The Journal of Nutrition
Nutrient Requirements and Optimal Nutrition

The Bioavailability of Vitamin D from Fortified Cheeses and Supplements Is Equivalent in Adults[1,2]

Dennis Wagner,[3,4]* Gloria Sidhom,[5] Susan J. Whiting,[6] Dérick Rousseau,[7] and Reinhold Vieth[3,4]

[3]Department of Nutritional Sciences, University of Toronto, M563E2 Toronto, Canada; [4]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, M5G1X5 Toronto, Canada; [5]Department of Clinical and Chemical Pathology, National Research Centre, PO Box 5216 Cairo, Egypt; [6]College of Pharmacy and Nutrition, University of Saskatchewan, S7N5C9 Saskatoon, Canada; and [7]School of Nutrition, Ryerson University, M5B2K3 Toronto, Canada

Abstract

There is a need to increase the options for vitamin D fortification. We have developed a method to fortify hard cheese with vitamin D. Our aim was to characterize the bioavailability of vitamin D from fortified cheeses. Eighty adults were randomized to weekly servings of fortified cheddar cheese (DC) (34 g; $n = 20$); fortified low-fat cheese (DLF) (41 g; $n = 10$); liquid vitamin D supplement (1 mL), taken with food (DS+) ($n = 20$) or without food (DS−) ($n = 10$); placebo cheddar cheese ($n = 10$); or placebo supplement ($n = 10$). The treatments contained 28,000 IU cholecalciferol (vitamin D3), equivalent to 4000 IU (100 μg/d). The primary outcome was the comparison of vitamin D bioavailability, as measured by the serum 25-hydroxyvitamin D [25(OH)D] response, between fortified cheeses and supplement. In the placebo groups, initial 25(OH)D, 55.0 ± 25.3 nmol/L, declined over the 8-wk winter protocol, to 50.7 ± 24.2 nmol/L ($P = 0.046$). In the vitamin D-treated groups, the mean increases in 25(OH)D over 8 wk were: 65.3 ± 24.1 (DC), 69.4 ± 21.7 (DLF), 59.3 ± 23.3 (DS+), and 59.3 ± 19.6 nmol/L (DS−); these changes differed from the placebo groups ($P < 0.0001$) but not from one another ($P = 0.62$). Compared with baseline, serum parathyroid hormone decreased with both fortification ($P = 0.003$) and supplementation ($P = 0.012$). These data demonstrate that vitamin D is equally bioavailable from fortified hard cheeses and supplements, making cheese suitable for vitamin D fortification. J. Nutr. 138: 1365–1371, 2008.

Introduction

Vitamin D insufficiency constitutes a largely unrecognized and widespread public health problem (1–8). Chronic vitamin D insufficiency prevents the proper mineralization of bone, leading to rickets in children and osteomalacia or osteoporosis in adults. Recent evidence indicates that low levels of 25-hydroxyvitamin D [25(OH)D],[8] the accepted clinical measure of vitamin D status, are associated with an increased risk of incident cancers, along with a higher mortality from these cancers (9–13). Evidence also exists of a beneficial effect of vitamin D on other diseases, including multiple sclerosis (14–16), diabetes (17–19), cardiovascular disease (20,21), rheumatoid arthritis (22), and microbial infections (23–25). Evaluation of most relationships of vitamin D with health and disease lead to the conclusion that a sufficient 25(OH)D concentration is ≥75 nmol/L (2,26), suggesting that the prevalence of vitamin D insufficiency is higher than anticipated.

There is strong evidence indicating that current vitamin D intakes in adults are inadequate (27–29). Cutaneous synthesis of vitamin D by UV-B sunlight, the major contributor to vitamin D status, is limited by environmental, cultural, and physiologic factors, including latitude, season, clothing, sunscreen, glass shielding, age, and skin pigmentation (30–34). Vitamin D is difficult to obtain from the diet, because it is not naturally present in many foods. Thus, populations in North America often rely on fortified foods and dietary supplements to meet their vitamin D needs during times of insufficient sunlight. Vitamin D fortification is mandatory in Canada for beverage milk (100 IU/250 mL)[9] and margarine (53 IU/10 g) (35) and optional in the United States for milk, breakfast cereals, and calcium-fortified fruit juices (40–140 IU/serving) (36). However, cross-sectional studies suggest that current North American fortification practices are not effective in preventing vitamin D insufficiency, because fortified foods provide inadequate amounts of vitamin D and are often underfortified (1,27,37–39). Furthermore, milk consumption has declined considerably since the 1980s (40) and lactose intolerance is a common problem, particularly among

[1] Supported by grants from the Dairy Farmers of Canada and the Natural Sciences and Engineering Research Council of Canada.
[2] Author disclosures: D. Wagner, G. Sidhom, S. J. Whiting, D. Rousseau, R. Vieth, no conflicts of interest.
[8] Abbreviations used: ALP, alkaline phosphatase; CTX, c-telopeptide; DC, fortified cheddar cheese; DLF, fortified low-fat cheese; DS+, vitamin D supplement taken with food; DS−, vitamin D supplement taken without food; 25(OH)D, 25-hydroxyvitamin D; PC, placebo (i.e. unfortified) cheddar cheese; PBO, placebo cheddar cheese and placebo supplement groups combined; PS, placebo supplement; PTH, parathyroid hormone.
* To whom correspondence should be addressed. E-mail: dennis.wagner@utoronto.ca.

[9] 1 μg = 40 IU.

0022-3166/08 $8.00

Manuscript received 21 January 2008. Initial review completed 15 March 2008. Revision accepted 7 May 2008.

populations that are at greatest risk of vitamin D deficiency (e.g. Blacks, Asians, Native Americans) (41,42). Clearly, additional foods need to be fortified with vitamin D to increase the availability of this essential nutrient for the general population.

