Fortification of orange juice with vitamin D: a novel approach for enhancing vitamin D nutritional health[1–3]

Vin Tangpricha, Polyxeni Koutkia, Suzanne M Rieke, Tai C Chen, Alberto A Perez, and Michael F Holick

ABSTRACT

Background: Fortification of milk with vitamin D may not be adequate for satisfying the vitamin D requirement because of variability in vitamin D content after fortification and because many persons have milk allergy or lactose intolerance. Additional foods need to be fortified with vitamin D.

Objective: We determined whether vitamin D, a fat-soluble vitamin, is bioavailable in orange juice and skim milk, 2 nonfat beverages.

Design: On 3 separate occasions, 18 adults ingested 25 000 IU vitamin D_2 in 240 mL whole milk or skim milk or in 0.1 mL corn oil applied to toast. A separate, double-blind, randomized, controlled trial investigated whether the consumption of orange juice fortified with vitamin D_3 would increase serum 25-hydroxyvitamin D [25(OH)D] concentrations: 14 subjects ingested 240 mL orange juice fortified with 1000 IU vitamin D, and 12 subjects ingested a control orange juice daily for 12 wk.

Results: Peak serum vitamin D_2 concentrations did not differ significantly after the ingestion of vitamin D_2 in whole milk, skim milk, or corn oil on toast. After subjects consumed orange juice fortified with 1000 IU vitamin D_3 daily for 12 wk, serum $25(OH)D_3$ concentrations increased by 150%, and serum parathyroid hormone concentrations decreased by 25% compared with baseline; control subjects had a seasonal increase of 45% in 25(OH)D and no significant change in serum parathyroid hormone.

Conclusions: The fat content of milk does not affect vitamin D bioavailability. Vitamin D fortification at 1000 IU/240 mL orange juice for 12 wk safely increased $25(OH)D_3$ concentrations in adults. *Am J Clin Nutr* 2003;77:1478–83.

KEY WORDS Vitamin D deficiency, milk, orange juice, vitamin D, sunlight, vitamin D fortification, vitamin D–fortified milk, lactose intolerance, milk allergy, vitamin D requirement

INTRODUCTION

Prevention of vitamin D deficiency and insufficiency remains an international health care priority (1–17). Rates of vitamin D deficiency and insufficiency are highest among elderly and institutionalized adults (2, 5–7, 9–14). Adolescents and young adults are at risk of vitamin D insufficiency as well (3, 8). Young adults aged 18–29 y had a 32% prevalence of vitamin D insufficiency at the end of the winter in Boston (3). In addition, darker-pigmented persons and Asians have a higher prevalence of vitamin D insufficiency because their skin is unable to produce vitamin D_3 efficiently (4, 18, 19). Vitamin D insufficiency results in secondary hyperparathyroidism and causes rickets in children and osteomalacia and osteoporosis in adults (1–17, 20, 21). Increasing evidence indicates that vitamin D insufficiency is associated with an increased risk of colon cancer (22–24), breast cancer (25), prostate cancer (26–29), and other cancers (30).

Vitamin D is difficult to obtain from the diet because it is not naturally present in many foods. In the 1930s, food and beverage manufacturers began to fortify milk, breads, hot dogs, sodas, and even beer with vitamin D (4). However, the outbreak of vitamin D intoxication in Europe in the 1950s and the strict regulations issued by the US Food and Drug Administration limited fortification to only milk and cereals in the 1950s; these policies have persisted to this day (4, 31). In most European countries, fortification of dairy products is forbidden. However, fortified milk is not suitable for preventing vitamin D insufficiency in the general population because of the high prevalence of lactose intolerance in Asians, blacks, and Native Americans (32) and because of milk allergies (33). In addition, the vitamin D content of fortified milk is highly variable; some tested samples contained < 50% of the amounts stated on the containers (34–36).

