Fortification of orange juice with vitamin D_2 or vitamin D_3 is as effective as an oral supplement in maintaining vitamin D status in adults[1–4]

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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 D_2 and vitamin D_3 from orange juice with that from vitamin D_2 and vitamin D_3 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 D_3, 1000 IU vitamin D_2, 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$). No significant difference in serum 25(OH)D_3 was observed between subjects who consumed vitamin D_3–fortified orange juice and vitamin D_3 capsules ($P > 0.1$). Similarly, no significant difference in serum 25(OH)D_2 was observed between subjects who consumed vitamin D_2–fortified orange juice and vitamin D_2 capsules ($P > 0.1$). No significant overall difference in parathyroid hormone concentrations was observed between the groups ($P = 0.82$).

Conclusion: Vitamin D_2 and vitamin D_3 are equally bioavailable in orange juice and capsules. *Am J Clin Nutr* 2010;91:1621–6.

INTRODUCTION

Vitamin D (D_2, D_3, 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 Crohns 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 D_3/236.6 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 D_3 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 D_3/100 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 D_3/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 D_2 (41).

Whether vitamin D_2 is equally as effective as vitamin D_3 at maintaining blood concentrations of 25(OH)D is still under discussion. A study of the bioavailability of 4000 IU vitamin D_2 and vitamin D_3 ingested in alcohol for 2 wk (42) or as a single 50,000-IU dose (43) suggested that vitamin D_2 was less effective than vitamin D_3 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 D_2 or 1000 IU vitamin D_3 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 D_2 weekly experienced an elevation in blood 25(OH)D concentrations equivalent to concentrations observed in children who received 2000 IU vitamin D_3 daily (45).

It is unknown whether vitamin D_2 and vitamin D_3 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 D_2 and vitamin D_3 from orange juice with that from vitamin D_2 and vitamin D_3 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 D_2 and vitamin D_3 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 D_3/236.6 mL, *3*) placebo capsule + orange juice containing 1000 IU vitamin D_2/236.6 mL, *4*) 1000 IU vitamin D_3 capsule + placebo orange juice, or *5*) 1000 IU vitamin D_2 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)D_2, and 25(OH)D_3 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 1
Demographic characteristics of the subjects[1]

Characteristics	Placebo (n = 15)	Vitamin D_3 in OJ (n = 18)	Vitamin D_2 in OJ (n = 17)	Vitamin D_3 in capsules (n = 20)	Vitamin D_2 in capsules (n = 16)
Age (y)					
Mean ± SD	40.8 ± 10.8	41.4 ± 12.6	40.1 ± 15.6	40.1 ± 18.0	38.9 ± 12.3
Range	24–59	22–65	19–73	22–81	18–59
Sex [n (%)]					
Female	13 (86.7)	15 (83.3)	9 (52.9)	12 (60)	10 (62.5)
Male	2 (13.3)	3 (16.7)	8 (47.1)	8 (40)	6 (37.5)
BMI (kg/m^2)	27.8	29.9	27	29.1	30.4
Race [n (%)]					
Asian	1 (6.7)	1 (9.1)	1 (5.9)	4 (20)	1 (6.25)
American Indian	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.25)
Black	7 (46.7)	11 (61.1)	9 (52.9)	8 (40)	9 (56.25)
Hispanic	0 (0)	2 (11.1)	0 (0)	2 (10)	1 (6.25)
White	6 (40)	2 (11.1)	6 (35.3)	6 (30)	4 (25)
Other	1 (6.7)	2 (11.1)	1 (5.9)	0 (0)	0 (0)
Multivitamin use [n (%)]	5 (33.3)	5 (27.8)	6 (35.3)	4 (20)	5 (31.3)
Vitamin D supplement use (n)	0	0	0	0	0
Dropouts [n (%)]	5 (21.7)	2 (9.1)	3 (15)	0 (0)	4 (20)
Compliance (%)	95.6	95.0	94.0	95.3	94.1

[1] OJ, orange juice.

