Consumption of Yogurts Fortified in Vitamin D and Calcium Reduces Serum Parathyroid Hormone and Markers of Bone Resorption: A Double-Blind Randomized Controlled Trial in Institutionalized Elderly Women

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Context: Nutritional prevention of bone deterioration with fortified foods seems particularly suitable in institutionalized elderly women at risk of vitamin D deficiency, secondary hyperparathyroidism, increased bone resorption, and osteoporotic fracture.

Objective: The objective was to evaluate whether fortification of yogurts with vitamin D and calcium exerts an additional lowering effect on serum PTH and bone resorption markers as compared with isocaloric and isoprotein dairy products in elderly women.

Design: A randomized double-blind controlled-trial, 56-day intervention was conducted in institutionalized women (mean age 85.5 years) consuming 2 125-g servings of either vitamin D- and calcium-fortified yogurt (FY) at supplemental levels of 10 μg/d vitamin D_3 and 800 mg/d calcium or nonfortified control yogurt (CY) providing 280 mg/d calcium.

Main Outcomes: The endpoints were serum changes from baseline (day 0) to day 28 and day 56 in 25-hydroxyvitamin-D (25OHD), PTH, and bone resorption markers tartrate-resistant acid phosphatase isoform-5b (TRAP5b), the primary outcome, and carboxyl-terminal cross-linked telopeptide of type I collagen (CTX).

Results: At day 56, serum 25OHD increased (mean $\pm$ SEM) by 25.3 $\pm$ 1.8 vs 5.2 $\pm$ 2.5 nmol/L in FY (n = 29) and CY (n = 27), respectively ($P < .0001$). The corresponding changes in PTH were $-28.6\% \pm 7.2\%$ vs $-8.0\% \pm 4.3\%$ ($P = .0003$); in TRAP5b, $-21.9\% \pm 4.3\%$ vs $3.0\% \pm 3.2\%$ ($P < .0001$); and in CTX, $-11.0\% \pm 9.7\%$ vs $-3.0\% \pm 4.1\%$ ($P = .0146$), in FY and CY, respectively. At day 28, these differences were less pronounced but already significant for 25OHD, PTH, and TRAP5b.

Conclusions: This study in institutionalized elderly at high risk for osteoporotic fracture suggests that fortification of dairy products with vitamin D_3 and calcium provides a greater prevention of accelerated bone resorption as compared with nonfortified equivalent foods. **(*J Clin Endocrinol Metab* 98: 2915–2921, 2013)**

In the elderly, there is a need to prevent the accelerated loss of bone mass with its associated microstructure deterioration and thereby to reduce the risk of fragility fracture. Institutionalized women are at particularly high risk of osteoporotic fractures. An elevated incidence of hip fracture has been documented in nursing home residents (1). This severe consequence of osteoporosis is frequently associated with inadequate nutrition (2). Therefore, with

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Abbreviations: BAP, bone alkaline phosphatase; CTX, carboxyl-terminal cross-linked telopeptide of type I collagen; CY, control (nonfortified) yogurt; DRI, Dietary Reference Intake; FY, vitamin D- and calcium-fortified yogurt; 25OHD, 25-hydroxyvitamin D; P1NP, amino-terminal propeptide of type I procollagen; TRAP5b, tartrate-resistant acid phosphatase isoform-5b.

or without the use of pharmacological agents known to reduce the incidence of osteoporotic fracture, improving the nutritional conditions of the elderly represents a reasonable option because the health benefit extends beyond the musculoskeletal system.

Vitamin D, calcium, and proteins are important nutrients contributing to reduce the negative bone balance often observed in the elderly. Evidence for a beneficial influence of these 3 nutrients has been documented on bone remodeling, bone mineral mass, and fracture risk (3–10).

