Fortification of orange juice with vitamin D₂ or vitamin D₃ is as effective as an oral supplement in maintaining vitamin D status in adults¹⁻⁴


ABSTRACT

Background: Vitamin D has been added to calcium-fortified orange juice. It is unknown whether vitamin D is as bioavailable from orange juice as it is from supplements.

Objectives: The objective was to compare the bioavailability of vitamin D₂ and vitamin D₃ from orange juice with that from vitamin D₂ and vitamin D₃ supplements. A secondary aim was to determine which form of vitamin D is more bioavailable in orange juice.

Design: A randomized, placebo-controlled, double-blind study was conducted in healthy adults aged 18–84 y (15–20/group) who received 1000 IU vitamin D₃, 1000 IU vitamin D₂, or placebo in orange juice or capsule for 11 wk at the end of winter.

Results: A total of 64% of subjects began the study deficient in vitamin D (ie, 25-hydroxyvitamin D [25(OH)D] concentrations <20 ng/mL). Analysis of the area under the curve showed no significant difference in serum 25(OH)D between subjects who consumed vitamin D-fortified orange juice and those who consumed vitamin D supplements (P = 0.084). No significant difference in serum 25(OH)D was observed between subjects who consumed vitamin D₂-fortified orange juice and vitamin D₁ capsules (P > 0.1). Similarly, no significant difference in serum 25(OH)D was observed between subjects who consumed vitamin D₂-fortified orange juice and vitamin D₂ capsules (P > 0.1). No significant overall difference in parathyroid hormone concentrations was observed between the groups (P = 0.82).

Conclusion: Vitamin D₂ and vitamin D₃ are equally bioavailable in orange juice and capsules. Am J Clin Nutr 2010;91:1621–6.

INTRODUCTION

Vitamin D (D₂, D₃, or both) deficiency is an international health concern (1–10) that has been associated with rickets, osteomalacia, muscle weakness, osteoporosis (11–15), and an increased risk of wheezing diseases, autoimmune diseases (eg, type 1 diabetes, multiple sclerosis, rheumatoid arthritis, and Crohn disease), and cancer, such as of the prostate, breast, and colon (16–30).

The major source of vitamin D is exposure to sunlight (31, 32). A secondary yet limited source of vitamin D is through the diet (33). Oily fish such as salmon, cod liver oil and sun-dried mushrooms are the only natural food sources of vitamin D (33, 34).

In the 1930s, fortification of dairy products with vitamin D eradicated rickets (35). Whereas milk is a commonly fortified food source of vitamin D, many children and adults have lactose maldigestion and avoid drinking milk (35–37). According to the US Department of Agriculture, 49% of Americans older than 2 y drink more than one glass (236.6 mL; 8 fluid oz) of juice every day. Tangpricha et al (38) reported that orange juice fortified with 1000 IU vitamin D₂/236.6 mL increased the serum 25-hydroxyvitamin D [25(OH)D] concentrations of adults by >150% over 12 wk, which indicated that the fortification of orange juice with vitamin D₃ is an effective way to increase vitamin D intake in adults.

Bread has been fortified with vitamin D since the 1930s (1). It was observed that fortifying wheat and rye bread with 400 IU vitamin D₁/100 g per serving resulted in a significant increase in serum 25(OH)D concentrations but no significant change in parathyroid hormone (PTH) concentrations after 3 wk compared with a control group (39). However fortification of bread with 5000 IU vitamin D₂/serving for 1 y not only increased serum 25(OH)D concentrations but also caused significant reductions in the PTH concentrations (40). A 3-wk bioavailability study showed comparable elevations in blood 25(OH)D concentrations between subjects who ingested wild mushrooms and those who ingested 400 IU vitamin D₂ (41).