Other foods that are consumed by most children and adults should also be fortified with vitamin D to increase the availability of this important nutrient and hormone. Recently, fortification of orange juice with calcium was introduced, making orange juice a potential good source of calcium for children and adults who do not drink milk. Because vitamin D is a fat-soluble vitamin, it was thought that only beverages containing fat could be fortified with vitamin D. In the current study, we performed experiments investigating whether the fat content of milk influenced the bioavailability of vitamin D in healthy adults. We discovered that fat content was not important for vitamin D absorption and went on to determine whether vitamin D added to orange juice was bioavailable. We measured serum concentrations of 25-hydroxyvitamin D [25(OH)D] in healthy adults who consumed either unfortified orange juice or orange juice fortified with 1000 IU vitamin D_3; subjects consumed the orange juice daily for 12 wk at the end of the winter.

[1] From the Vitamin D, Skin, and Bone Research Laboratory, Section of Endocrinology, Diabetes, and Nutrition, Department of Medicine, Boston University School of Medicine, Boston.

[2] Supported in part by NIH grant M01RR00533 and the Coca Cola Company, Atlanta.

[3] Address reprint requests to MF Holick, Boston University School of Medicine, M-1013, 715 Albany Street, Boston, MA 02118. E-mail: mfholick@bu.edu.

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SUBJECTS AND METHODS

We obtained approval from the Institutional Review Board at Boston University School of Medicine to conduct our studies. All study subjects gave written informed consent for participation in the studies.

Bioavailability of vitamin D_2 in whole milk, skim milk, and corn oil on toast

Subjects

Nineteen healthy adults with an average age of 36.3 ± 10.0 y (range: 19–68 y) underwent a basic physical examination and biochemical profile to evaluate their eligibility for this study. Potential subjects were excluded if they had a history of vitamin D deficiency, intestinal malabsorption, severe medical illness, hypercalcemia, cigarette smoking, or excessive alcohol use. Potential subjects were also excluded if they were pregnant or if they took medications known to interfere with vitamin D metabolism.

Protocol

Each subject came to the General Clinical Research Center on 3 separate occasions (≥ 2 wk apart) for studies designed to measure the bioavailability of vitamin D in milk. Subjects were asked to drink 240 mL whole milk or skim milk that contained 25 000 IU oral vitamin D_2 (ergocalciferol) or 25 000 IU vitamin D_2 that had been dissolved in 0.1 mL corn oil and applied to toast. The sequence in which the subjects ingested the 3 different fortified foods was randomized. Serum was obtained 0, 2, 4, 8, 12, 48, and 72 h after ingestion of the fortified food to measure the blood concentrations of vitamin D_2. Vitamin D_2 concentrations were determined by using a method described by Chen et al (37). This assay has an intraassay CV of 8% and an interassay CV of 12%.

Bioavailability of vitamin D_3 in orange juice

Subjects

Thirty adults with an average age of 29.0 ± 9.0 y (range: 22–60 y) were recruited for this double-blind, randomized study. Potential subjects were excluded if they were taking multivitamins, drank > 16 oz (480 mL) milk daily, took medications that interfered with vitamin D metabolism, had significant sun exposure within the past month, planned to travel to a sunny climate during the study, or had a history of hypercalcemia.

Protocol

The protocol began in the second week of March. The orange juice was provided by The Minute Maid Co (Houston). Minute Maid did not provide the details on how the vitamin D was dispensed into the orange juice. Each subject was randomly assigned to 1 of 2 groups. A computer-generated randomization code was used to randomly assign the subjects in sequential order. The subjects and researchers were blinded to the group assignment. One group consumed 240 mL orange juice fortified with 350 mg Ca and the other group consumed 240 mL orange juice fortified with 350 mg Ca and 1000 IU vitamin D_3 (Hoffman-La Roche, Nutley, NJ) daily for 12 wk. Subjects obtained their orange juice weekly from our General Clinical Research Center. A blood sample was obtained weekly from each subject for measurement of serum 25(OH)D. Serum calcium, phosphorus, and alkaline phosphatase were measured monthly. Serum parathyroid hormone (PTH) and urine *N*-telopeptide were measured at the beginning and end of the 12-wk study.

Calcium, phosphorus, alkaline phosphatase, intact PTH, and urine *N*-telopeptide were measured by Quest Diagnostics (San Clemente, CA). Serum 25(OH)D concentrations were determined by using a method described by Chen et al (38). The limit of detection was 12.5 nmol/L; values below the limit of detection were assigned a value of 12.5 nmol/L. The assay has an intraassay CV of 8% and an interassay CV of 12%.