Dairy foods provide both calcium and proteins, but their usual content of vitamin D is low, notably insufficient to meet the Dietary Reference Intake (DRI) (11). Consequently, several countries fostered the fortification of foods, particularly milk and other dairy products, with vitamin D (12). In addition to vitamin D, supplementing dairy foods with calcium may appear less warranted as far as the whole population is considered, i.e., for all age and sex groups (13). However, such a supplementation strategy is of interest for subpopulations with low spontaneous intake of calcium. Several surveys brought to light a progressive decrement in calcium intake with aging (2, 14). In the elderly, a calcium intake lower than the recommended supply can be particularly detrimental because the capacity of the intestine to absorb calcium is diminished (14). Furthermore, taking into account the physiology of the skeletal mineral metabolism, it appears obvious that a certain supply of calcium has to be associated with vitamin D to observe a beneficial impact on bone integrity and reduce the risk of hip or other fragility fracture in the elderly (7).

Food fortification systems can differ not only in terms of bioavailability (15) but, more importantly, also on their impact on bone metabolism. Several studies have demonstrated the favorable effects of vitamin D- and calcium-fortified dairy foods on bone metabolism (8, 16, 17). However, to our knowledge, whether dairy products fortified with vitamin D and calcium exert an effect on bone metabolism superior to the nonfortified foods has not been documented in clinical trials specially designed to examine this question.

In the present study carried out in institutionalized elderly women, we report a randomized double-blind controlled trial aimed at testing the hypothesis that yogurts fortified with vitamin D and calcium induce greater inhibitory effects on secondary hyperparathyroidism and bone resorption markers than the identical nonfortified dairy products.

Subjects and Methods

Subjects

A total of 89 institutionalized women ≥60 years old living in 10 French nursing homes were assessed for eligibility. The nursing homes were selected because they provided both medical supervision and adequate monitoring of food consumption. The recruitment for eligibility started after the ethics committee approved the study protocol. At recruitment time, the subjects had been living in nursing homes from a mean stay of 3.3 years (median 2.1 years). Of this initial group, 60 women responded to the inclusion criteria with low vitamin D status (25-hydroxyvitamin-D [25OHD] level >10 and ≤30 nmol/L) and moderately elevated serum PTH level (>46 and <150 ng/L). They were enrolled between December 20, 2010, and February 10, 2011, all having given their informed consent. The end of the intervention took place on March 25, 2011, with the day-56 visit of the latest enrolled participant. The age ranged from 68 to 99 years with mean ± SD age 85.5 ± 6.6 years.

Other inclusion/exclusion criteria were Mini Nutritional Assessment score >20 (18); no consumption during the last 6 months of food enriched with vitamin D and/or calcium; no treatment during the last 6 months for osteoporosis or other bone diseases, including pharmaceutical agents such as bisphosphonates, raloxifen, teriparatide, strontium ranelate, and denosumab; no glucocorticoid therapy; no disease with poor prognosis at short term; no participation in a clinical trial during the 3 months preceding the entry into the study; no osteoporotic fracture during the year preceding the study; no confinement to bed; and no consumption of meals in private room.

Design and conducted trial

It was a double-blind randomized study in which subjects were allocated to consume daily 2 yogurts of 125 g each, providing either 10 μg (400 IU) vitamin D_3 and 800 mg calcium (fortified yogurt [FY]) or 0 μg supplemental vitamin D_3 and 280 mg calcium (control yogurt [CY]) during 8 weeks. To fortify one type of yogurt (FY), vitamin D_3 was added in powder form and calcium (+260 mg/serving) as citrate salt. Both vitamin D_3 and calcium citrate were mixed with fruits (strawberry or apricot) used as flavoring components to the 2 types (FY and CY) of yogurts. Before the fortification process, both FY and CY contained 140 mg milk calcium per serving. Both types of yogurts were provided by Yoplait France.

