study, several methods were examined to measure vitamin D metabolites in a selective, sensitive, and accurate manner. Even though the radioimmunoassay has been widely used,^{15,29} we chose LC-MS/MS to interpret our key observations because it is now considered a gold-standard assay.³⁰ Discrepancies among antibody-based methods in cross-method comparisons^{31,32} and our findings question the usefulness of antibody-based methods for infant assessments.³³ In agreement with others,¹⁷ an abundance of 3-epimer-25(OH)D was observed during the first year of life, although its biological significance is unclear. In addition, 24,25(OH)₂D was equivalent to 15% of the 25(OH)D pool in infant samples. To our knowledge, the present study represents the first analyses of the several key vitamin D metabolites during infancy; these metabolite concentrations mirror vitamin D intake.

Even though this study has many strengths, including rigorous methods, the data may not represent all Canadian infants because the sample consisted of a large proportion of well-educated, high-income mothers, and baseline plasma 25(OH)D concentrations at 1 month of age were robust, with an average of 59 nmol/L (95% CI, 55-63 nmol/L). The population studied was underrepresented in participants with darker skin pigmentation, who are at higher risk of deficiency.^{3,4} Moreover, the study may have been underpowered to test for some secondary outcomes and too short to assess for benefits to bone. A larger, more heterogeneous group would need to be studied.

In conclusion, only the 1600-IU/d dosage of vitamin D met the 25(OH)D concentration target of 75 nmol/L or greater, confirming the guidelines of the Endocrine Society.² However, it also led to plasma 25(OH)D concentrations that exceeded the healthy population target range of 50 to 125

nmol/L.⁴ Furthermore, dosages of vitamin D exceeding 400 IU/d provide no additional benefits for bone mineral accretion up to 1 year of age. Additional studies are required before conclusions can be made regarding higher targets or the needs of high-risk groups.

Author Contributions: Drs Rodd and Weiler, the senior authors of this article, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Sharma, Jones, L'Abbé, Khamessan, Rodd, Weiler.

Acquisition of data: Gallo, Comeau, Vanstone, Agellon, Jones, Weiler.

Analysis and interpretation of data: Gallo, Sharma, Jones, L'Abbé, Rodd, Weiler.

Drafting of the manuscript: Gallo, Vanstone, Rodd, Weiler.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Gallo, Sharma, Rodd, Weiler.

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Administrative, technical, or material support: Gallo,

Comeau, Vanstone, Agellon, Jones, Khamessan, Rodd, Weiler.

Study supervision: Gallo, Vanstone, Agellon, L'Abbé, Rodd, Weiler.

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