Stability of vitamin D in orange juice

To be certain that the vitamin D was stable in orange juice, an HPLC analysis of the orange juice for vitamin D_3 was performed at the time that the vitamin D_3 was added to the orange juice and after 30 d of storage at 4 °C.

Statistical analyses

The results are presented as means ± SEMs. The data were analyzed with MICROSOFT EXCEL (Office 2000) and ANALYSE-IT software (Analyse-It Software Ltd, Leeds, United Kingdom). Differences in the mean changes in calcium, phosphorus, alkaline phosphatase, and urine *N*-telopeptide were analyzed with independent-sample two-tailed Student's *t* tests. Changes in serum PTH from baseline to the end of the 12-wk study were analyzed with a paired two-tailed Student's *t* test. The serum vitamin D_2 concentrations after ingestion of the corn oil on toast, skim milk, or whole milk were analyzed with a two-factor (vitamin D_2 × vehicle food) analysis of variance (ANOVA). The serum 25(OH)D concentrations in the orange juice study were analyzed by one-way ANOVA in both the vitamin D–fortified and control groups. Further analyses were performed with Bonferroni techniques to determine differences in serum 25(OH)D concentrations at several time points compared with baseline values.

With regard to sample size, for the study on bioavailability of vitamin D in milk we chose a sample size that would provide 80% power to detect a > 33% difference between groups at $P = 0.05$. We used the same sample size calculations for the study on bioavailability of vitamin D in orange juice (www.stat.ucla.edu; power calculator from the University of California, Los Angeles).

RESULTS

Bioavailability of vitamin D_2 in whole milk, skim milk, and corn oil on toast

Eighteen of the 19 subjects completed the study. After the subjects ingested vitamin D_2 in whole milk, skim milk, or corn oil on toast, their serum vitamin D_2 concentrations began to increase within 4 h and peaked at 12 h (maximum concentration = 74 nmol/L). Concentrations returned to near baseline values by 72 h (**Figure 1**). Repeated-measures two-way ANOVA applied to these data showed that the main effect of treatment (ie, the vehicle food in which the vitamin D_2 was placed) was not significant ($P = 0.62$). A two-way ANOVA showed that the vitamin D_2 concentrations rose and fell significantly from baseline to 72 h (time effect: $P < 0.05$) and there was no significant interaction between treatment and time ($P = 0.87$) (Figure 1). None of the subjects reported any adverse events.

Bioavailability of vitamin D_3 in orange juice

Of the 30 subjects, 3 subjects did not complete the study (2 in the control group and 1 in the vitamin D–fortified group) and 1 subject in the control group was withdrawn because he traveled to

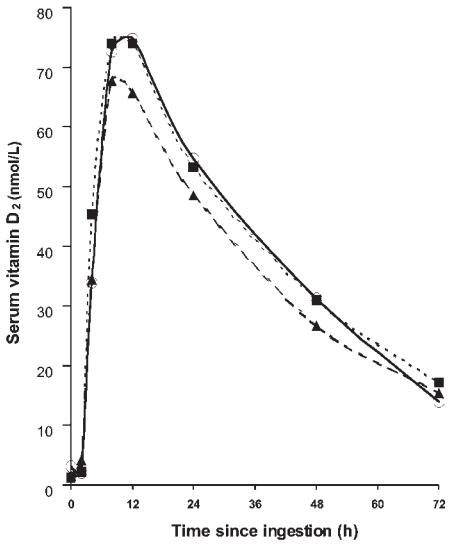

FIGURE 1. Serum vitamin D_2 concentrations in subjects given 25 000 IU vitamin D_2 in corn oil on toast (○), skim milk (■), and whole milk (▲); each subject ingested the 3 foods on 3 different occasions. For all 3 foods, $n = 18$ at 0, 4, 24, 48, and 72 h; $n = 14$ at 2 and 12 h; and $n = 17$ at 8 h. The two-way ANOVA showed a significant time effect ($P < 0.05$) but no significant treatment effect ($P = 0.62$) or interaction of treatment and time ($P = 0.87$).