A random number table was used to determine the allocation sequence. The random allocation was centralized, computer-generated, and stratified by investigating center. Presenting the 2 types of yogurts in identical packs designated according to A and B codes insured the blinding. The identification of the 2 types of yogurts, i.e., FY or CY corresponding to the 2 codes, remained unknown by all persons involved in the trial, including the patients, the nursing staff, and the investigators, until the statistical analysis was fully completed. The 2 servings of 125 g of either type of yogurt daily provided similar amounts of proteins (FY, 7.8 g; CY, 8.2 g), energy (FY, 221 kcal; CY, 248 kcal), lipids (FY, 6.5 g; CY, 6.5 g), and carbohydrates (FY, 33 g; CY, 39 g). One was consumed at lunch time and the other at dinner instead of equivalent dairy foods (e.g., yogurts or creamy desserts).

The primary endpoint was changes after 4 and 8 weeks in serum tartrate-resistant acid phosphatase isoform-5b (TRAP5b), selected as a sensitive marker of osteoclastic bone resorption. The secondary endpoints were the changes in serum 25OHD, PTH, carboxyl-terminal cross-linked telopeptide of type I collagen (CTX), and IGF-I.

Clinical assessment

Fracture risk evaluation

The 10-year risk of hip and major osteoporotic fracture of each participant was computed by using the probability model FRAX (19) calibrated to the French population. The prediction of fracture with the use of clinical risk factors alone in FRAX, ie, without a dual-energy x-ray absorptiometry test, is comparable to the bone mineral density measurement alone (20).

Dietary survey at baseline and during the intervention

Calcium, proteins, and energy intake were assessed at baseline (day 0) and monitored during the intervention for the 2 tested yogurts and at day 28 from all other sources. Food consumption was recorded by the nursing staff of each participating home using 2 3-day dietary diaries both at the onset and at day 28 of the intervention. The protein and energy consumption, as analyzed from the diaries, are reported in Results. Calcium intake was recorded using a food frequency questionnaire including 20 foods containing calcium and frequently consumed in France, inquiring on serving size and validated by a 5-day dietary diary (21).

Sun exposure

The whole period of intervention was essentially run during wintertime, limiting the opportunity of sun exposure. Nevertheless, in the follow-up record, the staff of each nursing home was responsible for indicating whether the participants spent daily more than 20 minutes with their uncovered arms exposed to the sun.

Adherence and acceptability

Observance and persistence were daily monitored throughout the intervention period. The acceptability of the 2 types of yogurts, FY and CY, was evaluated on a score scale from 0 (worse) to 10 (best) at days 28 and 56, inquiring about the following satisfactory criteria: 1) tastiness, 2) portion size suited to subjects' appetite; and 3) absence of progressive weariness during the food testing period.

Biochemical analysis

The blood samples were collected in the morning after an overnight fast and stored at −70°C until analysis. Serum calcium and inorganic phosphate were measured by colorimetry (Roche Diagnostics, Rotkreuz, Switzerland). Serum 25OHD, PTH, CTX, TRAP5b, bone alkaline phosphatase (BAP), amino-terminal propeptide of type I procollagen (P1NP), and IGF-I were measured by ELISA on the Bio-Rad Microtech microplate reader (Hercules, California). The following immunoassay kits were used: 25OHD (Immunodiagnostic Systems, Fountain Hills, Arizona), PTH (human bioactive PTH Elisa kit; Immunotopics, San Clemente, California), CTX (Immunodiagnostic Systems), TRAP5b (bone TRAP assay; Immunodiagnostic Systems), BAP (Ostease BAP, immunoenzymetric assay; Immunodiagnostic Systems), P1NP (human P1NP immunoassay kit; Cusabio Biotech Co, Newark, Delaware), and IGF-I (IGF-I Elisa; Immunodiagnostic Systems). The intra- and interassay variations, including the variations for TRAP5b, were less than 6.0% and 8.0%, respectively.

