

Long-term effects of giving nursing home residents bread fortified with 125 μg (5000 IU) vitamin D₃ per daily serving¹⁻⁴

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ABSTRACT

Background: In older adults, a serum 25-hydroxyvitamin D [25(OH)D] concentration >75 nmol/L lowers the risk of fracture. An oral intake of 125 μg (5000 IU) vitamin D₃/d may be required to achieve this target.

Objective: The objective was to characterize the safety and efficacy of fortifying bread with a biologically meaningful amount of vitamin D₃.

Design: In a single-arm design, 45 nursing home residents consumed one bun daily that had been fortified with 125 μg (5000 IU) vitamin D₃ and 320 mg elemental calcium.

Results: The initial mean (\pm SD) serum 25(OH)D concentration was 28.5 ± 10.8 nmol/L. After 12 mo, the 25(OH)D concentration was 125.6 ± 38.8 nmol/L, and it exceeded 74 nmol/L in 92% of the patients. At every 3-mo follow-up, serum parathyroid hormone was lower than at baseline ($P = 0.001$). No changes in serum calcium or cases of hypercalcemia were observed at the follow-up assessments. Both mean total urinary calcium and the mean urinary calcium-creatinine ratio increased from baseline at one follow-up time point ($P < 0.05$). Between baseline and the 12-mo visit, z scores for bone mineral density at the lumbar spine and the hip both increased significantly ($P < 0.001$).

Conclusions: Fortification of bread with much more vitamin D than used previously produced no evident adverse effects on sun-deprived nursing home residents and improved bone density measures. Fortification of bread with 5000 IU vitamin D₃/d provided reasonable assurance that vitamin D-deficient older adults attained a serum 25(OH)D concentration greater than the desirable objective of >75 nmol/L. This trial was registered at clinicaltrials.gov as NCT00789503. *Am J Clin Nutr* 2009;89:1132-7.

INTRODUCTION

Elderly persons in Europe, and nursing home residents in particular, are commonly deficient in vitamin D and have osteomalacia (1-4). One recent meta-analysis concluded that, to prevent fractures in the elderly, serum 25-hydroxyvitamin D [25(OH)D] concentrations should exceed 72 nmol/L (5). A group of experts concluded that, to prevent fractures, the serum 25(OH)D concentration should exceed 75 nmol/L (6). None of the randomized trials that used doses of vitamin D <17.5 μg (700 IU)/d succeeded in preventing fractures (5-7). Although trials of the effects of 20 μg (800 IU) vitamin D/d have failed to show evidence of fracture prevention (8, 9), the participants in

these trials had a poor compliance rate and failed to achieve the serum 25(OH)D concentrations reached in trials that showed a beneficial effect (7). One gap in knowledge stems from the fact that few studies of vitamin D fortification >10 $\mu\text{g}/\text{d}$ have been published.

To address the problem of vitamin D deficiency in Europe, the OPTIFORD (Optimizing the Fortification of vitamin D in the European Union) project was conducted. One of the aims of this project was to examine the potential of fortifying bread with vitamin D (10 μg vitamin D₃ in 3 slices of bread per day) (10), which is expected to raise the mean 25(OH)D concentration by ≈ 10 nmol/L (11, 12).

We undertook an examination of the safety and efficacy of food fortification of bread to provide high amounts of vitamin D and calcium to nursing home residents deficient in these compounds. We fortified bread to provide 125 μg (5000 IU) vitamin D₃/d—the same dose that was recently calculated by Aloia et al (13) as being optimal for increasing serum 25(OH)D beyond 75 nmol/L in American adults with initial serum 25(OH)D concentrations <55 nmol/L.

SUBJECTS AND METHODS

Subjects

We studied 45 patients (28 women and 17 men) aged 58-89 y (mean \pm SD: 71 ± 6.9 y) who were living in a nursing home for elderly people in Iasi, Romania (latitude: 47°N). The trial was conducted between November 2003 and December 2004.

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² This publication is dedicated to the memory of Paul Stitt, who died shortly before the publication of this research that he made possible.

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Excluded from the study were patients with malignant disease; with renal, hepatic, or gastrointestinal disorders; with endocrine disease associated with abnormal calcium metabolism that required therapy (diabetes mellitus type 2 or hypothyroidism were permitted for inclusion); who consumed >4 alcoholic drinks/d; or who had used estrogen, progesterone, glucocorticoids, anti-convulsants, vitamin D supplements, or other medications known to affect calcium or bone metabolism during the previous 12 mo. Other medications were continued during the study. Patients who had experienced fractures in the past were not excluded. The protocol was approved by the Research-Ethics Board of the Romanian Physicians' Council, and all patients gave written informed consent.