Whether vitamin D₂ is equally as effective as vitamin D₃ at maintaining blood concentrations of 25(OH)D is still under discussion. A study of the bioavailability of 4000 IU vitamin D₂ and vitamin D₃ ingested in alcohol for 2 wk (42) or as a single 50,000-IU dose (43) suggested that vitamin D₂ was less effective than vitamin D₃ in raising and maintaining blood concentrations of 25(OH)D. However, elevations in blood 25(OH)D concen-

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trations were identical between healthy adults given 1000 IU vitamin D2 or 1000 IU vitamin D3 in capsule form at the end of the winter for 3 mo (44). Similarly, children who received 2000 IU daily or 50,000 IU vitamin D2 weekly experienced an elevation in blood 25(OH)D concentrations equivalent to concentrations observed in children who received 2000 IU vitamin D3 daily (45).

It is unknown whether vitamin D2 and vitamin D3 are equally bioavailable from the same fortified food source or whether vitamin D in orange juice is as bioavailable as it is in a capsule. The purpose of our study was to compare the bioavailability of vitamin D2 and vitamin D3 from orange juice with that from vitamin D2 and vitamin D3 supplements.

**SUBJECTS AND METHODS**

**Subjects**

A total of 105 subjects aged 18–79 y were enrolled in a double-blind study that began on 14 February 2007 (Table 1). The subjects were randomly assigned into 1 of 5 groups by using a computer-generated randomization code. Potential subjects were excluded if they had a history of intestinal malabsorption, a severe medical illness, allergies, or an intolerance or dislike of orange juice or were taking a supplement containing >400 IU vitamin D/d. The subjects signed a consent form approved by the Institutional Review Board at Boston University Medical Center.

**Methods**

All of the vitamin D and placebo capsules were manufactured by Tishcon Corp (Salisbury, MD) and contained lactose (98.75%), magnesium stearate (1.0%), and silicon dioxide (1.25%). All of the calcium-fortified orange juices were prepared by Coca-Cola North America (Apoka, FL).

Cold water–soluble vitamin D (1000 IU) was added to every 236.6 mL calcium-fortified orange juice (39). The vitamin D was miscible, although labels instructing subjects to shake well before each use were placed on each orange juice container to ensure that the vitamin D was evenly distributed.

**Stability of vitamin D in orange juice and capsules**

HPLC was used to determine the amount and stability of vitamin D2 and vitamin D3 in the orange juice and capsules. The orange juice and capsules were found to contain either no vitamin D (placebo) or vitamin D within 10% of their specified concentrations.

**Design**

The study began in mid-February 2007. The subjects were randomly assigned into 1 of 5 groups: 1) placebo capsule + orange juice without vitamin D (placebo orange juice), 2) placebo capsule + orange juice containing 1000 IU vitamin D3/236.6 mL, 3) placebo capsule + orange juice containing 1000 IU vitamin D2/236.6 mL, 4) 1000 IU vitamin D3 capsule + placebo orange juice, or 5) 1000 IU vitamin D2 capsule + placebo orange juice. All orange juice contained 350 mg Ca/236.6 mL. Subjects consumed one capsule and one 236.6-mL glass of orange juice daily. The subjects were instructed to drink the orange juice in the morning and to ingest the capsule at night. Blood was collected once weekly for a total of 11 wk. Calcium, albumin, parathyroid hormone (PTH), 25(OH)D2, and 25(OH)D3 were measured (Table 2).

**Analytic methods**

Serum 25(OH)D was ascertained by liquid chromatography tandem mass spectroscopy at Quest Diagnostic Laboratory (San Juan Capistrano, CA) as reported previously (5). The assay has an

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Demographic characteristics of the subjects</th>
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<tbody>
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<td>Compliance (%)</td>
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1 OJ, orange juice.
RESULTS

Of the 105 subjects who started the study, 86 subjects completed the study (18 in the vitamin D3 orange juice group, 20 in the vitamin D1 capsule group, 17 in the vitamin D2 orange juice group, 16 in the vitamin D2 capsule group, and 15 in the placebo group). Sixty-four percent of all subjects were vitamin D deficient [25(OH)D < 20 ng/mL] and 21% were insufficient [25(OH)D 21–30 ng/mL]. No significant changes in serum calcium and albumin AUCs from baseline to week 11 were observed in any of the treatment groups.