In both Canada and the United States, industrial milk destined for processing (e.g. baking or making yogurt, ice cream, and cheeses) is not fortified with vitamin D and fortification of the final product is not required (27). Cheese is a good source of calcium and has only traces of lactose, making it a viable source of vitamin D and calcium for individuals with lactose intolerance and those who do not otherwise drink milk. Furthermore, cheese is potentially an ideal vehicle for vitamin D, because its dietary fat content might promote the stability and absorption of fat-soluble vitamin D (43). Lastly, cheese is a widely consumed food and its per capita consumption has increased steadily since the 1980s and is expected to continue to do so in the future (40,44). In a previous phase of our work, we demonstrated that a cheese-like matrix could be efficiently fortified with vitamin D (45). However, cheese contains certain components (e.g. the milk proteins β-lactoglobulin A and β-casein) that can bind strongly to vitamin D (46,47) and this may affect its bioavailability.

We investigated in a double-blind, randomized, controlled trial whether vitamin D is bioavailable from fortified hard cheeses and whether accompanying food might affect vitamin D bioavailability.

Materials and Methods

The study protocol was approved by the Research Ethics Boards of the University of Toronto and of Mount Sinai Hospital (Toronto, Canada). All study subjects signed a form indicating their informed consent.

Subjects and recruitment criteria. Subjects were recruited through the use of an Internet posting on the University of Toronto email-log-on notice board. Healthy men and women between 18 and 60 y of age were candidates for inclusion in the study. They were not admitted to the study if any of the following criteria were present: *1*) a history of any medical disorders that might affect vitamin D or mineral metabolism; *2*) use of vitamin D supplements in excess of 400 IU/d; *3*) use of medications that could interfere with vitamin D metabolism; *4*) potential for significant sun exposure (e.g. travel to a sunny locale or use of tanning beds) within the month prior to or during the study; or *5*) unwillingness to return for follow-up. Between November 2006 and January 2007, we recruited 40 men and 40 women meeting the eligibility requirements, most of whom were students at the University of Toronto (latitude 43°N).

Study design. The 80 eligible subjects were randomly assigned to 1 of 6 interventions (outlined in Fig. 1): *1*) vitamin D-fortified regular-fat cheddar cheese (DC) (33.6 g/serving); *2*) vitamin D-fortified low-fat cheese (DLF) (41.4 g/serving); *3*) vitamin D supplement (as an ethanolic solution) to be taken with food (i.e. during a meal) (DS+); *4*) vitamin D supplement (as an ethanolic solution) to be taken without food (i.e. just before bedtime) (DS−); *5*) placebo cheese, a regular-fat cheddar cheese (33.6 g/serving) containing no vitamin D (PC); and *6*) placebo supplement, an ethanolic solution containing no vitamin D (PS). Fortified cheeses and supplements contained 28,000 IU of cholecalciferol (vitamin D3) per serving or dose. Each serving or dose was consumed orally once a week at home for a total of 8 wk; this weekly dose is equivalent to a daily dose of 4000 IU cholecalciferol (48).

The protocol began in the fourth week of January and ended in the first week of April, a time of the year during which cutaneous synthesis of vitamin D from UV-B sunlight would be negligible (30). Subjects attended Mount Sinai Hospital biweekly for a total of 5 visits. At each visit, we obtained venous blood and urine samples from each subject for biochemical testing and provided each subject with individual servings of cheese or supplement.

The serum 25(OH)D metabolite is the appropriate measure of the success of vitamin D supplementation or fortification (49). Thus, we measured serum 25(OH)D at 2-wk intervals for 8 wk to assess bioavailability from the various vehicles and methods for providing vitamin D. The primary outcome was the comparison of serum 25(OH)D concentrations among groups at the 8-wk time point. The primary outcome comparison was between the DC group and the DS+ group. Two smaller groups (i.e. DS− and DLF) were designed to investigate subgroup effects, namely whether accompanying food or differences in the fat content of cheese, respectively, might affect vitamin D bioavailability. Secondary outcome measures included serum calcium, creatinine, phosphate, parathyroid hormone (PTH), alkaline phosphatase (ALP), and c-telopeptide (CTX), as well as urine calcium, creatinine, and phosphate. We tested the following hypotheses, which were set a priori: *1*) the change in serum 25(OH)D (from baseline to 8 wk) will be significantly greater in the vitamin D-treated groups compared with placebo; and *2*) the change in serum 25(OH)D will not differ among any of methods for providing vitamin D.

To have an 80% chance of detecting as significant (at the 2-sided 5% level) a 18-nmol/L difference in the mean change in serum 25(OH)D (from baseline to 8 wk) between the 2 main treatment groups (i.e. DC vs. DS+), assuming a SD of 20 nmol/L, 20 subjects in each group (40 in total) were required (50).

The randomization sequence was generated by the principal investigator, who was not involved in the implementation of the assignments, using computer software to produce randomly permutated blocks of 6 allocations [Random Allocation Software 1.0 (2004), by M. Saghaei]. With these blocks, random assignment was stratified by gender, so that equal numbers of males and females were allocated to each intervention group. Subject names and their corresponding cheese or supplement were numerically coded so that both the investigators and the subjects did not know whether or not the cheese or supplement samples provided to each subject contained vitamin D. The code was revealed to the researchers once recruitment, data collection, and laboratory analyses were complete.

