Supplementation with 1000 IU vitamin D/d leads to parathyroid hormone suppression, but not increased fractional calcium absorption, in 4–8-y-old children: a double-blind randomized controlled trial¹–⁴

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ABSTRACT

Background: The effects of vitamin D supplementation in healthy prepubertal children on physiologic outcomes have not been investigated.

Objective: The objective was to evaluate the effects of supplementation with 1000 IU vitamin D₃/d on calcium absorption.

Design: In a double-blind, placebo-controlled trial, we randomly assigned 64 children to 1000 IU vitamin D₃/d (n = 32) or placebo (n = 32) for 8 wk. Stable isotopes were used to assess calcium absorption. The main outcome measure was calcium absorption before and after supplementation.

Results: All of the data are shown as means ± SDs. At baseline, vitamin D intake was 221 ± 79 IU/d and calcium intake was 830 ± 197 mg/d. Baseline serum 25-hydroxyvitamin D [25(OH)D] was not significantly correlated with fractional or total calcium absorption. After 8 wk, with baseline values used as a covariate, no differences were seen in fractional or total calcium absorption based on supplementation group (P = 0.75 and 0.36, respectively). Supplemented children had a significant increase in 25(OH)D concentrations (from 27.7 ± 7.4 to 36.0 ± 10.3 ng/mL; P < 0.0001) and a decrease in parathyroid hormone (from 21.4 ± 10.4 to 12.9 ± 7.1 pg/mL; P < 0.001); no significant changes in the placebo group were observed. No adverse side effects were noted in either group.

Conclusions: Vitamin D₃ supplementation at 1000 IU/d increases 25(OH)D and decreases parathyroid hormone in children with average vitamin D intakes below the dietary recommendations of the Institute of Medicine. However, no significant effects of this change on calcium absorption occurred. This trial was registered at clinicaltrials.gov as NCT 00868738. Am J Clin Nutr doi: 10.3945/ajcn.112.046102.

INTRODUCTION

Recently, the Institute of Medicine (IOM)⁵ revised its guidelines for vitamin D intake in children and adults in the United States and Canada (1). A Recommended Dietary Allowance (RDA) of 600 IU/d was set for children older than 12 mo with the goal of achieving a serum 25-hydroxyvitamin D [25(OH)D] concentration of ≥20 ng/mL. Although others have targeted a higher serum 25(OH)D concentration (2–4), the IOM committee has stood by its perspective indicating that there was inadequate evidence to support higher concentrations of serum 25(OH)D in a healthy population (5). However, the IOM noted that there are few outcome data related to vitamin D intakes in prepubertal children in the United States (1).

In prepubertal children and older adolescents, recent reports have generally failed to find a close relation between calcium absorption and serum 25(OH)D across a broad range of 25(OH)D concentrations, although few subjects with 25(OH)D <12 ng/mL have been studied (6, 7). Except for one very small study in adolescents, virtually all pediatric data relating vitamin D intake and calcium absorption are cross-sectional in nature (8).

Achieving serum 25(OH)D concentrations >20 or 30 ng/mL in all children would clearly be difficult without either a comprehensive food-fortification strategy exceeding the current one or the widespread use of vitamin D supplements (1). Because these strategies would not be simple or inexpensive to implement, continuing evaluation of the basis for these recommendations is necessary.

Changes in calcium absorption during adolescence, such as those during pregnancy, are likely mediated primarily by hormonal factors and less by changes in vitamin D status, excluding adolescents who are severely vitamin D deficient. As such, evaluating the effects of vitamin D directly on calcium absorption in children may best be done in those who are prepubertal. Although it would be ideal to evaluate a range of intakes and vitamin D status in each child studied, this is impractical. Therefore, we chose to evaluate...

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⁵Abbreviations: BMC, bone mineral content; IOM, Institute of Medicine; PTH, parathyroid hormone; RDA, Recommended Dietary Allowance; 25(OH)D, serum 25-hydroxyvitamin D; 1,25(OH)₂D, serum 1,25-dihydroxyvitamin D.

a single dose, 1000 IU/d, that reflects a supplement amount that is easily obtainable in the marketplace and commonly recommended.

We hypothesized that consumption of a 1000-IU/d supplement of vitamin D for 8 wk would significantly increase serum 25(OH)D without increasing calcium absorption in a population of healthy 4–8–y-old children not identified as having a high risk of vitamin D insufficiency. We conducted a randomized, double-blind, placebo-controlled trial to ensure that changes over time in a supplemented group could be compared with those in a nonintervention group.