The AUC of serum concentrations against time is the best indicator of the total bioavailability of an administered agent. No significant difference in the AUC for serum 25(OH)Dtotal was observed between the subjects who received vitamin D2 in orange juice (279.2 ± 80.6 ng · wk/mL) and those who received vitamin D3 in orange juice (307.6 ± 82.6 ng · wk/mL). The overall difference in the AUC for serum 25(OH)Dtotal between all subjects who received either 1000 IU vitamin D2 or vitamin D3 in orange juice or in a capsule was not significant (P = 0.084) (Figure 1, A and B).

Subjects who received vitamin D3 in orange juice had an AUC for serum 25(OH)D3 of 296.4 ± 74.4 ng · wk/mL, which was not significantly different from the AUC for serum 25(OH)D3 in the group who received vitamin D1 in a capsule (302.3 ± 120.8 ng · wk/mL) (Figure 2A). The AUC for serum 25(OH)D3 was not significantly different (P > 0.05) between the group who received vitamin D1 in orange juice and those who received placebo in orange juice (209.1 ± 104.4 ng · wk/mL), whereas the AUC for serum 25(OH)D3 was significantly different (P < 0.0001) between the group who received vitamin D1 in capsules and those who received placebo in orange juice (Figure 2A).

No significant difference (P > 0.05) in the AUC for serum 25(OH)D2 was observed between the subjects who received vitamin D2 in orange juice (127.3 ± 57.9 ng · wk/mL) and the subjects who received vitamin D2 in capsules (118.0 ± 38.4 ng · wk/mL) (Figure 2B). However, the AUC for serum 25(OH)D2 was significantly different (P < 0.0001) between the subjects who received vitamin D2 in orange juice and those who received placebo in orange juice (11.4 ± 28.7 ng · wk/mL) (Figure 2B). No significant overall difference in PTH was observed between the groups (P = 0.82).

DISCUSSION

The bioavailability of vitamin D in orange juice and capsules was determined by analyzing the AUCs of serum 25(OH)D2 and serum 25(OH)D3. It was determined that the bioavailability of
vitamin D was equivalent in orange juice and capsules. The AUC analysis showed that the bioavailability of vitamin D2 and of vitamin D3 from orange juice was similar to that from capsules. The results indicate that vitamin D in orange juice is as bioavailable as is vitamin D in capsules. Furthermore, it was shown that vitamin D2 and vitamin D3 in orange juice were equally effective as vitamin D in capsules at raising serum 25(OH)D concentrations.

The results of the weekly blood analysis indicated that serum 25(OH)D2 concentrations were significantly greater in subjects who consumed orange juice fortified with 1000 IU vitamin D2 than in those who consumed the placebo plus orange juice without vitamin D. As expected, baseline 25(OH)D2 concentrations were very low or undetectable in all subjects. Because vitamin D2 can only be obtained through the diet in a limited amount of fortified foods, most persons who do not eat large quantities of these foods (eg, sun-dried mushrooms), do not take vitamin D2 supplements, or do not take prescription vitamin D2 do not have measurable concentrations of 25(OH)D2. Whereas 25(OH)D2 concentrations seemed to increase more rapidly in the subjects who consumed orange juice containing vitamin D2 than in the subjects who consumed vitamin D2 capsules, the increase was not statistically significant and peaked at week 5 (13.8 ± 4.8 ng/mL) in both groups (Figure 2B).