Materials. USP-grade cholecalciferol (vitamin D3) was purchased in crystalline form from Sigma and dissolved in USP-grade ethanol. The molar concentration of vitamin D was adjusted to 1823 μmol/L (28,000 IU/1-mL dose). Quantitation of vitamin D was based on an absorbance at 265 nm (33.36 absorbance units based upon a molar extinction coefficient of 18,300 absorbance units$\cdot$mol$^{-1}\cdot$L^{-1}). Spectroscopy was performed with an 8352A diode array spectrophotometer (Hewlett-Packard) using a 1-cm quartz cuvette. Appropriately blanked UV absorption spectra of the ethanolic solution of vitamin D supplements were measured before and after the study and the concentrations remained identical. PS consisted of USP-grade ethanol without vitamin D. DS+, DS−, and PS were physically identical and unidentifiable. They were consumed weekly by each subject by mixing 1 mL of the ethanolic solution into juice or water just before drinking it.

All cheeses were industrially manufactured by Agropur Cooperative using standard cheese-making methodologies. The preliminary production step involved the addition of vitamin D to the whole milk [3.8% fat (wt:wt)] or skim milk [0.8% fat (wt:wt)] destined for cheddar or low-fat cheese manufacture, respectively. We fortified each kind of milk to a level of 111 IU vitamin D/g using Vitamin D3 Premix (208,000 IU/mL), a conventional emulsified concentrate of cholecalciferol that is used in the dairy industry (Kingsway Chocolate). The vitamin D content of cheese samples was measured as previously described (45), with some modifications: *1*) the mass of cheese samples and distilled water was reduced 5-fold; *2*) an internal standard, USP-grade 25(OH)D (Sigma) was used; and *3*) sample preparation, heated saponification, and lipid extraction steps were all performed in a single test tube.

The mean vitamin D concentration of the DC was 833.88 IU/g and of the DLF was 676.09 IU/g. The PC contained no vitamin D. Random sites were sampled from 2-kg cheese blocks and measured vitamin D content was confirmed to be homogeneous throughout the cheeses. After analysis, the cheese was portioned into weekly servings of DC (33.6 g/serving) and DLF (41.4 g/serving), corresponding to 28,000 IU vitamin D/serving. All cheeses were vacuum-packaged in plastic food bags,

numerically coded so as not to disclose dosage on the package, and kept refrigerated at 4–8°C. The vitamin D content of the cheeses did not change over the course of the study. The tastes of the fortified cheeses were indistinguishable from that of their unfortified counterparts, as was the physical appearance of all the cheeses.

Measurements. We measured the anthropometrics of each subject (i.e. age, weight, and height) at the baseline visit. At each visit, we collected the empty vials and plastic cheese packages from each subject to monitor compliance. At the end of each study month, subjects completed a FFQ, which was used to calculate the mean background daily intake of vitamin D and calcium from food and supplements. The food composition database used for the FFQ was the most recent version of the Canadian Nutrient File (51). Validation of the FFQ was performed against 7-d food records and 25(OH)D concentrations of 104 subjects during the winter (S. J. Whiting, H. Wu, A. Godzik, and E. Parra, unpublished data). Subjects were instructed to maintain their typical dietary habits throughout the study.

Serum 25(OH)D was measured by radioimmunoassay (DiaSorin). The assay has a limit of detection of 3.75 nmol/L, an intra-assay CV of 8%, and an interassay CV of 16%. For serum 25(OH)D, all samples from a single subject were measured within the same run to minimize assay variation.

Serum calcium, phosphate, creatinine, PTH, ALP, and CTX, as well as urine calcium, phosphate, and creatinine were measured on the Modular Analytics Serum Work Area (Roche) immediately after obtaining the blood and urine samples. Urinary calcium excretion was calculated as the ratio of millimolar concentrations of urine calcium and urine creatinine.

Statistical analyses. The results are means ± SD. All data were analyzed with SPSS software (version 13.0). Graphs were created with GraphPad Prism 5 for Windows. One-way ANOVA were used to compare the baseline characteristics among the groups. Associations between biochemical measures were examined by means of the Pearson correlation coefficient. Within-group changes in serum and urine biochemical variables over time were analyzed with paired 2-tailed *t* tests. Between-group differences in mean serum and urine biochemical variables at various time points, as well as between-group differences in the changes in serum and urine biochemical values, were analyzed with 1-way ANOVA followed by Tukey's honestly significant difference post hoc testing. Differences between genders in mean 25(OH)D concentrations at various time points were analyzed with independent-sample 2-tailed *t* tests. The criterion for significance was set at $P < 0.05$.

Results

Characteristics of subjects. The flow of participants through each stage of the study is illustrated (**Fig. 1**). All enrolled subjects completed the entire protocol, with 0 missed visits. Compliance, measured by counts of empty vials and cheese packages returned by the subjects, was 98%. The analysis was intention-to-treat and involved all 80 subjects who were randomly assigned to groups. The demographics and baseline characteristics of the participants were very similar for the different intervention groups (**Table 1**). Intake of vitamin D and calcium (from food and supplements) did not differ among the groups. At baseline, serum 25(OH)D and CTX values ($r = -0.23$; $P = 0.044$) were correlated, as were serum ALP and serum CTX values ($r = 0.23$; $P = 0.041$). In the total group ($n = 80$), the baseline serum 25(OH)D concentration was 54.2 ± 24.0 nmol/L. Of these subjects, 65 (81.3%) had less than desirable 25(OH)D concentrations (<75 nmol/L), 39 (48.8%) had low concentrations (<50 nmol/L), and 5 (6.3%) had concentrations that could indicate osteomalacia (<25 nmol/L).

