The Change in Plasma 25-Hydroxyvitamin D Did Not Differ between Breast-Fed Infants That Received a Daily Supplement of Ergocalciferol or Cholecalciferol for 3 Months\(^1\)-\(^4\)

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Abstract

The biological equivalency of ergocalciferol (D2) and cholecalciferol (D3) has been debated; several comparisons have appeared in the adult literature but are scarce in pediatrics. The objective of this study was to compare increases in plasma 25-hydroxyvitamin D \(^{25}(\text{OH})\text{D}\) concentrations and attainment of 50 and 75 mol/L status cutoffs following 3 mo of daily supplementation with D2 compared with D3. Healthy, breast-fed, 1-mo-old infants \((n = 52)\) received 10 \(\mu\)g \((400 \text{ IU})\) of either D2 or D3 daily. At 1 and 4 mo of age, plasma 25-hydroxyergocalciferol and 25-hydroxycholecalciferol concentrations were determined by liquid chromatography tandem MS \((\text{LC-MS/MS})\) and total \(25(\text{OH})\text{D}\) by chemiluminescent immunoassay \((\text{DiaSorin Liaison})\). Data were analyzed using \(t\) tests and \(x^2\) by intent to treat. A total of 23\% of infants were deficient \((\geq 24.9 \text{ nmol/L})\) at baseline and 2\% at follow-up on the basis of LC-MS/MS. At 4 mo, 96\% were breastfed and there were no differences in compliance, breastfeeding rates, or sun exposure among groups. The change in total \(25(\text{OH})\text{D}\) measured by LC-MS/MS did not differ between the D2 \((17.6 \pm 26.7 \text{ nmol/L})\) and D3 \((22.2 \pm 20.2 \text{ nmol/L})\) groups. In the combined groups, the baseline plasma \(25(\text{OH})\text{D}\) concentration was inversely related to the change in total \(25(\text{OH})\text{D}\) \((r = -0.52; P < 0.001)\). Overall, 86\% of infants met the 50 nmol/L cutoff at follow-up; however, fewer infants in the D2 group \((75\%)\) met this level compared with the D3 group \((96\%)\) \((P < 0.05)\). Similar results were obtained by immunoassay. In conclusion, the increase in the \(25(\text{OH})\text{D}\) concentration among the D2 and D3 groups did not differ, suggesting daily intake of either isoform is acceptable for infants <4 mo. J. Nutr. 143: 148–153, 2013.

Introduction

Most countries around the world recommend supplementing breast-fed infants with 10 \(\mu\)g \((400 \text{ IU})\) of vitamin D daily \((1–6)\). This dosage is thought to maintain circulating 25-hydroxyvitamin D \(^{25}(\text{OH})\text{D}\)\(^8\) concentrations of 40–50 nmol/L as suggested by the Institute of Medicine \((\text{IOM})\) for bone health \((3)\); however, other organizations advise using a higher cutoff of 75 nmol/L \((7)\). Standard infant preparations in Canada are mammalian cholecalciferol \((\text{D3})\), but most research studies tested plant-derived ergocalciferol \((\text{D2})\) \((8–10)\). These isomers differ in side chain structure and were originally thought to exhibit identical biological responses \((3,11,12)\). There is controversy in the adult literature regarding the equivalency of the 2 isoforms. A single dose of 1250 \(\mu\)g \(\text{D3}\) increased 25(\text{OH})D based on AUC during 28 d with 3–10 times greater efficacy than D2 \((13)\). Furthermore, 100 \(\mu\)g/d \(\text{D3}\) resulted in a 75\% greater change in 25(\text{OH})D \((15)\). Similar results were observed in elderly women taking supplements containing either D2 or D3 \((<12.5 \mu\text{g/d})\) for an average 5–6 y \((16)\). It appears that dosage and sustained supplementation may explain the differences observed in these adult studies.

A recent meta-analysis concluded that D2 is less effective than D3 at raising the 25(\text{OH})D concentration \((17)\) and highlighted that too few studies exist to verify if this is the
situation in children (18,19). In neonates, there are additional physiological differences in intestinal absorption (19) and vitamin D metabolism (20) from adults. Whether differences in the absorption and binding to the vitamin D-binding protein between isoforms affect plasma 25(OH)D concentrations in infants is yet undetermined. A study was designed with the objective to compare the effectiveness of 10 μg/d of D2 compared with D3 to increase or maintain circulating 25(OH)D concentrations during a 3-mo period in healthy, breast-fed newborns. Our secondary objective was to compare the proportion of infants able to meet the 50 (3,6) or 75 (7) nmol/L cutoffs at follow-up.