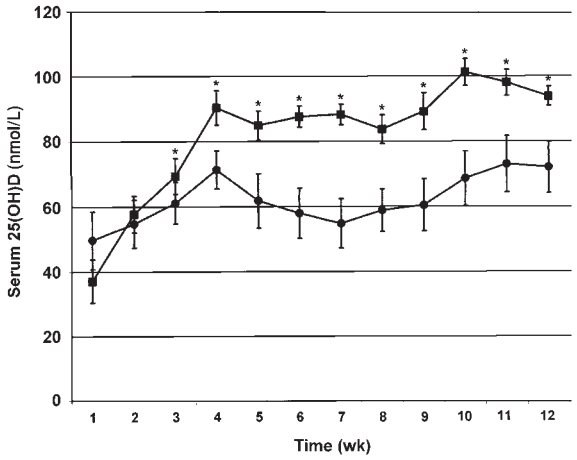

FIGURE 2. Mean (±SEM) serum 25-hydroxyvitamin D [25(OH)D] concentrations in subjects who ingested vitamin D–fortified (■) and unfortified (●) orange juice; 25(OH)D concentrations changed significantly over time in the group who ingested orange juice fortified with 1000 IU vitamin D (one-way ANOVA, $P < 0.0001$). *Significantly different from baseline, $P \leq 0.01$ (Bonferroni analysis at $\alpha = 0.01$). There were no significant changes in serum 25(OH)D concentrations over time in the group who ingested the unfortified orange juice (one-way ANOVA, $P = 0.38$).

the Caribbean during the study. Thus, 26 subjects completed the study, 14 in the vitamin D–fortified group and 12 in the control group. At the beginning of the study, 7 (58%) in the control group and 11 (79%) in the vitamin D–fortified group were vitamin D insufficient, defined as having 25(OH)D ≤ 50 nmol/L. There were no significant changes in serum calcium, phosphorus, or alkaline phosphatase from baseline values in either group (**Table 1**). None of the subjects reported any significant adverse effects. No subject developed hypercalcemia.

The subjects who consumed the vitamin D_3–fortified orange juice had a 150% increase in serum 25(OH)D concentrations from baseline to 12 wk (37.0 ± 8.0 to 94.0 ± 20 nmol/L; $P < 0.01$); control subjects had a 45% increase in 25(OH)D concentrations from baseline to 12 wk (50.0 ± 10 to 73.0 ± 8.0 nmol/L; $P < 0.01$). The subjects who consumed the vitamin D_3–fortified orange juice had significantly higher 25(OH)D concentrations at the end of the study compared with the control subjects and also had greater increases from baseline 25(OH)D concentrations (**Figure 2**). The mean increase in 25(OH)D in the group that consumed the vitamin D_3–fortified orange juice was 57.0 ± 7.0 nmol/L, compared with a mean increase of 22.5 ± 5.0 nmol/L in the control group ($P < 0.001$). Despite the presumed sun-induced synthesis of vitamin D in the control group, 25% of the subjects had vitamin D insufficiency at the end of the study, whereas none of the subjects who ingested 1000 IU vitamin D_3 had vitamin D insufficiency.

Subjects who consumed vitamin D_3–fortified orange juice had a 25% decrease in serum PTH concentrations compared with baseline (34.1 ± 3.3 to 25.6 ± 1.9 pg/mL; $P < 0.05$). There was no significant change in PTH concentrations in the control group (28.7 ± 3.2 to 31.4 ± 4.2 pg/mL). The mean individual change in PTH was −9.0 ± 10 and −1.6 ± 10 in the vitamin D-treated and control groups, respectively ($P = 0.05$) (Table 1). Urine *N*-telopeptide decreased 20% from baseline [41 ± 5 to 32.9 ± 4 nmol bone collagen equivalents (BCE)/mmol creatinine] in the vitamin D-treated group ($P = 0.07$). There was no significant change in urine *N*-telopeptide in the control group (38 ± 3 to

TABLE 1
Baseline values and mean individual changes (from baseline to 12 wk) in biochemical variables in subjects ingesting vitamin D–fortified and unfortified orange juice daily for 12 wk[1]