Serum 25(OH)D concentrations. In the combined placebo groups (PBO) ($n = 20$), serum 25(OH)D decreased from baseline to 8 wk (55.0 ± 25.3 to 50.7 ± 24.2 nmol/L; $P = 0.046$) (**Fig. 2**). In all vitamin D-treated groups, serum 25(OH)D increased from baseline to 8 wk by over 100% ($P < 0.0001$) (Fig. 2). Serum 25(OH)D concentrations in all vitamin D-treated groups were higher in 3 comparisons: *1*) at each visit (excluding baseline) compared with PBO ($P < 0.005$); *2*) at each visit compared with baseline ($P < 0.005$); and *3*) at each visit compared with the previous visit ($P < 0.05$). The 25(OH)D concentration attained at each visit did not differ among any of the groups that received vitamin D ($P > 0.50$). Serum 25(OH)D concentrations did not differ between men and women at any time point ($P > 0.10$). Upon completion of the 8-wk intervention, a 25(OH)D concentration of ≥75 nmol/L was attained in 18 of the 20 (90%) subjects consuming DC, 9 of the 10 (90%) subjects consuming DLF, 19 of the 20 (95%) subjects consuming DS+, and 9 of the 10 (90%) subjects consuming DS−.

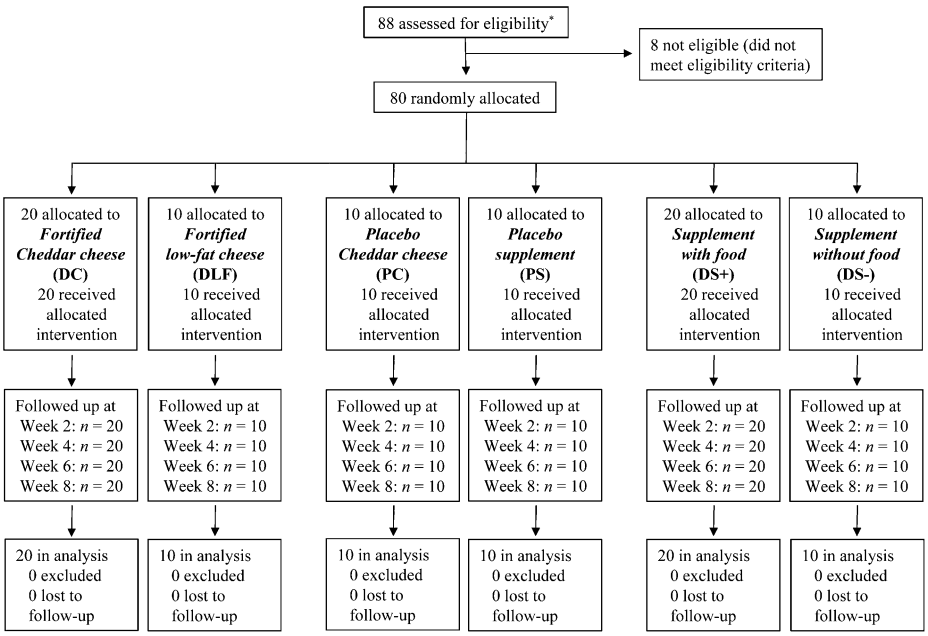

FIGURE 1 Trial profile. *Enrollment was limited to 40 subjects per gender.

TABLE 1 Baseline characteristics in each group of subjects[1]

	Fortified cheese		Supplement		Placebo	
	DC	DLF	DS+	DS−	PC	PS
n	20	10	20	10	10	10
Age, *y*	28.7 ± 11.4	30.6 ± 11.7	31.8 ± 12.5	23.6 ± 3.5	26.2 ± 6.5	24.6 ± 4.5
BMI, *kg·m*$^{-2}$	25.2 ± 5.0	24.2 ± 3.3	23.4 ± 3.8	23.8 ± 5.2	22.9 ± 3.9	21.7 ± 2.5
Vitamin D intake,[2] *IU/d*	119.5 ± 104.6	232.4 ± 156.9	207.8 ± 196.0	102.7 ± 77.6	135.5 ± 83.8	176.9 ± 150.5
Calcium intake,[2] *mg/d*	599.5 ± 278.4	886.7 ± 379.8	630.3 ± 305.2	713.7 ± 502.9	687.0 ± 268.2	952.6 ± 874.7
Serum 25(OH)D, *nmol/L*	50.7 ± 18.9	57.5 ± 18.4	55.7 ± 21.2	53.4 ± 40.1	52.4 ± 22.7	57.6 ± 28.7
<75 nmol/L, *n (%)*	16 (80)	8 (80)	16 (80)	9 (90)	8 (80)	8 (80)
<50 nmol/L, *n (%)*	11 (55)	3 (30)	8 (40)	7 (70)	4 (40)	6 (60)
<25 nmol/L, *n (%)*	1 (5)	0 (0)	1 (5)	1 (10)	1 (10)	0 (0)
Serum calcium, *mmol/L*	2.38 ± 0.08	2.35 ± 0.06	2.37 ± 0.07	2.40 ± 0.08	2.42 ± 0.09	2.42 ± 0.06
Urine calcium, *mmol/L*	2.77 ± 2.20	2.04 ± 2.34	3.34 ± 2.44	3.63 ± 1.50	5.22 ± 4.37	2.51 ± 1.71
Serum creatinine, *μmol/L*	71.90 ± 9.85	70.5 ± 9.12	76.05 ± 11.52	71.6 ± 11.32	74.6 ± 18.33	71.8 ± 13.70
Urine creatinine, *mmol/L*	11.42 ± 6.50	7.98 ± 7.63	13.12 ± 7.6	15.03 ± 8.85	13.52 ± 6.56	7.35 ± 4.85
Urinary calcium excretion,[3] *mmol:mmol creatinine*	0.28 ± 0.23	0.34 ± 0.33	0.33 ± 0.26	0.31 ± 0.15	0.38 ± 0.26	0.39 ± 0.17
Serum phosphate, *mmol/L*	1.24 ± 0.17	1.2 ± 0.13	1.19 ± 0.13	1.14 ± 0.13	1.21 ± 0.19	1.24 ± 0.11
Urine phosphate, *mmol/L*	17.01 ± 10.50	12.32 ± 14.87	18.03 ± 10.92	12.9 ± 15.75	20.15 ± 11.41	10.53 ± 8.93
Serum PTH, *pmol/L*	5.11 ± 3.32	3.9 ± 0.73	4.57 ± 2.11	3.77 ± 1.29	4.44 ± 2.47	4.34 ± 1.50
Serum ALP, *U/L*	66.85 ± 19.94	59.9 ± 13.66	61.4 ± 13.11	70.7 ± 25.10	66.1 ± 15.98	68.3 ± 18.29
Serum CTX, *μg/L*	0.27 ± 0.17	0.26 ± 0.12	0.22 ± 0.17	0.25 ± 0.10	0.30 ± 0.16	0.32 ± 0.24