TABLE 2
Measured outcomes[1]

Measured outcome	Placebo (n = 15)	Vitamin D_3 in OJ (n = 18)	Vitamin D_2 in OJ (n = 17)	Vitamin D_3 in capsules (n = 20)	Vitamin D_2 in capsules (n = 16)
25(OH)D (ng/mL)					
Initial	19.8 ± 9.6	17.9 ± 11.1	15.8 ± 10.0	19.6 ± 11.1	16.6 ± 9.9
Final	18.1 ± 6.4	30.7 ± 8.5	26.4 ± 7.4	28. ± 11.0	27.4 ± 10.5
Difference	−1.7 ± 5.8	12.8 ± 10.1	10.6 ± 7.2	9.3 ± 7.1	10.8 ± 5.9
	(−7.5, 1.2)	(8.1, 17.5)	(7.2, 14.0)	(6.2, 12.7)	(7.9, 13.9)
PTH (pg/mL)					
Initial	44.3 ± 27.1	37.1 ± 23.2	35.7 ± 17.4	42.0 ± 31.0	29.0 ± 18.7
Final	41.1 ± 19.4	25.6 ± 14.7	25.7 ± 14.9	34.2 ± 24.6	36.2 ± 22.9
Difference	−3.2 ± 20.3	−11.5 ± 19.9	−10.0 ± 17.5	−7.8 ± 22.9	7.2 ± 18.7
	(−13.5, 7.1)	(−20.7, −2.3)	(−18.3, −1.7)	(−17.8, 2.2)	(−2.0, 16.4)
Calcium (mg/dL)					
Initial	9.4 ± 0.3	9.4 ± 0.3	8.8 ± 2.1	9.3 ± 0.4	9.5 ± 0.4
Final	9.4 ± 0.3	9.4 ± 0.4	9.6 ± 0.3	9.4 ± 0.4	9.5 ± 0.3
Difference	0.0 ± 0.3	0.0 ± 0.4	−0.8 ± 2.3	0.1 ± 0.3	0.0 ± 0.3
	(−0.1, 0.1)	(−0.2, 0.2)	(−1.9, 0.3)	(−0.2, 0.2)	(−0.2, 0.2)
Albumin (g/dL)					
Initial	4.4 ± 0.3	4.3 ± 0.4	4.4 ± 0.4	4.3 ± 0.3	4.3 ± 0.3
Final	4.3 ± 0.3	4.1 ± 0.3	4.4 ± 0.3	4.2 ± 0.4	4.3 ± 0.2
Difference	−0.1 ± 0.3	−0.2 ± 0.2	0.0 ± 0.2	−0.1 ± 0.3	0.0 ± 0.2
	(−0.2, 0.0)	(−0.3, −0.1)	(−0.1, 0.1)	(0.0, 0.2)	(−0.1, 0.1)

[1] All values are means ± SDs; 95% CIs in parentheses. OJ, orange juice; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

intraassay CV of 9% and an interassay CV of 12%. Serum PTH was assessed by using an Immutopics International PTH (1-84) enzyme-linked immunosorbent assay (San Clemente, CA). The assay has an intraassay CV of from 2.2% to 2.3% and an interassay CV of from 5.6% to 8.6%.

Statistical analyses

Statistical calculations were performed by using SAS version 9.1 (SAS Institute, Cary, NC). Mean differences and 95% CIs for calcium, albumin, 25(OH)D, and PTH from baseline to week 11 were calculated for all subjects (Table 2). The mean (± SD) areas under the curve (AUCs) for serum $25(OH)D_2$, $25(OH)D_3$, $25(OH)D_{total}$, PTH, calcium, and albumin concentrations from baseline to week 11 were calculated for each treatment group. One-factor analysis of variance was used to detect overall significant differences in AUCs between treatment groups. To detect significant differences in AUCs between specific treatment groups, Tukey's honestly significant differences test was used. Tukey's honestly significant differences test was used regardless of the outcome of the analysis of variance. All significant differences were measured at the $P = 0.05$ level.

RESULTS

Of the 105 subjects who started the study, 86 subjects completed the study (18 in the vitamin D_3 orange juice group, 20 in the vitamin D_3 capsule group, 17 in the vitamin D_2 orange juice group, 16 in the vitamin D_2 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 $25(OH)D_{total}$ was observed between the subjects who received vitamin D_2 in orange juice (279.2 ± 80.6 ng · wk/mL) and those who received vitamin D_3 in orange juice (307.6 ± 82.6 ng · wk/mL). The overall difference in the AUC for serum $25(OH)D_{total}$ between all subjects who received either 1000 IU vitamin D_2 or vitamin D_3 in orange juice or in a capsule was not significant ($P = 0.084$) (**Figure 1**, A and B).