Study protocol

In this 1-y trial, all subjects received bread (buns) daily that had been fortified with 800 mg CaCO₃/d (320 mg elemental calcium) and 125 μg (5000 IU) vitamin D₃/d. Subjects were assessed initially by physical examination, blood was sampled, and urine was collected for 24 h. Bone mineral density (BMD) was measured at 2 sites to assess appendicular and axial changes to the skeleton. Vertebral fractures were identified by X-rays of the thoracic and lumbar spine. Subjects were seen every 3 mo to collect blood and urine. Urine was collected for 24 h to measure calcium and creatinine. Because of the poor reliability of 24-h urine collections in the elderly, data were obtained and presented as both the total urine volume of both compounds and as the ratio of calcium to creatinine, as was done previously (14), and was validated clinically as a sensitive measure of hypercalciuria (15–18).

Dietary records

At the beginning of the study, calcium intake in the group of institutionalized patients was assessed on the basis of the 10-d food records that were provided by the nursing home, which provided the same food to all patients; food records were not obtained from the patients individually. The evaluation of dietary intake was repeated at 4-mo intervals throughout the study.

Fortification of food

All subjects received daily one bread bun (100 g) fortified with 800 mg CaCO₃/d (320 mg elemental calcium) and 125 μg (5000 IU) vitamin D₃/d. We added 0.5 mL of an oil solution containing vitamin D₃ (20,000 IU/mL, Vigantol; Merck KGaA, Darmstadt, Germany) into the dough used per bun. The dough was baked at 260–270°C for 15 min. Estimated loss during processing was 40–50%. The vitamin D₃ in samples of the bread was measured by solvent extraction (19) followed by HPLC and ultraviolet detection. The mean amount of vitamin D₃ was 5062.2 ± 459.7 IU/bun. The subjects were asked to consume all of the bread they were given.

Adherence and completion rate

Of the 45 patients enrolled, 40 completed the study (89%). Of those who withdrew, 1 did so for personal reasons, 1 left the nursing home, and the other 3 died (2 patients died of chronic heart failure and 1 of acute myocardial infarction). Seven

additional patients were not available at the time the final dual-energy X-ray absorptiometry scans were performed (they had left the nursing home shortly after the final blood collection to be with relatives or were hospitalized elsewhere). Hence, initial and final pairs of BMD data were available for 33 subjects. Adherence to the study protocol was assessed by using a questionnaire at the end of the study and by measuring plasma 25(OH)D concentrations.

Analytic methods

Fasting blood and urine samples were collected at baseline and every third month during the 12-mo fortification period. Serum, plasma, and urine samples were kept frozen until analyzed. Serum calcium, phosphate, and creatinine were measured by using standard laboratory methods. Serum 25(OH)D was measured by immunoassay (Liaison Analyzer; DiaSorin, Stillwater, MN). Serum intact parathyroid hormone (PTH) was measured by an enzyme-amplified “2-step” sandwich-type immunoassay (DSL, Webster, TX) with an interassay CV that ranged from 6.0% to 6.3% (normal range for adults aged 40–70 y). Serum osteocalcin was measured with the use of an enzymatically amplified “one-step” sandwich-type immunoassay (DSL) with an interassay CV between 3.7% and 10.1% (normal range in adults). Serum C-terminal telopeptides of type I collagen were assessed by using an enzyme-linked immunosorbent assay (CrossLaps CTX–Serum, Osteometer; BioTech, Herlev, Denmark) based on 2 highly specific monoclonal antibodies against the amino acid sequence of EKAHD-β-GGR, for which the aspartic acid residue (D) is β-isomerized. To obtain a specific signal in this assay, 2 chains of EKAHD-β-GGR have been cross-linked. The interassay CV was between 6.5% and 8.1%.

The BMD of the lumbar spine, femoral neck, and trochanter were measured by dual-energy X-ray absorptiometry (Delfi A; Hologic, Waltham, MA). The bone densitometry measurements were calibrated against the European Spine Phantom, and the same experienced technicians performed all of the bone measurements.