[1] Values are means ± SD. Baseline values did not differ among groups, $P > 0.05$.
[2] Mean total intake from the diet and supplements (excluding intervention), as assessed by monthly FFQ.
[3] Expressed as a ratio of millimolar concentrations of urine calcium and urine creatinine.

Changes in serum 25(OH)D concentrations [Δ25(OH)D; nmol/L], calculated as the difference between baseline and wk 8, were higher in the DC (65.3 ± 24.1), DLF (69.4 ± 21.7), DS+ (59.3 ± 23.3), and DS− (59.3 ± 19.6) groups than in the PBO group (−4.3 ± 9.0) ($P < 0.0001$; **Fig. 3**). The Δ25(OH)D did not differ among any of the groups that received vitamin D ($P = 0.62$) and did not differ between genders in those groups ($P = 0.53$). It was not correlated with BMI ($r = 0.09$; $P = 0.49$), but in groups that received vitamin D, it was negatively correlated with the serum 25(OH)D concentration at baseline ($r = -0.38$; $P = 0.003$).

Secondary outcome measures. Because both types of fortified cheeses and both routes of vitamin D supplementation exhibited equal bioavailability, data were recoded into 3 groups (i.e. fortified cheese, vitamin D supplement, and placebo) for the assessment of secondary outcome measures. The changes (from baseline to 8 wk) in biochemical variables in these groups are summarized (**Table 2**). Serum creatinine, ALP, and CTX and urine biochemical measures did not differ from baseline to 8 wk within or between any of the groups ($P > 0.05$). Serum phosphate decreased from baseline to 8 wk in the placebo and vitamin D supplement groups ($P < 0.05$), but not in the fortified cheese group ($P = 0.29$); however, the changes in serum phosphate did not differ between any of the groups ($P = 0.59$). Serum PTH decreased by 25% in the fortified cheese group ($P = 0.003$) and by 21% in the vitamin D supplement group ($P = 0.012$) compared with baseline values (Table 2). There was no change in PTH concentrations in the placebo group ($P = 0.75$). At the final visit, serum 25(OH)D and PTH values were negatively correlated ($r = -0.34$; $P = 0.002$). Serum calcium decreased from baseline to 8 wk in the placebo group ($P = 0.004$) but remained unchanged in the fortified cheese and vitamin D supplement groups ($P > 0.5$). The change in serum calcium in the fortified cheese group was not different from the change in the vitamin D supplement group ($P = 0.82$), but it was greater than the change in the placebo group ($P = 0.02$); however, the latter difference was due to a decrease in the placebo group, not a change in the treatment group. In all subjects ($n = 80$), serum calcium concentrations remained within the normal reference range (2.2–2.6 mmol/L) at each time point. No subject developed hypercalcemia (serum calcium > 2.75 mmol/L) or hypercalciuria (millimolar ratio of urine calcium:urine creatinine > 1). None of the subjects reported any adverse effects.

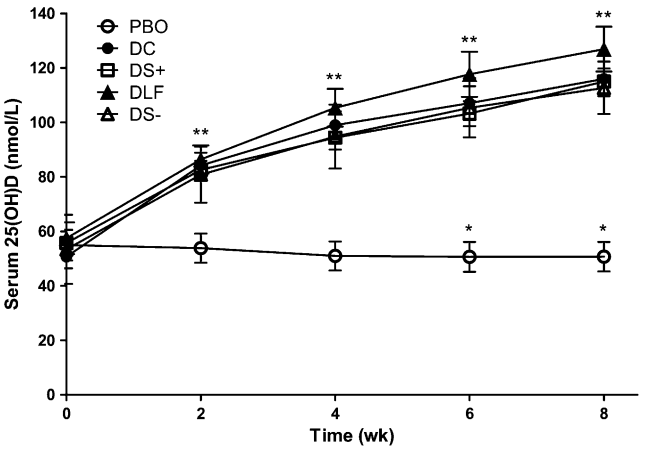

FIGURE 2 Serum 25(OH)D concentrations in subjects consuming fortified cheese, a vitamin D supplement with or without food, or placebo for 8 wk. Values are means ± SEM, $n = 10$ (DLF, DS−) or 20 (DC, DS+, PBO). **Different from baseline, $P < 0.005$, and from the previous time point, $P < 0.05$, in all vitamin D-treated groups; *different from baseline, $P < 0.05$, in the placebo group).