Statistical analysis

Determination of the sample size was estimated from the effects on changes in serum TRAP5b. This bone resorption marker was the primary outcome of the trial. It was expected that a difference of 10% in serum TRAP5b changes would be detected between the 2 groups with a power of 80% and 2-sided α of 0.05. Such a 10% difference in change in TRAP5b required a sample of 15 participants per group, taking into account a SD of interindividual difference of 9.3% (22). A total of 32 and 28 subjects in the FY and CY groups, respectively, were eventually enrolled into the study and randomized. One woman randomized in the CY group did not start the study, withholding any blood sampling. Three women randomized in the FY group did not pursue the study for the following reasons: one experienced a hip fracture after day 0, one was hospitalized after day 28 and died, and one eventually withdrew her informed consent after day 28.

The data are expressed as mean ± SEM. Differences between quantitative data measured at day 0 and changes from day 0 to day 28 and from day 0 to day 56 were evaluated by the Mann-Whitney *U* test. The differences in time courses (from day 0 to day 28 and to day 56), type of yogurt, and interactions between time course and type of yogurt for serum 25OHD, PTH, CTX, TRAP5b, BAP, P1NP, and IGF-I were evaluated by repeated-measures ANOVA with adjustment by the Tukey's test. The χ^2 or Fisher tests were used for the qualitative response, predefined as a decline in serum TRAP5b ≥10% after 28 days of intervention. Statistical analysis of the data was conducted by using SAS version 9.2 (SAS Institute Inc, Cary, North Carolina). The *P* value <.05 was considered statistically significant.

Results

Baseline characteristics

At baseline, anthropometric characteristics and dietary intake did not differ between the FY and CY groups (Table 1). No past prevalence of pathologic conditions or medications for extraskeletal diseases differed between the 2 groups (data not shown). Previous fractures did not significantly differ between the 2 groups (FY, n = 8; CY, n = 6). Using the FRAX questionnaire, the estimated 10-year risk of major osteoporotic fractures, or hip fractures alone, was, not surprisingly, high in these institutionalized women in their mid-80s (Table 1). Nevertheless, it did not differ between the FY and the CY groups (Table 1). Similarly, there was no difference between the 2 groups in the baseline serum biochemical variables (Table 2).

Quantitative response to fortified foods

The time-dependent changes in serum 25OHD were much more pronounced in the FY than in the CY groups (Table 2). After 28 days, it was (mean ± SEM) 20.4 ± 2.6 and 4.0 ± 2.4 nmol/L ($P < .0001$) in FY and CY, respectively. From day 28 to day 56, the changes in serum 25OHD was 4.7 ± 1.9 and 0.5 ± 05 nmol/L ($P = .0659$) in FY and CY, respectively. The greater rise serum 25OHD in FY than CY was associated with a greater time-dependent decrease in

Table 1. Participants' Characteristics at Baseline[a]

	CY Group (n = 27)	FY Group (n = 32)	*P*
Age, y	85.1 (1.3)	85.8 (1.2)	.610
Standing height, cm	157.7 (1.6)	155.1 (1.1)	.195
Body weight, kg	66.6 (3.1)	63.2 (1.9)	.532
BMI, kg/m^2	26.6 (1.0)	26.2 (0.7)	.945
MNA score	24.1 (0.4)	24.6 (0.3)	.389
Calcium intake, mg/d[b]	745 (44)	796 (57)	.626
Protein intake, g/d[b]	60.4 (3.9)	61.3 (3.1)	.734
Energy intake, kcal/d[b]	1905 (69)	1857 (69)	.451
10-y risk of major fractures, %[c]	25.4 (1.8)	22.9 (1.3)	.295
10-y risk of hip fracture, %[c]	12.9 (1.2)	11.3 (0.8)	.393

Abbreviations: BMI, body mass index; MNA, Mini Nutritional Assessment.

[a] Values of control and treated groups are means (SEM). *P* values for differences between CY and FY group were calculated by Mann-Whitney *U* test.

[b] Calculated from FFQ at day 0.

[c] Calculated from the World Health Organization FRAX rating scale.

serum PTH, TRAP5b, the primary endpoint of the study, and CTX, the other measured bone resorption marker (Table 2). For 25OHD, PTH, and TRAP5b, the interaction between the time course evolution and the type of yogurt markedly differed between the FY and the CY groups (Table 2). Such an interaction difference was close to statistical significance ($P = .0785$) for CTX (Table 2).