SUBJECTS AND METHODS

Subjects and research studies

The subjects for the study were selected to approximately match the ethnic distribution of the greater Houston, Texas, area. To be enrolled, subjects had to be healthy, to not be using any medications or multivitamins/minerals, and to have a usual dietary calcium intake of 600 to 1200 mg/d. Written informed consent was obtained from a parent or legal guardian for each subject. The Institutional Review Board of Baylor College of Medicine and Affiliated Hospitals approved the protocol.

The subjects were 4.0–8.9 y of age at the time they started the study. We targeted an enrollment of 32 subjects for both the placebo and supplement groups to ensure that 30 subjects completed the study. No meaningful changes were made to the protocol once the study commenced. The primary outcome was fractional and total calcium absorption and 25(OH)D concentrations before and after an 8-wk supplementation period with either 1000 IU vitamin D₃ or placebo measured by using a dual-stable-isotope technique.

Study procedures

This randomized, double-blind, controlled trial included 3 visits to the General Clinical Research Center of Texas Children’s Hospital in Houston, Texas. The first was an outpatient screening visit to confirm eligibility, the second an inpatient baseline study visit to determine calcium absorption, and the third an inpatient follow-up study visit after intervention to determine calcium absorption. To confirm eligibility, usual dietary nutrient intake was determined from 24-h telephone dietary recalls and a 3-d weighed dietary record at home that subjects performed immediately after the screening visit.

Vitamin D concentrations [25(OH)D and 1,25-dihydroxyvitamin D (1,25(OH)₂D)], parathyroid hormone (PTH) concentrations, and alkaline phosphatase activity were measured at the time of each calcium absorption study visit. Serum 25(OH)D samples were sent to ARUP Laboratories for analysis by using a Diasorin quantitative chemiluminescent immunoassay with an analytic sensitivity of 4 ng/mL (LIAISON; DiaSorin Inc) and a CV of 3% to 4%. This laboratory participates in the NIH Office of Dietary Supplements Vitamin D standardization program. The analyses included both 25(OH)D₁ and 25(OH)D₂. Serum 1,25(OH)₂D was measured by LabCorp with the use of a radioimmunoassay with a lower limit of detection of 2 pg/mL and a CV of 5% to 10%. Intact PTH was measured by Quest Diagnostics by using an immunochemiluminescent assay with a lower limit of detection of 3 pg/mL and a CV of 2%. Alkaline phosphatase activity was quantitatively measured by using the VITROS ALKP slide method by Texas Children’s Hospital with a CV of 2% to 3%.

Baseline studies of total-body bone mineral content (BMC) were performed by dual-energy X-ray absorptiometry with a Hologic Delphi Model (Hologic Inc). The dual-energy X-ray absorptiometry instrument undergoes regularly scheduled quality-control testing for phantom reproducibility and signal uniformity. Subject scanning does not take place unless all quality control results fall within acceptable limits. Values were compared with age-, sex-, and race-matched normal values for our institution (http://www.bcm.edu/bodycomplab).

Once the results from the baseline blood tests were available, the study dietitian reported the baseline 25(OH)D concentration to the Investigational Pharmacy of Texas Children’s Hospital, who assigned participants to the intervention groups. The subjects were randomly assigned by baseline 25(OH)D concentrations to receive 8 wk of 1000 IU vitamin D₃/d supplementation or placebo. The randomization scheme was generated by using the website randomization.com (http://www.randomization.com). The subjects were stratified by their baseline 25(OH)D concentrations as low (<20 ng/mL), middle (20–32 ng/mL), or high (>32 ng/mL). On the basis of the subject’s baseline vitamin D concentration, the pharmacist referred to the appropriate randomization table (low, medium, or high) and assigned the next treatment assignment on that table, either vitamin D supplement or placebo. Randomization tables were generated in blocks of 2 (vitamin D₃ and placebo) so that if one serum vitamin D concentration group enrolled significantly more than the others, the treatment assignments would still come out as 1:1. The subjects were assigned ~1 wk after the first inpatient visit once laboratory results were finalized so that baseline serum 25(OH)D concentrations could be reported to the Investigational Pharmacy. The principal investigator, study dietitian, study staff, nurses, and participants were all blinded throughout the study. Only the Investigational Pharmacists who were not involved in the data collection or analysis were aware of study group assignment.