	Vitamin D–fortified juice ($n = 14$)		Unfortified juice ($n = 12$)		
	Baseline	Change	Baseline	Change	P^2
Calcium (mg/dL)	9.3 ± 0.1[3]	−0.4 ± 0.1	9.2 ± 0.1	−0.05 ± 0.1	0.95
Phosphorus (mg/dL)	3.9 ± 0.2	−6.3 ± 5.0	3.4 ± 0.1	−0.2 ± 0.5	0.28
Alkaline phosphatase (U/mL)	72.7 ± 4.0	−2.0 ± 2.0	60.8 ± 6.0	−5.0 ± 5.0	0.59
Parathyroid hormone (pg/mL)	34.1 ± 3.3	−9.0 ± 10	28.7 ± 3.2	−1.6 ± 10	0.05
Urine *N*-telopeptide (nmol BCE/mmol creatinine)	41.0 ± 5.0	−8.0 ± 4.0	38.4 ± 3.0	−1.6 ± 5.0	0.33

[1] BCE, bone collagen equivalents.
[2] Difference between groups in mean individual change (paired Student's *t* test).
[3] $\bar{x}$ ± SEM.

37 ± 5 nmol BCE/mmol creatinine). The mean change in urine *N*-telopeptide for individual subjects was −8.1 ± 4 and −1.6 ± 5 nmol BCE/mmol creatinine in the vitamin D-treated and control groups, respectively; these values were not significantly different from each other.

DISCUSSION

When the same subjects ingested 25 000 IU vitamin D_2 in 3 different vehicles (whole milk, skim milk, and corn oil on toast), the increases in their blood vitamin D_2 concentrations were not significantly different. These results show that fat is not required for vitamin D to be bioavailable. On the basis of these results, it was reasonable to consider fortifying nonfat beverages such as orange juice with vitamin D. Orange juice is an ideal beverage to fortify with vitamin D because it is highly nutritious and is usually consumed at breakfast. Because orange juice has a pH of ≈4, there was concern that the vitamin D added to it would not be stable. However, we determined by analysis with HPLC that the concentration of vitamin D_3 remained unchanged after storage for 30 d at 4 °C.

To assess whether vitamin D was bioavailable in orange juice, we obtained weekly measurements of serum 25(OH)D concentrations, the most accurate marker of vitamin D status, in subjects who drank a daily glass of orange juice fortified with vitamin D_3. A separate control group of healthy subjects drank a glass of orange juice that was not fortified with vitamin D_3 for the same 12-wk period. We chose to add 1000 IU vitamin D_3, which is 5 times and 2.5 times the recommended adequate intake for children and adults aged 1–50 y and adults aged 51–70 y, respectively. We then looked for a statistically significant increase in serum 25(OH)D concentrations during the 12-wk study. We did not measure vitamin D concentrations because the blood concentrations were too low to be detected (8). Subjects who ingested 240 mL orange juice fortified with 1000 IU vitamin D_3 daily had significant increases in their serum 25(OH)D concentrations compared with subjects who ingested the same amount of orange juice that was not fortified with vitamin D_3. The subjects who ingested vitamin D–fortified orange juice not only increased their 25(OH)D concentrations by > 150% over a period of 12 wk but also had a significant 25% decrease in PTH concentrations that was associated with a 20% decrease in the concentration of urine *N*-telopeptide, a marker for bone turnover. The subjects who ingested vitamin D_3–fortified orange juice did not experience any untoward side effects.

There was also a significant increase in serum 25(OH)D concentrations from baseline to 12 wk in the subjects who drank orange juice that was not fortified with vitamin D. This was not unexpected, and resulted from the seasonal rise in 25(OH)D concentrations that occurs during the spring in Boston (4, 39). However, the serum 25(OH)D concentrations only increased by 45% from baseline in the control group compared with a 150% increase in the group that ingested vitamin D–fortified orange juice.