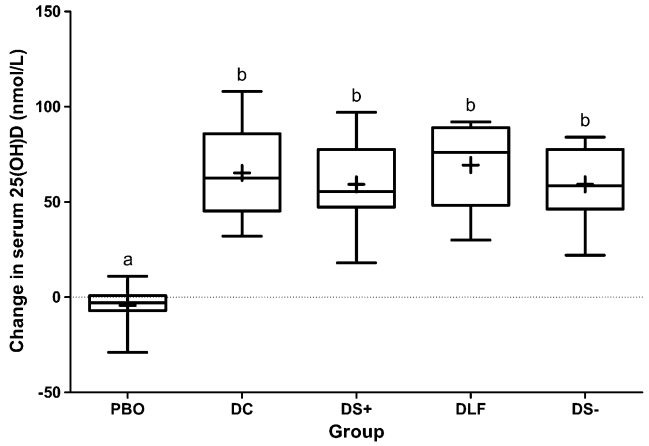

FIGURE 3 Changes in serum 25(OH)D concentrations in subjects consuming fortified cheese, a vitamin D supplement with or without food, or placebo for 8 wk. Boxes represent the range of the central 50% of the sample population, the whiskers show the highest and lowest values, the line indicates the median, and the + represents the mean, $n = 10$ (DLF, DS−) or 20 (DC, DS+, PBO). Means without a common letter differ, $P < 0.05$.

Discussion

Our results show that vitamin D is bioavailable from fortified cheddar and low-fat cheese. The change in 25(OH)D from baseline to 8 wk was significantly greater in the subjects consuming fortified cheddar or low-fat cheese compared with those consuming the placebo. Furthermore, the fortified cheeses produced a change in 25(OH)D that did not differ from vitamin D supplement, demonstrating that vitamin D is equally bioavailable from fortified cheese and supplement. These findings are similar to those of Natri et al. (52), who showed that bread fortified with cholecalciferol increases serum 25(OH)D as effectively as a supplement.

We also found that differences in the fat content of the fortified cheeses did not affect vitamin D bioavailability. The increases in serum 25(OH)D did not differ between subjects consuming fortified regular-fat cheddar cheese, comprised of ~33% fat, or fortified low-fat cheese, comprised of ~7% fat. These observations are consistent with those of Tangpricha et al. (53) in which peak serum vitamin D concentrations did not differ significantly after the ingestion of vitamin D in whole milk, skim milk, or corn oil on toast. We also did not observe any significant changes in serum 25(OH)D between subjects consuming vitamin D supplemented with food and those consuming it without food. We conclude that taking a vitamin D supplement together with food also does not alter its bioavailability.

All of the differences in the mean change in 25(OH)D among vitamin D-treated groups were less than one-half of what the present study had been powered to detect. For example, the largest difference in 25(OH)D response between treatment groups was 10 nmol/L (DLF compared with DS−). If this difference were true, an appropriately powered experiment to confirm this 10-nmol/L difference would require 2 groups of 71 study subjects (50). An efficacy difference requiring this large a sample size would not be clinically relevant. Taken together, our results demonstrate that vitamin D bioavailability is equivalent from the various methods for providing vitamin D that we tested.

Serum and urine calcium concentrations are the classic safety indices for vitamin D excess and those remained unchanged by the intervention. None of the subjects reported any untoward side effects. Ingestion of vitamin D from fortified cheese and vitamin D supplements lowered PTH concentrations over the period of 8 wk. Other markers of bone and mineral metabolism, including serum and urine phosphate, serum ALP, and serum CTX were not affected by the consumption of fortified cheese or vitamin D supplement. These bone variables should be expected to improve with vitamin D supplementation, but this would be more likely to occur in an older population than the one we studied. The results confirm previous reports of the safety and efficacy of vitamin D supplementation at doses ≥4000 IU/d (14,54,55).

We designed our study so that the vitamin D would be consumed once per week instead of daily, because the expected improvement in adherence to the protocol (56) would minimize variability. We achieved a 98% adherence to dose intake. Our weekly dosing protocol delivered 28,000 IU of cholecalciferol per weekly serving, which is equivalent to a daily vitamin D intake of 4000 IU. Weekly dosing with vitamin D is consistent with basic pharmacology. Suitable dosing intervals usually approximate the half-life of the administered agent (57). Depending on how it is characterized (tracer kinetics or decline upon discontinuation of input), serum 25(OH)D has a half-life of between 2 wk and 2 mo (48). Thus, weekly supplementation with cholecalciferol is effective and appropriate (48).

TABLE 2 Biochemical responses in subjects consuming fortified cheese, vitamin D supplement, or placebo over 8 wk[1]

	Fortified cheese, $n = 30$		Supplement, $n = 30$		Placebo, $n = 20$	
	Baseline	Change	Baseline	Change	Baseline	Change
Serum calcium, *mmol/L*	2.37 ± 0.07	0 ± 0.08[a]	2.38 ± 0.07	−0.01 ± 0.12[ab]	2.42 ± 0.07	−0.08 ± 0.11[†,b]
Urine calcium, *mmol/L*	2.53 ± 2.24	0.35 ± 2.74[a]	3.44 ± 2.15	−0.10 ± 2.66[a]	3.86 ± 3.52	−0.27 ± 3.04[a]
Serum creatinine, *μmol/L*	71.43 ± 9.48	1.0 ± 6.59[a]	74.57 ± 11.46	−0.60 ± 6.71[a]	73.2 ± 15.82	0.05 ± 5.79[a]
Urine creatinine, *mmol/L*	10.28 ± 6.96	0.20 ± 7.17[a]	13.76 ± 7.93	−1.46 ± 7.18[a]	10.43 ± 6.45	1.16 ± 8.66[a]
Urinary calcium excretion, *mmol:mmol creatinine*	0.30 ± 0.26	0.05 ± 0.23[a]	0.32 ± 0.22	0.02 ± 0.21[a]	0.38 ± 0.22	−0.03 ± 0.18[a]
Serum phosphate, *mmol/L*	1.22 ± 0.16	−0.04 ± 0.19[a]	1.17 ± 0.13	−0.06 ± 0.14[†,a]	1.22 ± 0.15	−0.09 ± 0.15[†,a]
Urine phosphate, *mmol/L*	15.45 ± 12.08	−3.07 ± 11.67[a]	16.32 ± 12.69	−3.79 ± 13.13[a]	15.34 ± 11.13	−1.87 ± 12.40[a]
Serum PTH, *pmol/L*	4.71 ± 2.78	−1.17 ± 1.97[†,a]	4.30 ± 1.89	−0.90 ± 1.83[†,a]	4.39 ± 1.99	0.22 ± 2.91[a]
Serum ALP, *U/L*	64.53 ± 18.15	0.53 ± 6.23[a]	64.50 ± 18.11	0.10 ± 7.25[a]	67.20 ± 16.75	2.05 ± 7.29[a]
Serum CTX, *μg/L*	0.27 ± 0.15	0.02 ± 0.20[a]	0.23 ± 0.15	−0.01 ± 0.10[a]	0.31 ± 0.20	−0.01 ± 0.11[a]