Dietary methods

At the screening visit, the study dietitian asked subjects what they usually ate on a normal day with the use of two 24-h dietary recalls, and food preferences were obtained. Inpatient menus for the overnight study visit were based on the usual daily calcium intake. All foods and beverages during the inpatient and outpatient visits were weighed before and after consumption to accurately determine intake. The subjects were provided food scales and were instructed to keep weighed food records for 3 d at home after the first inpatient calcium-absorption stable-isotope test and for 3 d at home before the second inpatient calcium-absorption stable-isotope test. To reflect the marketplace changes in dietary food contents during the study, dietary intake data were collected by using Nutrition Data System for Research software developed by the Nutrition Coordinating Center (University of Minnesota).

Supplement

Vitamin D₃ (1000 IU/mL) was added to almond oil (sweetened with 0.1% saccharin powder) and flavored with orange oil. The placebo solution was prepared the same way without the vitamin D. The 2 study solutions were provided by Greenpark Compounding Pharmacy. The amount of vitamin D in the supplements was measured by an independent laboratory (Professional Compounding Centers of America) to confirm that the solution
breakfast, the subjects were given a stable isotope of calcium (20 children were given a standard breakfast. Toward the end of was administered intravenously over ~2 min. Subsequently, the children were given a standard breakfast. Toward the end of breakfast, the subjects were given a stable isotope of calcium (20 μg 46Ca) that had been mixed with 120 mL Ca and vitamin D–fortified orange juice. Meals were identical for each subject at their 2 visits with a calcium load reflecting one-third of their usual dietary intake.

Beginning with breakfast, a complete 24-h urine collection was obtained. Urine samples were prepared for thermal ionization mass spectrometry as previously described by using an oxalate precipitation technique (9). The urine samples obtained were analyzed to determine their calcium isotope ratios by using a Finnigan MAT 261 (Thermoquest) magnetic sector thermal ionization mass spectrometer.

Fractional absorption of calcium was calculated as the relative recovered oral compared with intravenous isotope in a 24-h urine specimen collected at the time of the tracer administration as previously described (10, 11). Total calcium absorption was calculated as the product of calcium intake and fractional calcium absorption.

Sample size determination

Sample size determination was based on considering a difference in total calcium absorption (fractional absorption × dietary intake) of ~50 mg/d as being biologically significant, because this is the difference that might be seen with a typical calcium supplement in this age group. We hypothesized a mean calcium absorption efficiency of 30% and an SD of 30% of the mean (~9%), which resulted in total absorbed calcium of 240 ± 72 mg/d (10, 12). Assuming the smallest clinically significant difference in calcium absorption to be 50 mg/d (equivalent to the increment in calcium absorption from typical calcium supplements), a sample size of 30 in each group would have a power of >95% to detect such a difference at P < 0.05. This comparison was based on a change from baseline in the supplement group, assuming no change in the placebo group. We also preplanned to compare the changes between the start and end of the study in the 2 groups and to evaluate the relative changes in the 2 groups in serum 25(OH)D, 1,25(OH)2D, and PTH.

Statistical methods

The primary outcome was the effect of the intervention on calcium absorption variables over the 8 wk of the study. This was analyzed by general linear modeling with the use of the intervention as a group characteristic and baseline values to determine the effects of treatment group on outcomes. The relation between percentage and total calcium absorption and 25(OH)D was evaluated by linear regression analysis. Baseline differences in values between supplement and placebo groups were determined by unpaired t tests. Within-group endpoint differences were determined by paired t tests. All data were analyzed by using SPSS (version 16.0; SPSS Inc). Study data are reported as means ± SDs, except as specifically noted.