Statistical analysis

We performed an intention-to-treat analysis. Statistical calculations were performed by using SPSS version 13.5 (SPSS Inc, Chicago, IL). All data are expressed as means ± SDs. Initial comparisons between the male and female subjects were based on the independent-samples *t* test, without assuming that SD values are the same for both groups. Comparisons for variables measured at multiple time points were conducted by using the general linear model, with repeated-measures analysis of variance with post hoc testing, looking for interactions between month and sex. Effects of the intervention were examined by using a paired *t* test, comparing the initial and final values for each subject. Statistical significance was based on a *P* value < 0.05.

RESULTS

Dietary intakes of calcium and vitamin D (not including what was in the fortified bread) based on the group's 10-d food records of the institution, taken on three 10-d occasions during the study were 717 mg/d and 84 IU/d, respectively. Adherence to vitamin D intake from fortified bread was monitored by questionnaire and

was as follows: 91% of patients reported that they consumed the fortified bread every day. The proportion of each bun generally eaten by each subject was as follows: 76% of patients consumed the whole bun, 9% of patients consumed 75% of the bun, 12% of patients consumed 50% of the bun, and 3% (one person) regularly consumed 25% of the bun. There were no complaints about the taste of the bread.

Characteristics of the subjects at entry to the study are shown in **Table 1**. Women accounted for 64.4% of the group, and the mean age of the participants was 71.0 y. The results of biochemical testing at each time point during follow-up are summarized in **Table 2**, **Figure 1**, and **Figure 2**. The data were analyzed first by using repeated measures, looking for a significant interaction between month and sex. There were no sex or sex-by-time differences for any of the biochemical tests. Hence, the biochemical data were pooled for further analysis. For serum calcium, mean values did not differ significantly from the initial values at any of the 4 follow-up visits. In urine, the mean total concentration of calcium was not significantly higher than baseline according to general linear model statistical criteria for post hoc multiple comparisons (Table 2). However, an increase in urinary calcium should be expected when calcium and vitamin D are provided to adults who are relatively deprived in these nutrients. Therefore, to avoid a type II error, it is appropriate to report that, using Wilcoxon's signed-rank test, individual comparisons compared with the initial urinary calcium values were significant at 3, 6, and 9 mo of fortification: the *P* values were 0.016, 0.04, and 0.056, respectively (urine-calcium data did not support the parametric assumptions suitable for the paired *t* test). The ratio of urinary calcium to creatinine was significantly higher at the 6-mo follow-up visit than at baseline (Figure 1). Only at the 12-mo time point was osteocalcin, a serum marker of bone formation, significantly lower than at baseline. C-telopeptide, a serum marker of bone resorption, was not significantly lower than that at baseline. Mean serum PTH was initially near the top of the reference range for PTH, but at every subsequent time point tested (6, 9, and 12 mo) it was significantly lower (*P* < 0.001 for each, based on repeated-measures analysis of variance).

Individual extremes in urinary ratios of calcium to creatinine values, which reflect potential adverse events, are shown in Figure 1. Despite repeated measurements for each participant, there were only 3 instances (participants 30, 38, and 44) of a calcium-creatinine ratio higher than our hypercalciuria criterion of 1.0 (14). None of the participants exhibited either hypercalciuria or an extreme urinary calcium-creatinine ratio on more than one occasion during the fortification.

Serum 25(OH)D increased from its initial mean of 28.8 ± 9.9 nmol/L to the 12-mo mean of 126.4 ± 37.3 nmol/L. The

median increase in 25(OH)D during the year with bread fortified with 5000 IU/d was 98.0 nmol/L. There was no evidence of a sex difference in the 25(OH)D response to vitamin D. At every follow-up time point, serum 25(OH)D was significantly higher than that at baseline (*P* < 0.001 for each, based on repeated-measures analysis of variance). Post hoc, multiple-repeat comparison testing among all 4 of the 25(OH)D measurement time points indicated no significant difference at 3, 6, and 12 mo. However, because this statistical approach is prone to type II (β) error, especially for differences that had been expected, it is relevant to report that, based on conventional paired *t* test analysis, the 25(OH)D concentration increased between 3 and 6 mo (*P* = 0.021). However, there was no significant difference between 6 and 12 mo (*P* = 0.340). This suggests that a final plateau had been approached by 6 mo. The extremes of individual 25(OH)D responses to the fortified bread are shown in Figure 2. Despite the substantial fortification of a common food, 3 of 39 study participants (8%) did not reach the goal of ≥75 nmol/L after 12 mo; this finding resulted because of either biologic variation or poor compliance. Subjects who were provided 5000 IU vitamin D₃/d maintained 25(OH)D concentrations within the physiologic range (<240 nmol/L), as has been reported to be achieved through sun exposure.

