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NUTRITIONAL APPROACH FOR INHIBITING BONE RESORPTION IN INSTITUTIONALIZED ELDERLY WOMEN WITH VITAMIN D INSUFFICIENCY AND HIGH PREVALENCE OF FRACTURE

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Abstract: *Background:* Nutritional approach to the deterioration of bone integrity and increased fracture risk appears to be particularly appropriate in elderly women living in nursing homes. *Objective:* To investigate the beneficial effect of the consumption of soft plain cheese on bone resorption markers in institutionalized elderly women. *Design:* Prospective, randomized crossover controlled study. *Setting:* Six French nursing homes or other institutions for elderly. *Participants:* Institutionalized women ≥ 65 years old with low vitamin D status and calcium intake below 700 mg/day. *Intervention:* Consumption of soft plain cheese made of semi-skimmed milk which was fortified by both vitamin D3 (+1.25μg/100g) and milk extracted Ca, thus achieving a total Ca content of 151 mg/100g as compared to about 118 mg/100g for standard fresh cheese. Two servings were taken every day during the 6 weeks that preceded or followed a period of 6 weeks without soft plain cheese consumption. *Measurements:* The primary end point was the change in serum carboxy terminal cross-linked telopeptide of type I collagen (CTX) selected as a marker of bone resorption. *Results:* 29 women aged 73-94 yr were selected, 21 of them with mean age 87.2±6.1 years remained compliant. The intervention increased calcium and protein intakes by 51% (904±228 vs. 599±122 mg/d) and 33 % (74.2±17.1 vs. 55.6±12.7 g/d, mean±SD), respectively. The dietary intervention was associated with a statistically significant increase in serum levels of both 25OHD and IGF-I, while those of PTH, CTX and TRAP5b were significantly reduced. Compliance was 93,4 %. The daily consumption of two servings of soft plain cheese was well accepted in terms of tastiness and appetite suited portion size. *Conclusion:* This randomized crossover controlled trial demonstrates that in elderly women living in nursing homes, the consumption of soft plain cheese increasing the supply of vitamin D, calcium and proteins, could reduce bone resorption and thereby reduce the risk of incidental fragility fractures in the long term.

Key words: Elderly women, osteoporosis prevention, nutritional intervention, fortified cheese, bone resorption.

Introduction

Fragility fractures in elderly can be the long term consequence of an increase in bone remodeling, leading to bone loss with micro structural alterations of both cortical and trabecular structures (1, 2). In the primary prevention of osteoporosis, adequate nutrition and physical activity are important determinants of bone health throughout life. In the secondary prevention, several pharmaceutical agents have been shown to substantially reduce the risk of incident fragility fractures in postmenopausal women and in elderly (3-6). Secondary prevention of osteoporosis, however, cannot merely be limited to drug prescription. The adequate response to pharmaceutical treatment for preventing both further deterioration of bone structure and reducing the risk of falling is dependent upon other accompanying measures, among which nutrition is an essential contributor (7). Vitamin D, whenever cutaneous sources do not meet the needs, calcium and proteins are three nutrients influencing key processes in the acquisition and maintenance of bone structure. Several reviews on vitamin D, calcium (8-14) and proteins intakes (15-21) have underscored the importance of these three nutrients in the prevention of bone loss and, thereby in reducing the risk of fragility fracture in elderly.

Dairy products, by providing both calcium and proteins, can be expected to play a positive role on bone health. Milk consumption has been shown to favorably affect bone remodeling in postmenopausal women (22, 23) and in elderly (24). In an open study carried out in institutionalized vitamin D deficient women, we observed an apparent beneficial effect on bone turnover markers after 4 weeks of soft plain cheese consumption, providing about 17-25 % of the recommended daily intakes of vitamin D, calcium and proteins (25).

In the present report, we investigated, in another cohort of institutionalized elderly women, and using a crossover controlled study design whether the same intervention could significantly reduce bone resorption markers. In order to optimally capture any change in bone resorption rate, two circulating markers of this process were monitored atthe end of two sequential 6-week periods, with and without soft plain cheese consumption. One biochemical marker (CTX) was selected for its capacity to reflect more change in osteoclast function, whereas the other (TRAP 5b) would preferentially capture modification in the number of osteoclast (26). As designed the present study could also adequately assess how well the tested food was tolerated.

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Methods

Participants and Design

The study involved six French nursing homes or institutions for elderly. All of them were selected because they provided both medical supervision and adequate food consumption monitoring. The screening of the subjects for eligibility started on January 28 2008, after the study protocol had been approved by both the regional (Lyon) and national ("Direction Générale de la Santé) ethical committees.