As illustrated in Figure 1, the percent changes in serum PTH, TRAP5b, and CTX were greater in the FY than in the CY groups. These differences were already detectable at day 28 but were even more pronounced at day 56 (Figure 1).

Concerning the biochemical markers of bone formation, Table 2 indicates a slight difference in changes in serum BAP from day 0 to day 56 (FY, −1.4 μg/L; CY, −0.1 μg/L, $P = .0766$), but not in serum P1NP. No significant change in serum IGF-I was recorded (Table 2).

Qualitative response to fortified food

After 28 days of intervention, a decline ≥10% in serum TRAP5b was observed in 48.4% and 18.5% (χ^2 test, $P = .017$) of the participants in the FY and CY group, respectively.

Other monitored variables

The mean dietary calcium record at baseline (Table 1) corresponded to 66% and 62% of DRI (1200 mg/d), in the FY and CY groups, respectively. During the intervention, calcium intake rose up to 1450 mg/d (~120% of DRI) and 925 mg/d (~77% of DRI) in the FY and CY groups, respectively. Meanwhile, the consumption of energy (FY, 1956 ± 82 kcal/d; CY, 1934 ± 77 kcal/d) and proteins (FY, 63.9 ± 3.4 g/d; CY, 62.7 ± 4.1 g/d) did not differ between the 2 groups. As compared with the baseline level indicated in Table 1, the protein intake increased only by 4.2% ± 1.3% and 3.8% ± 1.2% in the FY and CY groups, respectively. Body weight (mean ± SEM) remained stable from day 0 to day 56: +0.03 ± 0.34 kg (n = 29) and −0.11 ± 0.36 kg (n = 27) in the FY and CY groups, respectively.

Table 2. Participants' Serum Biochemistry at Baseline (Day 0) and After 4 (Day 28) and 8 (Day 56) Weeks of CY or FY Consumption[a]

	CY Group				FY Group							
	d 0, n = 27	d 28, n = 27	d 56, n = 27	d 56 − d, 0, n = 27	d 0, n = 31	d 28, n = 31	d 56, n = 29	d 56 − d 0, n = 29	*P*[b]	*P*[c]	*P*[d]	*P*[e]
Ca, mmol/L	2.31 (0.02)	ND	2.29 (0.02)	−0.03 (0.02)	2.31 (0.02)	ND	2.31 (0.02)	0.00 (0.01)	.271	NA	NA	NA
Pi, mmol/L	1.14 (0.03)	ND	1.24 (0.03)	0.10 (0.02)	1.17 (0.03)	ND	1.25 (0.03)	0.08 (0.03)	.763	NA	NA	NA
Albumin, g/L	35.4 (0.8)	ND	35.3 (0.94)	−0.16 (0.75)	37.0 (0.6)	ND	36.6 (0.83)	−0.328 (0.60)	.861	NA	NA	NA
Prealbumin, g/L	0.23 (0.01)	ND	0.22 (0.01)	−0.011 (0.008)	0.23 (0.01)	ND	0.24 (0.01)	0.003 (0.006)	.143	NA	NA	NA
25OHD, nmol/L	16.2 (0.6)	20.1 (2.6)	21.4 (2.7)	5.2 (2.5)	19.2 (1.2)	39.5 (3.3)	44.6 (2.5)	25.3 (1.8)	<.0001	<.0001	<.0001	<.0001
PTH, ng/L	53.4 (6.3)	48.4 (5.1)	46.3 (4.6)	−7.1 (2.9)	60.8 (7.1)	42.1 (3.2)	32.4 (1.8)	−28.6 (7.2)	.0035	<.0001	.5134	<.0001
CTX, μg/L	0.53 (0.06)	0.50 (0.05)	0.49 (0.05)	−0.033 (0.023)	0.43 (0.03)	0.40 (0.04)	0.35 (0.05)	−0.073 (0.044)	.055	.0061	.0307	.0785
TRAP5b, U/L	3.66 (0.20)	3.56 (0.13)	3.65 (0.15)	−0.01 (0.13)	3.89 (0.27)	3.39 (0.25)	2.82 (0.17)	−1.06 (0.26)	.0209	.0003	.0222	<.0001
BAP, μg/L	20.2 (1.2)	19.4 (1.3)	20.1 (1.8)	−0.1 (1.2)	18.9 (1.2)	18.9 (1.6)	17.5 (1.8)	−1.4 (1.4)	.0766	.0200	.0229	.2047
P1NP, μg/L	12.1 (0.5)	12.3 (0.6)	12.4 (0.5)	0.3 (0.3)	11.6 (0.4)	11.6 (0.4)	12.1 (0.4)	0.5 (0.2)	.5922	.0709	.4425	.5661
IGF-I, μg/L	71.1 (4.8)	70.1 (4.3)	65.2 (5.1)	−5.8 (2.9)	84.9 (9.9)	90.8 (13.8)	81.8 (11.6)	−3.2 (10.7)	.6344	.0212	.5514	.4885