BMD readings taken at baseline and 12 mo after fortification are summarized in **Table 3**. BMD at the lumbar spine was significantly different between sexes; therefore, these data are presented separately for each sex. In women, the increase in mean lumbar spine BMD was statistically significant (4.6% from baseline; *P* = 0.037), but the change in men was not statistically significant. The *z* scores for lumbar spine BMD are standardized in relation to age and sex, based on reference data provided by the manufacturer of the densitometer. The *z* scores for lumbar spine BMD did not differ significantly between the male and female subjects; therefore, *z* score data are presented for the sexes combined. The rise in *z* scores for lumbar spine BMD was highly statistically significant (*P* < 0.001). The greater statistical significance with the *z* scores reflects improved precision for the variable because of adjustment for age and sex. Hip BMD measures were not significantly different between sexes; therefore, data for the hip are presented for the sexes combined. Hip BMD increased substantially (23.4% from baseline; *P* < 0.001). Likewise, the rise in *z* scores for hip BMD was highly statistically significant (*P* < 0.001).

DISCUSSION

We were well justified in exploring the utility of what may, at first glance, be perceived as a high dose of vitamin D₃. In the long-term, an incremental intake of 1 μg (40 IU)/d increased the mean serum 25(OH)D concentration by 0.5–1.5 nmol/L (20). Heaney (21) calculated that the average adult with an initial 25(OH)D concentration between 20 and 40 nmol/L requires 55 μg (2200 IU) vitamin D₃/d to reach a 25(OH)D target concentration of 80 nmol/L. This is not enough to ensure a concentration >80 nmol/L for all adults (13). Thus, ≈50% of the subjects taking Heaney's recommended dose will end up with concentrations below the target. Furthermore, in 22% of our subjects, the starting 25(OH)D concentration was <20 nmol/L. Aloia et al (13) recently concluded that if adults start with 25(OH)D concentrations in the range initially present in our subjects, then 125 μg (5000 IU) vitamin D/d would be required.

TABLE 1
Baseline characteristics of the study participants

Characteristic	Men	Women	Total
Subjects [n (%)]	16 (35.6)	29 (64.4)	45 (100)
Age (y)	70.7 ± 6.0 ¹	71.2 ± 7.4	71.0 ± 6.9
Weight (kg)	78.8 ± 12.4	72.7 ± 14.1	74.8 ± 13.8
Height (cm)	165.9 ± 8.2 ²	153.8 ± 5.5	158.0 ± 8.7

¹ Mean ± SD (all such values).

² Significantly different from women, *P* = 0.001 (independent-samples *t* test).

TABLE 2Biochemical values at baseline and 3, 6, 9, and 12 mo after the consumption of bread fortified with calcium and vitamin D₃¹

Variable	Baseline	3 mo	6 mo	9 mo	12 mo	Overall <i>P</i>
Serum calcium (normal: 2.15–2.55 mmol/L)	2.29 ± 0.15 ² (2.1–2.65) [38]	2.33 ± 0.17 (1.94–2.58) [43]	2.28 ± 0.13 (2.05–2.55) [41]	2.35 ± 0.15 (2.10–2.65) [40]	2.28 ± 0.15 (1.90–2.55) [39]	0.516
Urinary calcium (normal: 2.5–7.5 mmol/L)	3.7 ± 1.6 (1.7–8.0) [36]	4.7 ± 2.1 ³ (2.1–10.0) [42]	4.4 ± 1.8 ³ (1.9–10.5) [41]	4.8 ± 2.0 (1.7–9.0) [38]	3.4 ± 2.2 (0.65–10.6) [40]	0.001
Serum parathyroid hormone (normal: 16–62 pg/mL)	59.3 ± 38.2 (10–181) [37]	Not tested	25.3 ± 20.3 ⁴ (0.5–91) [41]	21.1 ± 13.4 ³ (1–74) [36]	19.0 ± 16.0 ⁴ (0.2–58) [38]	0.001
Serum osteocalcin (ng/mL)	20.1 ± 10.3 (7.7–59.5) [42]	Not tested	19.1 ± 10.3 (2.2–45.3) [41]	17.6 ± 7.4 (7.6–43.1) [32]	14.7 ± 9.0 ⁴ (4.6–47.4) [39]	0.001
Serum C-telopeptide (ng/mL)	0.48 ± 0.34 (0.08–1.65) [42]	0.39 ± 0.19 (0.09–1.05) [41]	Not tested	Not tested	0.39 ± 0.26 (0.07–1.29) [39]	0.146