RESULTS

Baseline findings for the study population are shown in Table 1. There were 27 white, 12 African American, 23 Hispanic, and 1 Asian subject, which closely represented the racial and ethnic distribution of the greater Houston area. One subject (supplement group) did not return for follow-up; therefore, the final number of enrolled subjects was 31 in the placebo group and 32 in the supplement group (Figure 1). Whereas intention-to-treat analysis using the last value carried forward might have been considered, it was not appropriate for this study because the subject who dropped out was in the vitamin D supplement group. Therefore, that subject was excluded from the final analysis. Subjects were recruited throughout the year. No significant difference in season of study was found between the placebo and supplement groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo group (n = 31)</th>
<th>Supplement group, 1000 IU/d (n = 32)</th>
<th>P-difference2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>6.4 ± 1.4</td>
<td>6.7 ± 1.4</td>
<td>0.96</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>21.7 ± 3.8</td>
<td>23.3 ± 4.6</td>
<td>0.37</td>
</tr>
<tr>
<td>BMI z score</td>
<td>−0.1 ± 0.8</td>
<td>0.0 ± 0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Dietary calcium intake (mg/d)</td>
<td>793 ± 185</td>
<td>864 ± 206</td>
<td>0.21</td>
</tr>
<tr>
<td>Dietary vitamin D intake (IU/d)</td>
<td>216 ± 80</td>
<td>224 ± 80</td>
<td>0.91</td>
</tr>
<tr>
<td>Serum 25(OH)D (ng/mL)</td>
<td>27.6 ± 7.3</td>
<td>27.7 ± 7.4</td>
<td>0.68</td>
</tr>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>9.8 ± 0.5</td>
<td>9.6 ± 0.4</td>
<td>0.19</td>
</tr>
<tr>
<td>Serum 1,25(OH)2D (pg/mL)</td>
<td>58.1 ± 16.3</td>
<td>53.7 ± 11.1</td>
<td>0.64</td>
</tr>
<tr>
<td>Total-body bone mineral content z score3</td>
<td>0.13 ± 0.80</td>
<td>0.15 ± 0.85</td>
<td>0.49</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs. 1,25(OH)2D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.
2 Determined by unpaired t tests.
3 Compared with internal age-, sex-, and race-matched population (http://www.bcm.edu/bodycomplab/Flashapps/AllDXAAreIsChartpage.html).
Furthermore, serum 25(OH)D was not significantly associated with the season of measurement at baseline \((P = 0.9)\); therefore, the season of measurement was not considered further in the analysis. Enrollment occurred continuously from July 2009 to August 2011. No adverse side effects were noted in either group. On the basis of the before and after weights of the supplement bottles as a marker of compliance, 86% \((n = 50)\) of the subjects consumed the supplement $75\%$ of the days during the 8-wk period. Six subjects \((10\%)\) did not return their supplement bottles at the end of the study. The percentage compliance was not related to study outcome. The trial ended after all study visits were completed.

Eight of the 63 subjects had a baseline 25(OH)D value <20 ng/mL, and 1 subject had a concentration <10 ng/mL \((at 9 \text{ ng/mL})\). No subject had a concentration >50 ng/mL at baseline, and the highest concentration was 44 ng/mL. None of the baseline PTH values were >50 pg/mL, and 8 were >30 pg/mL. Dual-energy X-ray absorptiometry scans showed that no subject had a total-body BMC $z$ score below $-1.5$, and 2 subjects had BMC $z$ scores above +2.0.

### Calcium absorption

Results for calcium intake, absorption, and urinary excretion are shown in Table 2. Although baseline values for both calcium intake and fractional absorption were not significantly different between the placebo and supplement groups, both were slightly higher in the supplement group. The product of the two, total calcium absorption, was significantly higher at baseline in the supplement group. The results of the analysis shown in Table 2 indicate that there were no significant differences in end of study fractional or total calcium absorption based on study group assignment (supplement or placebo) by using baseline values as covariates.

Of note, a significant correlation in both the placebo and supplement groups was found between the fractional absorption of calcium at the start and end of the study: whole group \((n = 63; r = 0.46, P < 0.001)\), supplement group \((n = 32; r = 0.50, P = 0.004)\), and placebo group \((n = 31; r = 0.36, P = 0.046)\).

### Vitamin D and parathyroid hormone

No significant relation was found between baseline fractional or total calcium absorption and serum 25(OH)D or 1,25(OH)$_2$D concentrations. The relation between fractional absorption and serum 25(OH)D at baseline is shown in Figure 2. The results were similar for the correlations between fractional absorption and 1,25(OH)$_2$D \((r < 0.01, P = 0.83)\). Values for total calcium absorption also showed no significant relation with either 25(OH)D \((r = 0.03, P = 0.80)\) or 1,25(OH)$_2$D \((r = 0.09, P = 0.48)\).

At the end of the 8-wk study, there was no relation overall between serum 25(OH)D and fractional calcium absorption \((r = 0.17, P = 0.18)\), nor was there a difference between subjects in the placebo group \((r = 0.03, P = 0.52)\) or the supplement group \((r = -0.31, P = 0.08)\), although a trend toward a negative relation was noted for the supplement group. Similar results were seen for the relation with total calcium absorption.

For 1,25(OH)$_2$D, although there was no significant relation with fractional calcium absorption at baseline, they were significantly related at the end of the study \((r = 0.29, P = 0.029)\)
Serum PTH was not significantly related to calcium absorption efficiency at the start ($r = 0.09, P = 0.48$) or end ($r = 0.16, P = 0.22$) of the study.

The changes in serum 25(OH)D and PTH during the study are shown in Table 3. A significant increase ($P < 0.001$) in 25(OH)D was associated with supplementation and with a significantly higher final 25(OH)D concentration in the supplement group than in the placebo group. Of note was the finding of significant suppression of PTH in the supplement group but not in the placebo group. The differences at the end of the study between the placebo and supplement group were significantly different ($P < 0.001$).