Participants and Methods

**Trial design.** Infants were randomly assigned to receive a 10-μg/d oral dose of either D2 or D3 in a 1:1 ratio stratified by sex. Both the D3- (NPN no. 80001869) and D2- (NPN no. 80003406) based products are commercially available (Ddrops Company), eliminating the need for Health Canada approval as a phase IV trial. There were no differences in appearance and both products were tasteless and odorless. These products are oil based (coconut and palm) and dosages were delivered in 1-drop volumes (0.03 mL) using a standardized Eurodropper. Compliance was assessed by weighing unused portions in bottles and parent self-report. Compliance was calculated as the number of dosages taken divided by the days since last visit and “compliant” was defined as 80–100% of doses taken. The study was approved by the Institutional Review Board of McGill University. Parents gave written informed consent.

**Participants.** Newborns were referred from a primary care hospital and birthing center located in greater Montréal between May 2010 and October) for Montréal located at latitude 45° (21). Infants were eligible to begin the study at 1 mo of age if they were healthy, singleton, term infants born the appropriate size for gestational age as assessed according to the WHO growth charts (between 5th and 95th percentile) and to healthy, breastfeeding women (consuming >80% of total foods from breast milk). Exclusion criteria included infants of mothers with a history of gestational diabetes or hypertension in pregnancy, malabsorption syndromes (celiac and Crohn’s disease), or taking medications that interfere with vitamin D metabolism (anticonvulsants and corticosteroids). Mothers taking ≥50 μg/d of vitamin D from supplementation were not included, because this value is above the current nutrition recommendations (22). Demographic information, including race, education, and income (in Canadian dollars), was reported by the mother. At baseline and follow-up visits, weight, length, and head circumference were measured. Data are expressed in absolute units and Z-scores using data from the 2006 WHO growth charts (23) at each time point.

**Dietary and endogenous vitamin D sources.** Information on infant breastfeeding status (feeds/day) and formula feeds (amount, frequency, brand) was collected at each visit. Skin pigmentation was measured on the constitutive upper underarm and facultative forehead, forearm, and outer lower leg using a portable computerized spectrophotometer (CM-600D, Konica Minolta). Based on the Commission Internationale de l’Eclairage colorimetry system (L*a*b*), the individual typological angle (ITA) [ITA = [arc tangent (L*a*b*))] 180/3.14159 was calculated (24). Infants were classified into 5 skin phototypes: dark (≤10°), olive (10–28°), medium (28–41°), fair (41–55°), and very fair (>55°). The ITA difference between the exposed (forearm) and unexposed (inner arm) skin sites was used to assess exposure to sun during the trial.

**Plasma 25(OH)D.** Capillary blood samples were collected from infants by heel or finger lance and mother’s fastest blood was collected by venipuncture (between 0800 and 1200 h). Samples were centrifuged (2235 × g for 20 min at 4°C) and stored frozen at −80°C for batch analysis. Plasma samples were analyzed using a sensitive (limit of quantification: 12 nmol/L) liquid chromatography tandem MS (LC-MS/MS) developed by Warnex Bioanalytical Services. This method uses derivatization of vitamin D metabolites with substituted triazolinediones in a Diels-Alder cycloaddition with chromatographic separation of

**TABLE 1** Infant and mother baseline characteristics

| Variable                             | Treatments       |
|--------------------------------------|------------------|
|                                     |                  |
| **Mothers**                          |                  |
| Plasma 25(OH)D,² nmol/L              | 68.3 ± 21.4      |
| Age at delivery, y                   | 31.0 ± 4.5       |
| Income ≥75,000 Canadian $, n (%)     | 16 (61.5)        |
| Education, ≥university, n (%)        | 20 (76.9)        |
| **Infants**                          |                  |
| Male, n (%)                          | 12 (46.2)        |
| Born during vitamin D-synthesizing period (April–October), n (%) | 15 (57.7) |
| Taking a vitamin D supplement at baseline, n (%) | 17 (65.4) |
| Age started vitamin D supplement, d  | 4 (3, 7)         |
| White, self-identified, n (%)        | 14 (58.3)        |
| Skin color (based on ITA)¹, n (%)    |                  |
| Very fair                            | 2 (7.7)          |
| Fair                                 | 12 (46.2)        |
| Medium                               | 7 (26.9)         |
| Olive                                | 3 (11.5)         |
| Dark                                 | 2 (7.7)          |

¹ Values are frequency and percent or mean ± SD, n = 26. Non-normally distributed data presented as median (25th, 75th percentile). D2, ergocalciferol; D3, cholecalciferol; ITA, individual typological angle; LC-MS/MS, liquid chromatography tandem MS; 25(OH)D, 25-hydroxyvitamin D.