Abbreviations: NA, not applicable; ND, not determined; Pi, inorganic phosphate.

[a] All biochemical values are means (SEM). Reference values are as follows: Ca, 2.20–2.60 mmol/L; inorganic phosphate, 0.85–1.40 mmol/L; albumin, 35–50 g/L; prealbumin, 0.10–0.40 g/L; 25OHD, 48–145 nmol/L; PTH, 10–46 ng/L; CTX, 0.11–0.74 μg/L; TRAP5b, 2.40–6.85 U/L; BAP, 4–15 μg/L; P1NP, 19–83 μg/L; and IGF-I, 56–191 μg/L. At day 0, none of the difference between CY and FY groups was significant (Mann-Whitney *U* test) with *P* ranging from .130 to .932.

[b] Probability level calculated by the Mann-Whitney *U* test for difference between CY and FY groups for day 56 − day 0 changes.

[c] Probability level by repeated-measures ANOVA for difference in time course (day 0 to day 56).

[d] Probability level by repeated-measures ANOVA for difference between type of yogurt.

[e] Probability level by repeated-measures ANOVA for interaction between time course (day 0 to day 56) and type of yogurt.

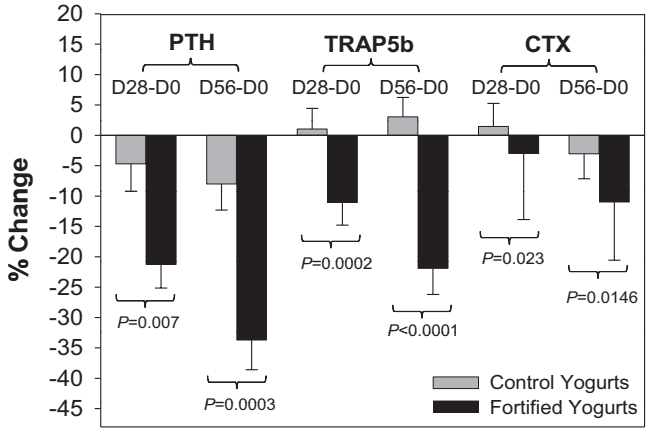

Figure 1. Percent changes in serum PTH, TRAP5b, and CTX after 28 and 56 days of consumption of CY as compared with FY. Values are mean ± SEM. Changes in percentage after 28 days (day 28 − day 0/day 0 × 100) and 56 days (day 56 − day 0/day 0 × 100). Regarding number of subjects, n = 27 in the group consuming the CY; n = 31 and n = 29 after 28 and 56 days, respectively, in the group consuming FY. From day 0 to day 28, the absolute changes (mean ± SEM) in serum 25OHD were +4.0 ± 2.4 and +20.4 ± 2.6 nmol/L ($P < .0001$) in subjects consuming the CY and the FY, respectively. From day 0 to day 56, the changes in serum 25OHD were +5.2 ± 2.5 and +25.3 ± 1.8 nmol/L ($P < .0001$) in subjects consuming the CY and the FY, respectively. *P* values were calculated by the Mann-Whitney *U* test.