No changes in serum 25(OH)D2 or 25(OH)D3 concentrations were observed in the placebo group, which indicated that sun exposure and diet had no significant effect on their vitamin D status. Subjects who consumed orange juice containing 1000 IU vitamin D3 had significantly greater 25(OH)D3 concentrations than the placebo group. Subjects who consumed orange juice containing vitamin D3 and those who consumed vitamin D3 capsules began the study with average 25(OH)D3 concentrations of 17.6 ± 6.4 ng/mL. Their serum 25(OH)D3 concentrations steadily increased until week 5, at which time they plateaued. The increases in 25(OH)D3 in these 2 groups were not significantly different, which suggests that serum 25(OH)D3 concentrations will increase similarly when 1000 IU vitamin D3 is consumed in orange juice or in capsule form.

Serum PTH concentrations decreased in subjects who consumed orange juice fortified with vitamin D and calcium.
D3 capsules, or placebo; however, the results were not statistically significant. Overall, there was no statistically significant difference in serum PTH concentrations between any of the groups (P = 0.82).

Two studies have suggested that vitamin D3 is more effective than vitamin D2 at maintaining serum 25(OH)D concentrations (42, 43). The results of our study indicate that consumption of 1000 IU vitamin D2 or vitamin D3 in orange juice was equally as effective as 1000 IU vitamin D2 or D3 in capsule form in raising and maintaining circulating concentrations of total 25(OH)D (Figure 1, A and B). The results are consistent with our previous observation that the consumption of 1000 IU vitamin D2 daily in capsule form was equally as effective as consuming a 1000 IU capsule of vitamin D3 in raising serum 25(OH)D2 and 25(OH)D3 (44).

Fortification of foods and drinks with vitamin D is an economical way to provide adequate vitamin D supplementation to adults who are at risk of a myriad of diseases ranging from type 1 diabetes to osteoporosis. Exogenous factors such as time of day, season, and latitude influence cutaneous production of vitamin D. The variability inherent in these factors makes relying on sun exposure as a primary method of obtaining vitamin D often impractical. Diet is a necessary component of ensuring sufficient 25(OH)D concentrations in the blood, especially for those living in the northern hemisphere during the winter months. However, studies that measured the vitamin D content in milk across the United States and parts of Canada showed variable amounts of vitamin D (35, 46, 47).

Also, lactose maldigestion causes many persons to avoid drinking milk regularly. Fortification of orange juice with vitamin D is as effective as oral supplementation in enhancing 25(OH)D concentrations in adults. Therefore, fortification of orange juice with vitamin D2 or vitamin D3 is a resourceful way of enhancing vitamin D status in children and adults.

Quest Diagnostics/Nichols Institute is a clinical laboratory that specializes in liquid chromatography tandem mass spectroscopy and performed the 25 (OH)D assays for this study. We thank Jeff Mathieu for measuring the serum concentrations of PTH in all of the specimens and the staff at the Mattapan Community Health Center for their help in recruiting the study subjects.

The authors’ responsibilities were as follows—MFM AH, DB, and RMB: participated in the study design, statistical analysis, recruitment of subjects, study visits, data collection, and preparation of the manuscript; MHC and MRW: helped in the statistical analysis of the study; EKK: participated in the study oversight and study design; AA: participated in the recruitment of the subjects, study visits, and data collection; TCC: participated in the design of the study and the analysis of the blood samples; and RR and WS: participated in the design of the assay methodology, performance of the assay, and interpretation of the data. MFM is on the Speaker’s Bureau for Merck, Proctor and Gamble, and Eli Lilly and is a consultant for Amgen, Novartis, Quest Diagnostics, Bayer, Abbott, Proctor and Gamble, and Merck. RMB, MHC, MRW, EKK, AA, WS, TCC, AHY, and DB had no conflicts of interest to declare. RR is Medical Director of Quest Diagnostics/Nichols Institute and has equity interests in Quest Diagnostics/Nichols Institute. The Beverage Institute for Health & Wellness, a Division of Coca-Cola North America, Atlanta, GA, funded the study but had no role in the design, implementation, analysis, or interpretation of the research.

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