¹ There were no significant differences between sexes for any of the variables; hence, the data were pooled for both sexes. For calcium concentrations in the 24-h urine samples, the overall result by general linear model repeated-measures analysis of variance indicated significant differences across time points; however, general linear model tests of within-subjects contrasts were not statistically significant. *P* values for contrasts are based on the results of within-subjects contrasts, compared with the initial value for the measurement variable, using the simple-difference option in the SPSS software (SPSS Inc, Chicago, IL) with control for multiple comparisons.

² Mean ± SD; range in parentheses and number of subjects in brackets (all such values).

³ Significantly different from baseline, *P* < 0.05 (Wilcoxon's test). This nonparametric test was performed because not all data matched the parametric assumptions required for paired *t* tests.

⁴ Significantly different from baseline, *P* < 0.001 (general linear model repeated-measures analysis of variance).

Efforts to correct vitamin D deficiency and secondary hyperthyroidism with doses of vitamin D >2000 IU/d have been severely constrained in adults by the tolerable upper intake level (UL) for vitamin D. The UL is 50 μg (2000 IU)/d in North America and Europe, whereas in the United Kingdom a similar safe intake limit—the guidance level—is 25 μg (1000 IU)/d (22). Because the UL is intended as a guide for nutrient intake by the

general public, the UL was never intended to restrict nutrient doses used in clinical research. The careful study of a wide range of doses has been encouraged so that data can exist that

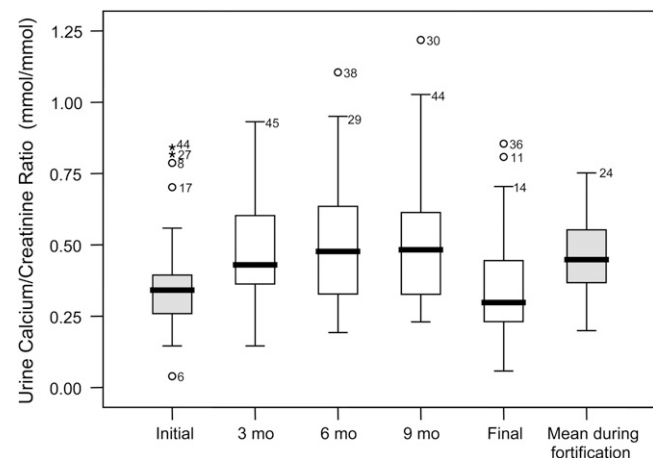


FIGURE 1. Ratios of urinary calcium to creatinine presented as box plots showing quartiles and individual extreme values during the consumption of bread fortified with vitamin D₃ and calcium. Shaded boxes represent initial values and mean values for each participant during the study. Means differed significantly between baseline and during fortification (*P* = 0.022). The ratios were significantly higher at 6 mo than at baseline (*P* = 0.045, post hoc repeated-measures analysis of variance). The numbers in the figure are unique identifiers for each participant who had an outlier (○) or extreme outlier (★) value [an outlier is a data point at or beyond the whisker that indicates the last data point that is within 1.5 box lengths beyond the 75th percentile as determined by the SPSS software (SPSS Inc, Chicago, IL)]. The sample size for each box is 36–44 study subjects, depending on the availability of the subjects for testing.