Sun exposure

During the intervention, none of the subjects spent daily more than 20 minutes outdoors with their uncovered arms exposed to the sun, even for one single day.

Acceptability and adherence

The 2 types of yogurt were well tolerated. The acceptability in terms of taste and portion size was satisfactory, in keeping with the absence of tiredness in consuming either dairy product. Participant adherence (compliance and persistence) throughout the intervention was 89% in both groups.

Discussion

In the elderly, dietary enhancement with both calcium and vitamin D prevents bone loss and reduces the risk of fragility fracture (7), hence the need for ensuring an adequate supply of calcium and vitamin D (23, 24). The intake of calcium and vitamin D considered as adequate (11, 25) exceeds the spontaneous consumption, particularly among the elderly population. The strategy to fortify some well-accepted foods with calcium and/or vitamin D appears to be an appropriate option (12, 15, 26). However, each fortified product should be thoroughly tested to establish its degree of bioavailability (15, 26).

Few studies have examined the efficacy of food fortification. Contrasting results have been obtained for either calcium- or vitamin D-fortified foods. Thus, the calcium bioavailability, as assessed by measuring the temporary increase in serum calcium, was shown to markedly differ between 2 commercially marketed orange juices fortified with distinct calcium salts (15). Regarding vitamin D, the efficacy of the fortification of foods, mostly dairy products, was assessed by measuring the increment in serum 25OHD (26). Measurement of calcium absorption (15) or serum 25OHD (26) in response to the consumption of fortified foods certainly conveys important information on the tested products, particularly when compared with other similar labeled food products. These measurements suggest but do not establish that the fortified foods exert beneficial effects on bone health by reducing secondary hyperparathyroidism and inhibiting bone resorption markers.

Several studies have documented a positive influence of calcium- and/or vitamin D-fortified dairy products on bone metabolism (8, 17, 22, 27). However, the part due to the added fortificant nutrients was not quantitatively assessed by comparing in parallel-group randomized trials the identical nonfortified food.

In the randomized controlled trial reported here, serum 25OHD level increased by about 25 nmol/L in response to 10 μg/d additional vitamin D_3. It is comparable to that estimated from the above-mentioned meta-analysis including studies testing the increment in serum 25OHD in response to vitamin D_3-fortified foods, mostly dairy products (26).

According to the vitamin D status classification, moderate vitamin D deficiency is assessed with serum 25OHD levels of 12.5 to 25 nmol/L and vitamin D replete as >50 nmol/L (28). In our study, after 56 days of fortified yogurt consumption, serum 25OHD increased from a moderate vitamin D deficiency state (19.2 ± 1.2 nmol/L) to a level (44.6 ± 2.5 nmol/L) slightly inferior to the vitamin D replete state (28). According to the 2011 Institute of Medicine report on DRI for calcium and vitamin D, a serum 25OHD level of 50 nmol/L would meet the needs of 97.5% of the population in North America (11). In this report, the vitamin D necessary to achieve such a serum 25OHD concentration was estimated to be 20 μg/d (11), a larger dose than that provided by the fortified yogurt tested in the foregoing study.

The time necessary for vitamin D supplements to reach a steady state in serum 25OHD was estimated to be about 3 months (26, 29). In our study, the greatest increment in serum 25OHD was monitored during the first month (about 20 nmol/L). It was much less (about 5 nmol/L) during the second month. Nevertheless, this increment during the second month of intervention, although attenuated as compared with the rise during the first month, suggests that the steady state was close to 50 nmol/L and that this level could have been reached after a third month of such a fortified dairy consumption providing 10 μg/d vitamin D_3. In the CY group, the slight rise of +5.2 nmol/L in serum 25OHD might be ascribed to a small additional dietary in-

take of vitamin D, taken into account the lack of even minimal sun exposure, as indicated in the follow-up report filled in by the staff of each participating nursing home.