[1] Values are means ± SD. Mean changes in a row with superscripts without a common letter differ, $P < 0.05$. [†]Different from baseline, $P < 0.05$.

The amount of vitamin D fortification we used for this study protocol was substantially higher than what would be added to foods. A more conventional dose would have been 100–400 IU vitamin D/30–50 g serving of cheese, the usual fortification level permitted by North American food regulations. However, Johnson et al. (58) demonstrated that in elderly subjects, daily consumption of fortified processed cheese containing 600 IU of vitamin D for 2 mo did not produce a detectable increase in serum 25(OH)D. Their results were surprising, because they provided the adequate intake of vitamin D for the elderly (> 70 y) (49), which should have produced a measurable change, at least in theory. Nevertheless, their findings are consistent with previous reports that suggest that 600 IU vitamin D/d is insufficient to increase serum 25(OH)D in the elderly (1,54,55). In contrast to Johnson et al. (58), we studied a younger population, fortified different types of cheeses, and administered a higher dose of cholecalciferol (equivalent to 4000 IU/d). The higher vitamin D intake was chosen to produce a rise in serum 25(OH)D that would increase the statistical power to detect possible differences in bioavailability among our treatment groups.

Ingestion of 4000 IU/d cholecalciferol from fortified cheese or supplement safely increased the vitamin D status of our subjects, ensuring a serum 25(OH)D concentration of ≥75 nmol/L in over 90% of the vitamin D-treated subjects, and significantly decreased mean PTH levels. We would not expect a lower dose to produce as effective a rise in 25(OH)D as that here. Even so, a daily dose of 400 IU vitamin D can improve vitamin D status, as was shown with vitamin D-fortified bread (52).

In conclusion, we found that fortified cheese enhances vitamin D status as effectively as a supplement. The extension of vitamin D fortification to cheese and other such foods that are widely distributed, frequently consumed, and exhibit good bioavailability is an inexpensive and effective approach for increasing the availability of vitamin D in the diet. This will improve vitamin D intakes in the population and could help to bring about the public health benefits that many experts are now proposing may result from a greater consumption of vitamin D.

Literature Cited

1. Vieth R, Cole DE, Hawker GA, Trang HM, Rubin LA. Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it. Eur J Clin Nutr. 2001;55: 1091–7.
2. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266–81.
3. Isaia G, Giorgino R, Rini GB, Bevilacqua M, Maugeri D, Adami S. Prevalence of hypovitaminosis D in elderly women in Italy: clinical consequences and risk factors. Osteoporos Int. 2003;14:577–82.
4. Moussavi M, Heidarpour R, Aminorroaya A, Pournaghshband Z, Amini M. Prevalence of vitamin D deficiency in Isfahani high school students in 2004. Horm Res. 2005;64:144–8.
5. Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, Allen C, Doughertly C, Gunter EW, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr. 2002;76:187–92.
6. Rucker D, Allan JA, Fick GH, Hanley DA. Vitamin D insufficiency in a population of healthy western Canadians. CMAJ. 2002;166:1517–24.
7. Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living healthy young adults. Am J Med. 2002;112:659–62.
8. Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, Vamvakas EC, Dick IM, Prince RL, et al. Hypovitaminosis D in medical inpatients. N Engl J Med. 1998;338:777–83.
9. Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). Cancer Causes Control. 2000;11:847–52.
10. Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). Cancer Causes Control. 2005; 16:83–95.
11. Gorham ED, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M, Newmark HL, Giovannucci E, Wei M, et al. Vitamin D and prevention of colorectal cancer. J Steroid Biochem Mol Biol. 2005;97:179–94.
12. Holick MF. Vitamin D: its role in cancer prevention and treatment. Prog Biophys Mol Biol. 2006;92:49–59.
13. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr. 2007;85:1586–91.
14. Kimball SM, Ursell MR, O'connor P, Vieth R. Safety of vitamin D3 in adults with multiple sclerosis. Am J Clin Nutr. 2007;86:645–51.
15. Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. J Neuroimmunol. 2003;134:128–32.
16. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA. 2006;296:2832–8.
17. Casteels K, Waer M, Bouillon R, Depovere J, Valckx D, Laureys J, Mathieu C. 1,25-Dihydroxyvitamin D3 restores sensitivity to cyclophosphamide-induced apoptosis in non-obese diabetic (NOD) mice and protects against diabetes. Clin Exp Immunol. 1998;112:181–7.
18. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr. 2004;79:820–5.
19. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet. 2001;358:1500–3.
20. Lind L, Hanni A, Lithell H, Hvarfner A, Sorensen OH, Ljunghall S. Vitamin D is related to blood pressure and other cardiovascular risk factors in middle-aged men. Am J Hypertens. 1995;8:894–901.
21. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, et al. Deficiency and risk of cardiovascular disease. Circulation. 2008;117:503–11.
22. Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. Arthritis Rheum. 2004;50:72–7.
23. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006;311: 1770–3.
24. Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, Packe GE, Davidson RN, Eldridge SM, et al. A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med. 2007;176:208–13.
25. Zasloff M. Fighting infections with vitamin D. Nat Med. 2006;12: 388–90.
26. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr. 2006;84:18–28.
27. Calvo MS, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. Am J Clin Nutr. 2004;80:S1710–6.
28. Moore C, Murphy MM, Keast DR, Holick MF. Vitamin D intake in the United States. J Am Diet Assoc. 2004;104:980–3.
29. Whiting SJ, Green TJ, Calvo MS. Vitamin D intakes in North America and Asia-Pacific countries are not sufficient to prevent vitamin D insufficiency. J Steroid Biochem Mol Biol. 2007;103:626–30.
30. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab. 1988;67:373–8.
31. Matsuoka LY, Wortsman J, Dannenberg MJ, Hollis BW, Lu Z, Holick MF. Clothing prevents ultraviolet-B radiation-dependent photosynthesis of vitamin D3. J Clin Endocrinol Metab. 1992;75:1099–103.
32. Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA, Holick MF. Sunscreens suppress cutaneous vitamin D3 synthesis. J Clin Endocrinol Metab. 1987;64:1165–8.
33. MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest. 1985;76:1536–8.

34. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. Lancet. 1982;1:74–6.
35. Health Canada. Consolidation of the Food and Drugs Act and the Food and Drug Regulations. 2007 [cited 2008 Jan 10]. Available from: http://www.hc-sc.gc.ca/fn-an/legislation/acts-lois/fdr-rad/index_e.html.
36. US FDA. Direct food substances affirmed as generally recognized as safe. 2008 [cited 2008 Jan 10]. Available from: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=184
37. Chen TC, Shao A, Heath H III, Holick MF. An update on the vitamin D content of fortified milk from the United States and Canada. N Engl J Med. 1993;329:1507.
38. Faulkner H, Hussein A, Foran M, Szijarto L. A survey of vitamin A and D contents of fortified fluid milk in Ontario. J Dairy Sci. 2000; 83:1210–6.
39. Murphy SC, Whited LJ, Rosenberry LC, Hammond BH, Bandler DK, Boor KJ. Fluid milk vitamin fortification compliance in New York State. J Dairy Sci. 2001;84:2813–20.
40. Putnam J, Allshouse J. Trends in U.S. per capita consumption of dairy products, 1909–2001. Amber Waves. 2003;1:12–3.
41. Carroccio A, Montalto G, Cavera G, Notarbatolo A. Lactose intolerance and self-reported milk intolerance: relationship with lactose maldigestion and nutrient intake. Lactase Deficiency Study Group. J Am Coll Nutr. 1998;17:631–6.
42. Vesa TH, Marteau P, Korpela R. Lactose intolerance. J Am Coll Nutr. 2000;19:S165–75.
43. Holmberg I, Aksnes L, Berlin T, Lindback B, Zemgals J, Lindeke B. Absorption of a pharmacological dose of vitamin D3 from two different lipid vehicles in man: comparison of peanut oil and a medium chain triglyceride. Biopharm Drug Dispos. 1990;11:807–15.
44. Sorensen HH. The world market for cheese. 5th ed. Brussels: International Dairy Federation; 2001
45. Kazmi SA, Vieth R, Rousseau D. Vitamin D fortification and quantification in processed dairy products. Int Dairy J. 2007;17:753–9.
46. Forrest SA, Yada RY, Rousseau D. Interactions of vitamin D3 with bovine beta-lactoglobulin A and beta-casein. J Agric Food Chem. 2005;53:8003–9.
47. Wang Q, Allen JC, Swaisgood HE. Binding of vitamin D and cholesterol to beta-lactoglobulin. J Dairy Sci. 1997;80:1054–9.
48. Vieth R. The pharmacology of vitamin D, including fortification strategies. In: Feldman D, Glorieux FH, Pike JW, editors. Vitamin D. New York: Academic Press; 2005. p. 995–1015.
49. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary reference intakes: calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy Press; 1997.
50. Schoenfeld D. Statistical considerations for clinical trials and scientific experiments. Harvard School of Public Health; 2007 [cited 2008 Jan 12]. Available from: http://hedwig.mgh.harvard.edu/sample_size/size.html
51. Canadian Nutrient File. Health Canada 2008; 2007b. Available from: http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/index_e.html
52. Natri AM, Salo P, Vikstedt T, Palssa A, Huttunen M, Kärkkäinen MU, Salovaara H, Piironen V, Jakobsen J, et al. Bread fortified with cholecalciferol increases the serum 25-hydroxyvitamin D concentration in women as effectively as a cholecalciferol supplement. J Nutr. 2006; 136:123–7.
53. Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA, Holick MF. Fortification of orange juice with vitamin D: a novel approach for enhancing vitamin D nutritional health. Am J Clin Nutr. 2003;77: 1478–83.
54. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr. 2003;77:204–10.
55. Vieth R, Chan PC, MacFarlane GD. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. Am J Clin Nutr. 2001;73:288–94.
56. Kruk ME, Schwalbe N. The relation between intermittent dosing and adherence: preliminary insights. Clin Ther. 2006;28:1989–95.
57. Brunton LL, Parker KL, Lazo JS. Goodman and Gillman's the pharmacological basis of therapeutics. New York: McGraw-Hill; 2006.
58. Johnson JL, Mistry VV, Vukovich MD, Hogie-Lorenzen T, Hollis BW, Specker BL. Bioavailability of vitamin D from fortified process cheese and effects on vitamin D status in the elderly. J Dairy Sci. 2005;88: 2295–301.