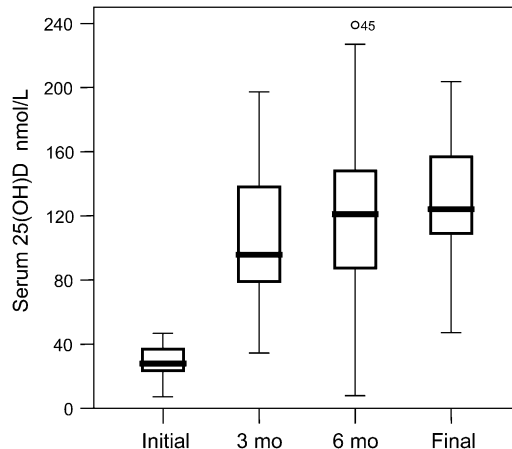


FIGURE 2. Serum 25-hydroxyvitamin D [25(OH)D] concentrations presented as box plots showing quartile values before fortification and during the consumption of bread fortified with vitamin D₃ and calcium. The numbers in the figure are unique identifiers for each participant who had an outlier (○) value [an outlier is a data point at or beyond the whisker that indicates the last data point that is within 1.5 box lengths beyond the 75th percentile as determined by the SPSS software (SPSS Inc, Chicago, IL)]. There were 45 study subjects, but the number available for testing at each of the time points from the initial to the final measurement was 37, 40, 41, and 39, respectively. Serum 25(OH)D increased at all time points after the start of the trial (overall significant change from initial baseline value, *P* < 0.001; general linear model, repeated-measures analysis of variance). The individual contrasts compared with initial 25(OH)D concentrations at each time point were all significant (*P* = 0.001) based on the results of within-subjects contrasts, compared with the initial value for the measurement variable, using the simple-difference option in the SPSS software with control for multiple comparisons.

TABLE 3Bone mineral density (BMD) before and 12 mo after the consumption of bread fortified with calcium and vitamin D₃¹

Bone density measures	Baseline	12 mo	Change ²	P ³
Lumbar BMD (g/cm ²)	0.830 ± 0.128 ⁴	0.863 ± 0.146	0.033 ± 0.086 (4.0)	0.037
Men (n = 10)	0.943 ± 0.148 ⁵	0.966 ± 0.178	0.024 ± 0.106 (2.4)	0.491
Women (n = 23)	0.782 ± 0.083	0.818 ± 0.105	0.036 ± 0.078 (4.6)	0.037
Lumbar z score	-0.403 ± 0.938	-0.036 ± 1.075	0.039 ± 0.79 (NA)	<0.001
Hip BMD (g/cm ²)	0.734 ± 0.126	0.906 ± 0.156	0.172 ± 0.83 (23.4)	<0.001
Hip z score	0.468 ± 1.041	0.8129 ± 0.965	0.278 ± 0.492 (NA)	<0.001

¹ Pooled data for sexes are shown for variables in which no significant sex differences were found.² Percentages in parentheses. NA indicates "not applicable," because the values were already standardized.³ Represents the comparison between baseline and 12 mo by paired *t* test.⁴ Mean ± SD (all such values).⁵ Significantly different from women, *P* = 0.002.

are appropriate for nutrient risk assessment and for a UL determination (23, 23).

Beyond the question of safety, it is plausible that vitamin D intakes higher than the current UL are necessary to provide a degree of assurance appropriate for a Recommended Dietary Allowance (RDA). An RDA is a nutrient intake that minimizes the risk of nutrient inadequacy for practically all who consume the RDA (24). If vitamin D adequacy is defined as a serum 25(OH)D concentration ≥ 75 nmol/L, then the dose used in the present study was probably appropriate. In contrast with a report of the adverse event of hypercalcemia in elderly subjects given 50 μ g vitamin D/d (25), we detected no evidence that 125 μ g vitamin D₃/d with calcium affected serum calcium. There was no statistically significant change in mean serum calcium and no evidence of hypercalcemia in the present study. As expected with higher intakes of calcium and vitamin D, urinary calcium excretion increased slightly. This is desirable because it reflects the increased calcium absorption expected when calcium and vitamin D are given. Hypercalciuria is the most sensitive marker of excessive calcium or vitamin D (18). Because adults are essentially in calcium balance, most of the net increase in calcium absorption from the gut is compensated for by increased calcium excretion into the urine. Hypercalcemia can eventually occur once the renal capacity to clear calcium is exceeded (18). We did detect hypercalciuria (urinary calcium-creatinine ratio: >1), but it was sporadic, occurring even at baseline; the effect was not persistent in any participant. Repetition of a measurement, as done here, will increase the probability of detecting transient fluctuations in urinary calcium across any clinical-decision limit. A meaningful adverse event would have been the persistence of hypercalciuria, which did not happen.

Our goal was to maintain a serum 25(OH)D concentration > 75 nmol/L. The fortification of bread achieved this goal for most of the study participants, and the highest 25(OH)D concentrations achieved were well within the physiologic range that could be obtained through vitamin D production in the skin (26).