Subjects who received vitamin D_3 in orange juice had an AUC for serum $25(OH)D_3$ of 296.4 ± 74.4 ng · wk/mL, which was not significantly different from the AUC for serum $25(OH)D_3$ in the group who received vitamin D_3 in a capsule (302.3 ± 120.8 ng · wk/mL) (**Figure 2**A). The AUC for serum $25(OH)D_3$ was not significantly different ($P > 0.05$) between the group who received vitamin D_3 in orange juice and those who received placebo in orange juice (209.1± 104.4 ng · wk/mL), whereas the AUC for serum $25(OH)D_3$ was significantly different ($P < 0.0001$) between the group who received vitamin D_3 in capsules and those who received placebo in orange juice (Figure 2A).

No significant difference ($P > 0.05$) in the AUC for serum $25(OH)D_2$ was observed between the subjects who received vitamin D_2 in orange juice (127.3 ± 57.9 ng· wk/mL) and the subjects who received vitamin D_2 in capsules (118.0 ± 38.4 ng · wk/mL) (Figure 2B). However, the AUC for serum $25(OH)D_2$ was significantly different ($P < 0.0001$) between the subjects who received vitamin D_2 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 $25(OH)D_2$ and serum $25(OH)D_3$. It was determined that the bioavailability of

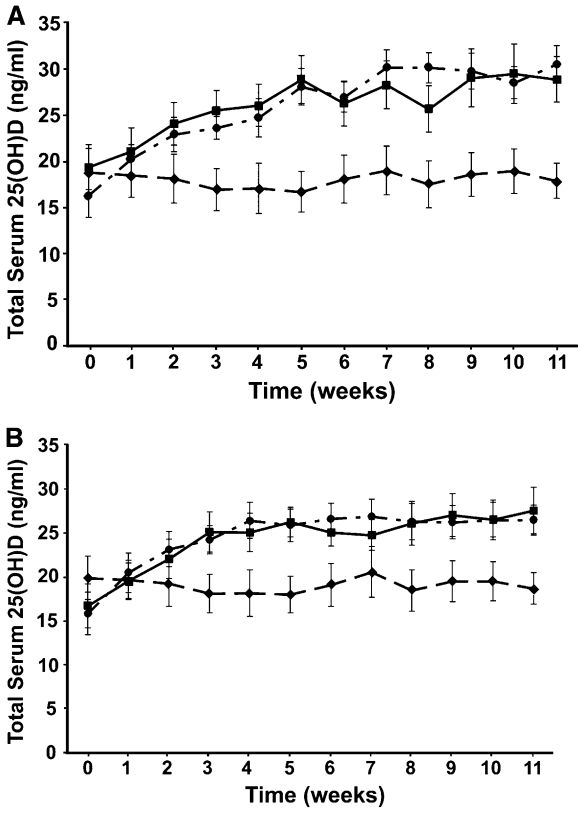

FIGURE 1. A: Mean (±SEM) total 25-hydroxyvitamin D [25(OH)D] concentrations over time after the oral administration of 1000 IU vitamin D_3 in orange juice (●; n = 18), 1000 IU vitamin D_3 in capsules (■; n = 20), or unfortified orange juice plus placebo capsules (♦; n = 15). No statistically significant differences were observed between areas under the curve for serum total 25(OH)D between the vitamin D_3 in orange juice and vitamin D_3 capsule groups (one-factor ANOVA, $P = 0.084$). B: Mean (±SEM) total 25(OH)D concentrations over time after oral administration of 1000 IU vitamin D_2 in orange juice (●; n = 17), 1000 IU vitamin D_2 in capsules (■; n = 16), or unfortified orange juice plus placebo capsules (♦; n = 15). No statistically significant differences were observed between areas under the curve for serum total 25(OH)D between the vitamin D_2 in orange juice and vitamin D_2 capsule groups (one-factor ANOVA, $P = 0.084$).