² Results were tested by LC-MS/MS. The results were similar to LC-MS/MS when tested by immunoassay: D2: 74.0 ± 21.7 vs. D3: 79.6 ± 27.4; P = 0.42.

³ Based on mother’s and father’s race.

⁴ ITA° = [arc tangent (L° – 50/b°)] 180/3.14159, classified in 5 skin phototypes: dark (≤10°), olive (10–28°), medium (28–41°), fair (41–55°), and very fair (>55°). 1 μg vitamin D = 40 IU.
epimers (25). The concentrations of 25-hydroxycholecalciferol [25(OH)D3] and 25-hydroxyergocalciferol [25(OH)D2] were calculated using Watson LIMS software, version 7.1.0.01. Pooling serum samples from the Vitamin D External Quality Assessment Scheme were used as quality control samples for 25(OH)D3. Deuterium-labeled 25(OH)D3 was used as internal standard. The intra-assay and inter-assay percent CVs for 25(OH)D2 and 25(OH)D3 were <6.2% and <1.0%, respectively, for all controls. The analytical ranges were 12.5–250.0 nmol/L (5–100 μg/L) for 25(OH)D3 and 25(OH)D2. At baseline, no subjects had detectable concentrations of 25(OH)D2. At follow-up, no subjects in the D3 group had detectable 25(OH)D2; therefore, the change in 25(OH)D for the D3 group was based only on 25(OH)D3 concentrations. For the D2 group, the sum of 25(OH)D2 and 25(OH)D3 at follow-up was subtracted from baseline 25(OH)D2 to calculate the change in 25(OH)D. Values below the quantification limit of the assay were replaced by zero (26,27). Plasma 25(OH)D concentrations were categorized by different thresholds: ≤24.9 (deficiency), 25.0–49.9, 50–74.9, and ≥75 nmol/L.

To make this data usable by others using immunoassays, we also measured the total 25(OH)D concentration using an automated chemiluminescent immunoassay system (Liaison, DiaSorin). This assay measures the total 25(OH)D in plasma and has a sensitivity of 10.0 nmol/L. The range of the assay was 10–375 nmol/L (4–150 μg/L). The intra-assay and inter-assay percent CVs were <5.9% and <10.7%, respectively, for all controls. All analyses were completed in a laboratory meeting the performance targets set by the Vitamin D External Quality Assessment Scheme.

**Sample size.** A previous study in infants (<6 wk old) that gave 10 μg of D2 daily found a Δ 35.7 ± 20.2 nmol/L during a 3-mo period (28). With a sample size of 26 participants/group, α = 0.05 and β = 0.20 (power 1 – β = 0.8) would allow the detection of a difference of 45% between treatments in the change in 25(OH)D during the 3-mo period. This translates to an effect size of 16 nmol/L, representing ~1 SD in the change in the 25(OH)D concentration (28).

**Statistical methods.** Subject characteristics were tested for baseline differences among treatments using a t test for continuous variables and χ² (with Fisher’s exact tests for small sample sizes) for categorical variables. Baseline characteristics with differences were included as covariates in the ANOVA model. The intent-to-treat principle was applied for all outcomes. The associations between mother’s and infant’s vitamin D status as well as the change in infant 25(OH)D concentrations and infant baseline vitamin D status were both tested using Pearson correlation. Plasma 25(OH)D concentrations at each time point as well as the mean change in 25(OH)D from baseline during the trial were compared between treatments using unpaired t tests. Differences in the change in 25(OH)D by treatment were also tested as a mixed-model ANOVA accounting for any baseline differences among the groups. To compare the proportion of infants of the D2 and D3 groups separately and over time. Data were checked for normality and equal variances using Shapiro-Wilk and Levene’s tests; appropriate nonparametric tests were used if assumptions were violated. Significance was set at P ≤ 0.05; all tests presented are 2 tailed. Data were analyzed using SAS version 9.2 (SAS Institute).

**Results.** A total of 52 healthy infants were enrolled and 73% were taking a vitamin D supplement at baseline. There was a trend (P = 0.08) for differences in race among the groups (Table 1); however, skin color did not differ (P = 0.23). Two infants in the D2 group were lost to follow-up (Fig. 1). Infants were growing; the mean weight-for-age and length-for-age Z-scores were within 1 SD from the WHO standard. However, the D3 group had a significantly higher length-for-age Z-score than the D2 group at baseline and follow-up (Supplemental Table 1). The supplementation period tended (P = 0.09) to be 10% longer in the D3 group than in the D2 group. There were no differences in compliance among groups as assessed by both bottle weighing (median 89, range 32–100% of doses taken) and reported intake (95, 31–100% of doses taken). Maternal vitamin D status at baseline was associated with neonatal values at both baseline (r = 0.41; P = 0.003) and follow-up (r = 0.31; P = 0.03) as measured using LC-MS/MS; similar results were obtained by immunoassay (data not shown).