The intervention increasing both vitamin D and calcium intake was associated with a significant reduction in the serum expression of secondary hyperparathyroidism. Before the onset of the fortified yogurt consumption, the serum concentration of PTH was 32% above the upper limit of the laboratory reference range. It significantly declined within the normal range after 4 weeks of fortified yogurt consumption and even more after 8 weeks. The observed inverse relationship between serum 25OHD and PTH is in agreement with data obtained over a large range of vitamin D status in the elderly (30, 31). The increase in calcium intake probably contributed to the substantial reduction in PTH and bone resorption markers, because high calcium intake can exert a sparing effect on the vitamin D status (32).

Both types of serum bone resorption markers, TRAP5b and CTX, responded to the fortified product. However, as compared with CTX, the relative reduction in TRAP5b was already observed after 4 weeks and was much greater after 8 weeks of intervention. This differential response is quite consistent with results obtained in 3 previous trials (8, 17, 22). TRAP5b appears to be a fit-for-purpose bone resorption marker better than CTX for relatively short-term interventions aimed at testing the capacity of food products to inhibit bone resorption. The present study corroborates this notion. It further documents the greater sensitivity of TRAP5b as compared with CTX for detecting with time the progressive effect of calcium- and vitamin D-fortified dairy products on bone resorption as compared with a virtually isocaloric and isoprotein type of food. This greater sensitivity of TRAP5b as compared with CTX may be explained by the distinct metabolic steps that these 2 markers express along the osteoclastic bone resorption process (33).

In contrast with previous studies investigating various dairy products (8, 22, 34–37), we did not observe a difference in serum IGF-I between the investigated groups. In former trials documenting a rise in serum IGF-I, the tested dairy products were not compared with other dairy foods containing the same amount of proteins. In the present study, the change in the protein intake was virtually the same in the calcium- and vitamin D-fortified yogurt group and the nonfortified ones. Moreover, the tested foods were consumed instead of and not in addition to other dairy products. Therefore, knowing the key role of protein intake to stimulate the hepatic production and circulating level of IGF-I (6), it is not surprising that no difference between the 2 groups was observed.

The strengths of our study include a randomized, double-blind, placebo-controlled design in subjects representative of the institutionalized female population among whom vitamin D status (28) is frequently deficient; a maximal adherence to the 2 tested foods, enabling us to analyze the results in intention to treat; a quantification of the beneficial effects of calcium and vitamin D_3 fortification as compared with nonfortified foods on vitamin D deficiency, secondary hyperparathyroidism, and increased bone resorption markers; and additional evidence that serum TRAP5b is a sensitive bone resorption marker that is particularly well suited to monitor the dynamic response to dietary intervention on bone resorption in subjects at risk of osteoporosis.

The limitations include a duration of intervention not long enough and/or a degree of fortification not high enough to fully normalize the status in vitamin D, as estimated at 50 nmol/L 25OHD in the 2011 Institute of Medicine report (11); furthermore, if to reduce the risk of fragility fractures, the appropriate serum 25OHD level to target is 75 nmol/L (10, 25, 38), a dose of about 20 μg/d vitamin D_3, i.e., approximately twice that supplemented in the present trial, will be required. Whether long-term fortified dairy consumption could be maintained remains to be assessed.

In conclusion, the results of this double-blind randomized controlled trial carried out in institutionalized elderly at high risk of osteoporotic fracture suggest that consumption of a vitamin D_3- and calcium-fortified dairy product, as compared with a nonfortified equivalent food, improves the vitamin D status, as assessed by a substantial rise of serum 25OHD level, corrects secondary hyperparathyroidism and reduces accelerated bone resorption.

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J.-P.B. and V.B. designed the study; J.-P.B., V.B., F.P., and M.K. analyzed the data; J.-P.B. and V.B. wrote the manuscript; J.-P.B. had the primary responsibility for final content. All authors read and approved the final manuscript.

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