The recommendation for adequate intake of vitamin D for children and adults ≤ 50 y is 200 IU vitamin D/d (40, 41). Our adult subjects were ingesting 1000 IU vitamin D_3 daily. This caused a significant increase in 25(OH)D concentrations after 3 wk that was sustained for an additional 2 mo. The circulating concentrations of 25(OH)D did not increase linearly over time, but plateaued after 4 wk and showed a gradual increase thereafter above 85 nmol/L. These results suggest that 1000 IU vitamin D_3 per day is not only safe but is very effective in maintaining serum 25(OH)D concentrations in the mid-normal range.

Adequate intakes of vitamin D and calcium are important for the prevention of rickets in children and osteomalacia and osteoporosis in adults. In addition, there is mounting evidence that adequate vitamin D nutriture and exposure to sunlight can decrease the risk of death from cancer of the colon (4, 22–24), breast (25, 30), and prostate (26–29). The average age at the onset of prostate cancer was 5 y higher in men who had the most exposure to sunlight (29). Finnish children who received vitamin D supplementation from the age of 1 y had an 80% reduction in the prevalence of type I diabetes (42). The mechanism by which sunlight exposure and vitamin D nutritional sufficiency decrease the risk of some common cancers and type I diabetes is not well understood. It is known that most organ systems, including the breast, prostate, gonads, large and small intestine, kidney, bone, brain, skin, and pancreas and the cells of the immune system possess vitamin D receptors and thus recognize and respond to $1,25(OH)_2D$ (4, 43, 44).

Besides its well known biological functions with regard to calcium metabolism, $1,25(OH)_2D$ is one of the most potent inhibitors of cellular growth and enhancers of cellular maturation (4, 44–48). Although the kidney is essential for the endocrine production of $1,25(OH)_2D$ for the purpose of maintaining calcium homeostasis, it cannot increase the production of this potent calciotropic hormone when there is an increase in the cutaneous production or ingestion of vitamin D. This is because $1,25(OH)_2D$ production is tightly regulated by serum calcium and PTH. It was only recently recognized that the colon, breast, prostate, and skin all have the enzymatic machinery (ie, 25-hydroxyvitamin D-1α-hydroxylase) to produce $1,25(OH)_2D$ locally for the likely purpose of modulating cell growth (4, 49–52). It is also known that $1,25(OH)_2D$ is a potent immunomodulatory factor (53) and it markedly reduces type I diabetes in mice with a high incidence of the disease (54).

Fortification of foods with vitamin D is an inexpensive approach to ensuring adequate vitamin D nutrition in all children and adults. The US Department of Agriculture reported that 49% of the US population aged > 2 y drinks ≥ 1 glass (240 mL) of a fruit juice daily. Sixty percent of children aged 9–18 y drink ≥ 1 glass of juice each day. Thus, fortifying juice products with vitamin D could have a significant effect on the vitamin D nutritional status of the population. We found that ingestion of orange juice containing 1000 IU vitamin D was very effective in enhancing the vitamin D status of adult subjects. However, it would be unrealistic to add 1000 IU vitamin D to 240 mL orange juice. It would be more reasonable to add 100 IU to 240 mL; this is the amount added to milk. We know with certainty that 1000 IU vitamin D in 240 mL orange juice is bioavailable. There is no reason to suspect that reducing the amount 10-fold to 100 IU/240 mL would alter its bioavailability. With this assumption, 1 glass of vitamin D–fortified juice (100 IU/240 mL) would represent 50% of the adequate intake recommended by the Institute of Medicine for all children and adults ≤ 50 y (39). Orange juice and other juice beverages that are now fortified with calcium should be considered for vitamin D fortification in a manner similar to the fortification of milk. Vitamin D fortification of orange juice and other juice products would increase vitamin D intake, which would help prevent osteomalacia and osteoporosis in adults and might provide additional potential health benefits, such as reduced risk of some common cancers and type I diabetes mellitus.

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VT, AAP, and MFH participated in the design of the study, the statistical analysis, the recruitment of subjects, and the preparation of the manuscript. PK and SMR participated in the recruitment of the subjects. TCC participated in the design of the study, the analysis of blood samples, and the preparation of the manuscript. MFH serves as a consultant for the Minute Maid Company, a division of the Coca-Cola Company. None of the other authors had any conflicts of interest.

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