Inclusion/exclusion criteria were as follow: women ≥ 65 years old living in nursing or elderly people home (Etablissement d'Hébergement pour Personnes Agées Dépendantes) having given their informed consent; calcium intake lower than 700 mg daily as assessed by frequency questionnaire (27); daily sun exposure of uncovered arms limited to less than 20 minutes; serum level of 25-hydroxyvitamin D (25OHD) ≥ 4 ng/ml in order to avoid enrolling subjects with severe vitamin D deficiency requiring appropriate medical management; serum PTH ≥ 46 ng/l and ≤ 150 ng/l; creatinine clearance, as calculated according to Cockroft formula, either normal or moderately reduced with value ≥ 30ml/min; appreciating to eat dairy foods; Mini Nutritional Assessment (MNA) with score ≥ 21 (28); no consumption during the last 6 months of food enriched withvitamin D and/or calcium; no treatment for osteoporosis or other bone diseases, including the following pharmaceutical agents: calcitonin, bisphosphonates, raloxifene, teriparatide, strontium ranelate; no disease with poor prognosis at short term; no participation in a clinical trial during the last 3 months preceding the entry into the study; no osteoporotic fracture during the 12 months preceding the study; not confined to bed or taking meals in her private room.

According to these inclusion/exclusion criteria 29, out of the 40 screened subjects were eventually enrolled into the study. Subjects were selected from the results recorded at a first visit that took place about 3 weeks before the beginning of the trial. The intervention consisted in the consumption of soft plain cheese made of semi-skimmed milk which was enriched by both vitamin D (+1.25 μg/100g) and milk calcium, thus achieving a total calcium content of 151 mg/100g, as compared to about 118 mg /100g for standard fresh cheese according to the French CIQUAL food database. Two servings of 100g each were taken every day during 6 weeks. They provided daily: 164 Kcal; 2.5 μg vitamin D; 302 mg calcium; 233 mg phosphorus 14.2 g proteins. Subjects were randomly assigned to consume the tested dairy product during the first or the second 6-week period. During the 12-week intervention phase, two blood samples were collected, i.e. by the end of the 6-week periods with and without soft plain cheese consumption.

The main endpoints were the differences in two serum markers of bone resorption, carboxy terminal cross-linked telopeptide of type I collagen (CTX) and tartrate resistant acid phosphatase, isoform 5b (TRAP 5b) between the end of the period with and without soft plain cheese consumption.

Biochemical measurements

Calcium was measured by colorimetry, sodium and potassium by indirect potentiometry with the use of specific electrodes, and creatinine by the Jaffe reaction (Roche Diagnostics, Meylan, France). Serum parathyroid hormone (PTH), osteocalcin, amino-terminal propeptide of type I procollagen (PINP), and carboxy terminal cross-linked telopeptide of type I collagen (CTX, Cross-laps) were measured by automated immunochemiluminescence on the Elecsys platform (Roche Diagnostics, Meylan, France), as previously described (29). For these 4-biochemical analyses, the within- and between-run coefficient of variations (CV) were lower than 5%, whatever the concentration tested. The reference range of these assays was established in a group of 59 premenopausal healthy women aged 35-48 years. All of them had regular menses associated with plasma FSH concentration below 12 mU/l. They received a single dose of 100'000 IU of vitamin D3 one week before the blood sampling. The reference ranges were 10-46 pg/ml, 13-32 ng/ml, 19-50 ng/ml, and 700-3000 pmol/L for PTH, osteocalcin, P1NP, and C-TX, respectively. Bone alkaline phosphatase (BAP) was measured by automated immunochemiluminescence on the Access II platform (Beckman-Coulter, Chaska, Minesota, USA). BAP within-run CV was 6.7 % at mean concentration of 13.2 μg/l and below 5% at concentration above 25 μg/l. The reference range established in healthy premenopausal women was 4-15 μg/l.

Tartrate resistant acid phosphatase, isoform 5b (TRAP 5b), a surrogate marker of osteoclast number (26), was determined by immunoassay using a monoclonal antibody raised against TRAP 5b purified from human osteoclasts, and recombinant human TRAP as a reference standard (Bone TRAP® Enzyme Assay kit, SBA Sciences). Intra and inter assay coefficient of variation are lower than 4.8 and 5.2 %, respectively.

The serum level of 25-hydroxyvitamin D (25OHD) was measured by RIA (DiaSorin, Stillwater, Minesota, USA) as previously reported (30). Vitamin D insufficiency was defined as a serum level of 25OHD lower than 30 ng/ml (42). Serum IGF-I was measured by an immunoradiometric assay (IGF-I RIA-CT, Schering-Cis Bio, Gif sur Yvette, France) based on the use of two monoclonal antibodies directed toward different IGF I epitopes. In this method, bound IGF I is displaced from IGFBPs by acidification. A large excess of IGF-II is then added to the acid-treated serum to prevent reassociation of IGF-I with its carrier proteins when buffer is added. The analytical properties of this assay was recently reported (29). The normal range of serum IGF-I in women aged 36-85 years was 68-247 ng/ml.