Overall, based on LC-MS/MS measurements, both supplements significantly increased the plasma 25(OH)D concentration during the 3-mo period, with a mean increase of 20.0 nmol/L (95% CI: 13.3–26.6 nmol/L) (Fig. 2). The mean difference between the increases (D2 – D3) was −4.5 nmol/L (95% CI: −17.9 to 8.9 nmol/L; P = 0.5). The difference between the slopes of the line [Δ in plasma 25(OH)D/Δ in time] of the 2 groups was not significant (D2: 24.4 ± 6.6 nmol/L; D3: 18.8 ± 6.2 nmol/L; P = 0.54). However, a higher (P = 0.05) proportion of infants in the D3 group (96.2%) than in the D2 group (75.0%) achieved the 50 nmol/L cutoff at follow-up (Table 2). No differences were noted among groups in the proportion that achieved the 75 nmol/L cutoff at follow-up. A larger proportion of infants in the D2 group (n = 8; 33%) had decreases in 25(OH)D concentration during the 3-mo period compared with infants in the D3 group (n = 2; 8%) (Fischer’s Exact test, P = 0.02). Similar results were observed using the immunoassay (Fig. 2; Table 2).
We observed a significantly larger proportion of infants in the D2 group achieved the 50 nmol/L cutoff at follow-up compared with infants in the D3 group. However, the mean decrease for 8 of the 24 infants in the D2 group was <10 nmol/L and compliance was questionable in 4 of these infants, who consumed <60% of doses (assessed either as reported or loss from bottle). Overall, the majority of infants (86%) achieved the 50 nmol/L cutoff at follow-up as suggested by the IOM for achievement of bone health (3). The results presented in this report complement the work of Gordon et al. (18), who found no differences in 25(OH)D concentrations after 6 wk of supplementation of 50 µg/d D2 compared with D3 for the treatment of low vitamin D status [25(OH)D ≤50 nmol/L] in older infants and toddlers. The increase in 25(OH)D after supplementation is largely dependent on the baseline 25(OH)D concentration and our sample consisted of 44% of infants with 25(OH)D ≤50 nmol/L at baseline. The observation that baseline 25(OH)D is a predictor of the change in 25(OH)D over time has been previously observed in adults (29, 30). This study shows that baseline status is an important factor in determining the

**TABLE 2** Plasma 25(OH)D concentrations and number (%) meeting 25(OH)D cutoffs of ≥50 and ≥75 nmol/L in infants that received 10 µg/d of D2 or D3 as assessed by immunoassay and LC-MS/MS

| Treatments | D2 | D3          | n | n |
|------------|----|-------------|---|---|
|ĸ coefficient between methods was 0.57 when infants were categorized according to plasma 25(OH)D concentrations at follow-up.

**Discussion**

Daily supplementation of either vitamin D isoform at 10 µg/d elevated the plasma 25(OH)D concentration from 1 to 4 mo of age in healthy, breast-fed infants. This was important to know, because both D3 and D2 are common in the North American marketplace. These results are in accordance with the IOM’s Dietary Reference Intakes for vitamin D (3), which state “the two isoforms appear to be equivalent and adequate for almost all infants.” However, a significantly higher proportion of
response to supplementation in healthy infants. When infants were categorized by baseline status, there were no differences in the change in 25(OH)D concentration between treatment groups.

Previous studies in adults established a magnitude difference of 1.7-fold in the change in 25(OH)D concentration among groups taking D2 compared with D3; however, this was based on administration of an equivalent dosage of 100 μg/d (14). A lower dosage of 10 μg/d might result in more modest differences among groups. A similar pattern was observed in this dataset compared with Trang et al. (14) [30% higher change in 25(OH)D concentration in the D3 vs. D2 group]; however, the present study was perhaps underpowered for this analysis. A larger sample size would have provided more confidence to detect a smaller difference between treatments. Although most equivalency studies testing D2 compared with D3 had a similar number of subjects [Armas et al. (13), n = 30; Holick et al. (15), n = 68; Trang et al. (14), n = 72], future studies testing daily low-dose vitamin D (10 μg) should aim for an effect size in the range of 10–20% between the groups.

One of the challenges in assessing vitamin D status in infants includes the potential for a large proportion of 25(OH)D being 10–20% between the groups.

In conclusion, this study provides novel findings regarding daily low-dose supplementation of the 2 vitamin D isoforms in breast-fed newborns. The increases in 25(OH)D concentration between the D2 and D3 groups did not differ, suggesting a sustained daily intake of either isoform is acceptable for infants 4 mo of age and younger.

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