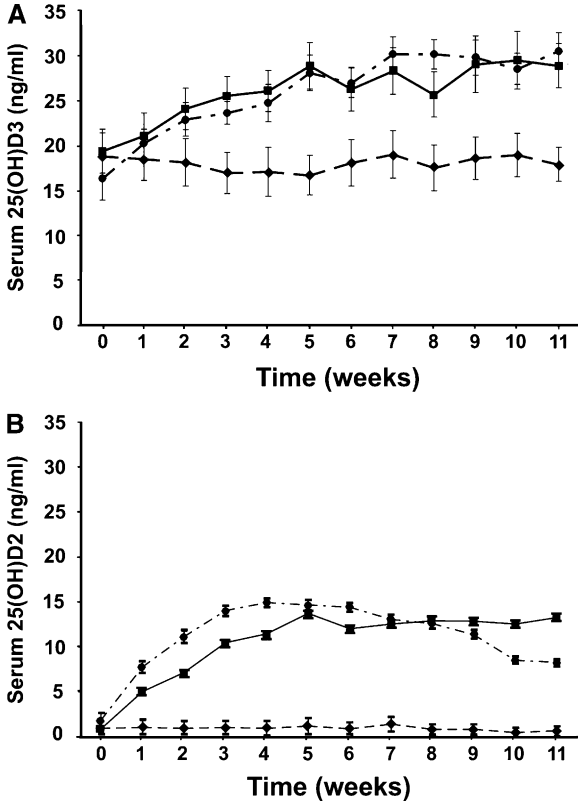

FIGURE 2. A: Mean (±SEM) serum 25-hydroxyvitamin D [25(OH)D_3] concentrations over time after oral administration of 1000 IU vitamin D_3 in orange juice (●; n = 18), 1000 IU vitamin D_3 in capsules (■; n = 20), or unfortified orange juice plus placebo capsules (♦; n = 15). The area under the curve for serum 25(OH)D_3 after consumption of vitamin D_3 in orange juice and after vitamin D_3 in capsules was not significantly different (one-factor ANOVA, $P > 0.05$). B: Mean (±SEM) serum 25(OH)D_2 concentrations over time after oral administration of 1000 IU vitamin D_2 in orange juice (●; n = 17), 1000 IU vitamin D_2 in capsules (■; n = 16), or unfortified orange juice plus placebo capsules (♦; n = 15). The area under the curve for serum 25(OH)D_2 after consumption of vitamin D_2 in orange juice and of the vitamin D_2 capsules was not significantly different (one-factor ANOVA, $P > 0.05$).

vitamin D was equivalent in orange juice and capsules. The AUC analysis showed that the bioavailability of vitamin D_2 and of vitamin D_3 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 D_2 and vitamin D_3 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)D_2 concentrations were significantly greater in subjects who consumed orange juice fortified with 1000 IU vitamin D_2 than in those who consumed the placebo plus orange juice without vitamin D. As expected, baseline 25(OH)D_2 concentrations were very low or undetectable in all subjects. Because vitamin D_2 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 D_2 supplements, or do not take prescription vitamin D_2 do not have measurable concentrations of 25(OH)D_2. Whereas 25(OH)D_2 concentrations seemed to increase more rapidly in the subjects who consumed orange juice containing vitamin D_2 than in the subjects who consumed vitamin D_2 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)D_2 or 25(OH)D_3 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 D_3 had significantly greater 25(OH)D_3 concentrations than the placebo group. Subjects who consumed orange juice containing vitamin D_3 and those who consumed vitamin D_3 capsules began the study with average 25(OH)D_3 concentrations of 17.6 ± 6.4 ng/mL. Their serum 25(OH)D_3 concentrations steadily increased until week 5, at which time they plateaued. The increases in 25(OH)D_3 in these 2 groups were not significantly different, which suggests that serum 25(OH)D_3 concentrations will increase similarly when 1000 IU vitamin D_3 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, vitamin

D_3 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 D_3 is more effective than vitamin D_2 at maintaining serum 25(OH)D concentrations (42, 43). The results of our study indicate that consumption of 1000 IU vitamin D_2 or vitamin D_3 in orange juice was equally as effective as 1000 IU vitamin D_2 or D_3 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 D_2 daily in capsule form was equally as effective as consuming a 1000-IU capsule of vitamin D_3 in raising serum 25(OH)D_2 and 25(OH)D_3 (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 D_2 or vitamin D_3 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—MFH AY, 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. MFH 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, AY, 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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