Desirable effects of the present fortification strategy included significant reductions in serum PTH and in biochemical markers of bone turnover (Table 2). Heaney et al (12) reported that 4 mo of supplementation with 5000 IU vitamin D₃/d lowered serum PTH by 24%. In the present study, the mean serum PTH concentration decreased from the initial 59 ng/mL to 19 ng/mL (a 58% decline) after 3 mo and by 68% after 12 mo. The more substantial decrease in PTH in the Romanian elderly participants was attributable to their much lower initial 25(OH)D concen-

trations compared with the younger men studied in Omaha, whose initial 25(OH)D concentrations were 28 and 70 nmol/L, respectively (12).

Substantial increases in BMD measures were observed after consumption of the fortified bread for 12 mo (Table 3). Lumbar spine BMD increased by 4% over the initial measure. Hip BMD increased by a surprising 23.4% over the initial reading. These findings are consistent with reports showing that osteoporosis patients nonresponsive to treatment with antiresorptive drugs gain bone when supplemented with vitamin D (27, 28), and they support the desirability of maintaining a serum 25(OH)D concentration >75 nmol/L (6). The increases in BMD were probably due to mineralization of osteoid in patients with vitamin D deficiency and osteomalacia (29); the serum 25(OH)D concentration was <20 nmol/L in 22% of our subjects and was <40 nmol/L in 87% of our subjects. The major weakness in this study was that there was no control group against which to compare the bone findings. However, we doubt that it would be ethical to revisit a group that is now known to be this vitamin D deficient, for a randomized clinical trial, and to withhold vitamin D from a placebo group to confirm the effect of vitamin D on bone. From the outset, the intent of our trial was to study bone responses. To our knowledge, our findings represent the best data available concerning the response of vitamin D-deprived adults to calcium plus sufficient vitamin D to achieve the desirable serum 25(OH)D concentration of >75 nmol/L (Table 2) (6).

We had initially designed this study to include both a control and a supplemented group by using fortified bread. Placebo buns had been produced that were different only in appearance—baked with a cut-top or smooth-top dough style—for the control and fortified groups. However, it became obvious quickly that the residents did not consistently consume the bun they were given; they often shared or traded the buns. This made randomization of a food within a single institution unreliable, and our experience highlights a major difficulty in conducting drug-style placebo-controlled research in clinical nutrition. After baseline testing showed low 25(OH)D concentrations, we quickly modified the protocol to provide fortified buns to all study participants. The single-arm design did not invalidate the key conclusions, ie, that fortification with 5000 IU vitamin D/d to vitamin D-deficient nursing home residents offers reasonable assurance that serum 25(OH)D concentrations will exceed 75 nmol/L while maintaining the 25(OH)D concentration within the physiologic range, <225 nmol/L (26, 30), without increasing the risk of hypercalcemia. Hypercalcemia due to vitamin D excess appears to



require a serum 25(OH)D concentration of ≥ 700 nmol/L (31). A further weakness of the study was that the intervention combined 2 nutrients, vitamin D and calcium; however, combining these nutrients is the conventional thing to do. Had we not fortified the bread with calcium, questions about the safety of vitamin D would have been raised. The full effects on urinary calcium, PTH, and bone reported in the present study probably reflected the combined actions of both nutrients. A longer trial would be needed to characterize the persistence of the changes in BMD and to determine whether there is improvement in rates of disease events, such as fractures or cancer, as suggested by Lappe et al (32).

In conclusion, fortification of bread with vitamin D₃ and calcium was effective at eliciting the desired suppression in PTH and bone-turnover markers, while improving BMD at the hip and spine. The present results provide a plausible rationale for conducting clinical trials of vitamin D₃ at intakes >800 IU/d.

The authors' responsibilities were as follows—VM: was the principal clinical investigator, participated in the design, and cowrote the manuscript; PAS: conceived of the study and participated in its design; ARS: participated in the design, carried out the clinical examination, and evaluated the patients; OV: conducted the food intake analysis; VL: guided the laboratory work; EZ: guided the clinical work; and RV: participated in the design, guided the practical work, measured the 25(OH)D concentrations, performed the data analysis, and cowrote the manuscript. All authors read and approved the final manuscript. PAS was an employee of Natural Ovens of Manitowoc, Manitowoc, WI. None of the other authors had a potential conflict of interest.

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