At the end of the study, 3 subjects had serum 25(OH)D values $>50$ ng/mL, 2 of which were in the supplement group (53 and 60 ng/mL) and 1 of which was the highest value and occurred in the placebo group (86 ng/mL). That subject was a 7-y-old Hispanic boy who was studied during early and late summer; his baseline 25(OH)D concentration was 35 ng/mL.

The relation between total vitamin D intake from the diet and supplement and serum 25(OH)D was evaluated by using the method from the IOM (1), in which the serum 25(OH)D concentration was divided by the natural log intake to identify a conversion constant and the range of that constant measured. For the entire 126 measurements, the mean ($\pm$SD) constant was 5.3 $\pm$ 1.7, or serum 25(OH)D = 5.3 $\times$ ln (total dietary vitamin D intake). Evaluation of the only subgroups led to little change in this value. For example, looking only at the follow-up postintervention measurements, the mean ($\pm$SD) constant was 5.4 $\pm$ 1.9.

**DISCUSSION**

Our study was the first of which we are aware to directly assess the effects in prepubertal children of a biologically meaningful dose of vitamin D$_3$ on calcium absorption by using a randomized, double-blind, parallel, placebo-controlled trial. We found no increase in calcium absorption fraction with this intervention over 8 wk but also found a significant increase in serum 25(OH)D concentration to a mean $>32$ ng/mL and a statistically significant decrease in serum PTH with intervention. Neither of these changes in serum 25(OH)D or PTH occurred in the placebo group.
These findings suggest that small children have responses (e.g., changes in PTH concentration) to vitamin D intake appropriate and similar to those in older children and adults. They do not, however, suggest any specific benefit in our population of children from the southern part of the United States to supplementation with 1000 IU vitamin D/d related to calcium metabolism or to achieving a serum 25(OH)D concentration >32 ng/mL.

Most of the scientific and medical literature related to vitamin D and bone health in children is from either infants or toddlers who are at high risk of rickets because of the very low content of vitamin D in breast milk and relatively low calcium concentration of human milk for older infants or in adolescents. Prepubertal children, however, are also an important group. Traumatic fractures occur frequently in this age group, and dietary patterns are established for adolescents and adulthood during this time period.

The estimated average requirement of 400 IU/d and the RDA of 600 IU/d were established for children largely based on data from infants or toddlers who are at high risk of rickets because of the very low content of vitamin D in breast milk and relatively low calcium concentration of human milk for older infants or in adolescents. Prepubertal children, however, are also an important group. Traumatic fractures occur frequently in this age group, and dietary patterns are established for adolescents and adulthood during this time period.

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The suppression of PTH with vitamin D supplementation is also of interest. This was expected based on cross-sectional (13) studies. However, because PTH concentrations were not elevated at the start of our study, it is uncertain what the functional consequences of this suppression might be in healthy children. It would be of interest to know whether lower PTH concentrations sustained over a longer period would lead to beneficial or harmful effects on calcium absorption and bone mineral accretion. No data are currently available to specifically answer these questions and long-term studies would be needed to evaluate these relationships in a meaningful manner.

Our data do not provide evidence for any specific dietary requirement for vitamin D, especially for a vitamin D–sufficient population. We selected a supplement of 1000 IU/d because this is an amount that has widely been recommended for all age groups, including children (14). Since then, a large range of vitamin D intakes have been recommended, from the RDA of 600 IU/d to much higher intakes (1–4).

Our study was limited by enrolling relatively healthy children who were not subject to very low 25(OH)D concentrations despite relatively low intakes. By design, our population was a diverse one in ethnicity and race, but did not include those who were obese or taking chronic medications. Still, our data provide no support for providing 1000 IU vitamin D/d to healthy children or specifically targeting 25(OH)D concentrations >30 ng/mL, at least as related to calcium absorption. Although it has been suggested that factors other than vitamin D primarily drive higher calcium absorption during puberty (15), these data indicate that higher serum 25(OH)D concentrations, at least those above the presumed target of 30 ng/mL, do not lead to increased calcium absorption in children from 4 to 8 y of age.

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The authors’ responsibilities were as follows—SAA: designed the research protocol, analyzed the data, wrote the manuscript, and had primary responsibility for the final content of the manuscript; KMH: assisted with the design of the research protocol, conducted the research, recorded and analyzed the data, assisted in writing the manuscript, and had secondary responsibility for the final content of manuscript; and ZC: analyzed all isotope recovery samples on the mass spectrometer and assisted with the manuscript preparation.

REFERENCES