Statistical analysis

The statistical power was estimated from the expected change in serum CTX that was a priori considered as the main endpoint of the study. By using a crossover design, it was expected that a difference would be detected in serum CTX between periods with and without soft plain cheese consumption by 20% with a power of 80% and a two-sided of

0.05. Twenty-nine subjects were eventually enrolled in order to secure a greater power to the study. Taking into account a measured intra-individual coefficient of variation (CV) of 10%, 15 % difference in serum CTX required a sample of 20 subjects in a crossover-designed -study.

The results are expressed as mean ± standard deviation (SD). Paired Student's t test was usedto assess differences between the values measured by the end of the two 6-week periods with and without soft plain cheese consumption, except osteocalcin for which a Wilcoxon rank-sum test was applied because of the skewed distribution of this variable. P values ≤ 0.05 were considered as statistically significant. Statistical analysis was made using the software SAS, version 8.02.

Results

The baseline demographic and biochemical characteristics, as assessed at the inclusion visit, are described in Tables 1 and 2.

Table 1
Baseline demographic characteristics of the 29 enrolled women, as assessed at the inclusion visit

Age (years)	86.9 ± 6.3
Age at menopause (years)	48.7 ± 4.2
Standing height (cm)	154 ± 8
Body weight (kg)	68.5 ± 15.6
BMI (kg/m²)	29.0 ± 6.4
Calcium intake (mg/d)	604 ± 132
Protein intake (g/d)	61.5 ± 12.3
MNA	25.8 ± 2.2

All values are means ± SD; BMI: Body Mass Index. ; MNA: Minimum Nutritional Assessment.

Out of the twenty-nine included subjects who fulfilled enrollment criteria, three women withdrew before the onset of the trial. In three others, blood was not collected in the fasting state, whereas for two women the dairy product was not taken during more than 4 days out of the 6 weeks defined in the study design. Therefore, the influence of the intervention was analyzed in 21 out of the 29 subjects initially enrolled at the inclusion visit. The anthropometric, nutritional and biochemical mean values of those 21 women kept in the analysis were not statistically different (data not shown) from the 29 selected subjects presented in Table 1 and 2.

Overall, 44.8 % of the enrolled women had already experienced at least one fracture with skeletal sites including wrist or forearm, spine and hip.

As compared to the reference ranges of serum biochemical analytes, mean albumin, and 25(OHD) values of the 29 enrolled subjects were low, whereas that of PTH was elevated (Table 2). To this high serum level of PTH was associated an increase in the bone markers, CTX and P1NP above the reference range established in premenopausal women, as assessed in the investigator laboratory (29). The intervention increased calcium and protein intakes by 51% (904±228 vs. 599±122 mg/d) and 33 % (74.2±17.1 vs. 55.6±12.7 g/d, mean±SD), respectively.

Table 2
Serum biochemical characteristics in the 29 enrolled women as assessed at the inclusion visit

		Reference Values
Calcium (mmol/L)	2.24 ± 0.07	2.20 - 2.60
Inorganic Phosphate (mmol/L)	1.12 ± 0.12	0.85 -1.40
Albumin (g/L)	31.8 ± 2.7	35 - 50
Prealbumin (g/L)	0.23 ± 0.037	0.10 - 0.40
Creatinine (μmol/L)	83.9 ± 25.9	44 - 80
TSH (mUI/L)	2.2 ± 1.4	0.4 - 4.4
25OHD (ng/mL)	8.8 ± 7.5	30 - 80
PTH (ng/L)	75.8 ± 24.2	10 - 46*
CTX (pmol/L)	4325 ± 1948	700 – 3000 *
TRAP 5b (ng/mL)	5.75 ± 1.50	2.40 - 6.85 #
Osteocalcin (ng/mL)	30.3 ± 13.0	13 - 32*
BAP (μg/mL)	13.6 ± 5.3	4 - 15*
P1NP (ng/mL)	79.9 ± 31.8	19 - 50*
IGF-I (ng/mL)	111.6 ± 32.4	68-247

TSH: Thyroid-stimulating hormone; 25OHD: 25-hydroxyvitamin D; PTH: Parathyroid hormone; CTX: Carboxy terminal crosslinked telopeptide of type I collagen; TRAP 5b: Tartrate resistant acid phosphatase, isoform 5b; BAP: Bone Alkaline Phosphatase; P1NP : Amino-terminal propeptide of type I procollagen; IGF-I: Insulin-Like Growth Factor-I; * Reference range established in healthy premenopausal women aged 35-48 years; # Reference range established in healthy premenopausal women aged 22-54 years.

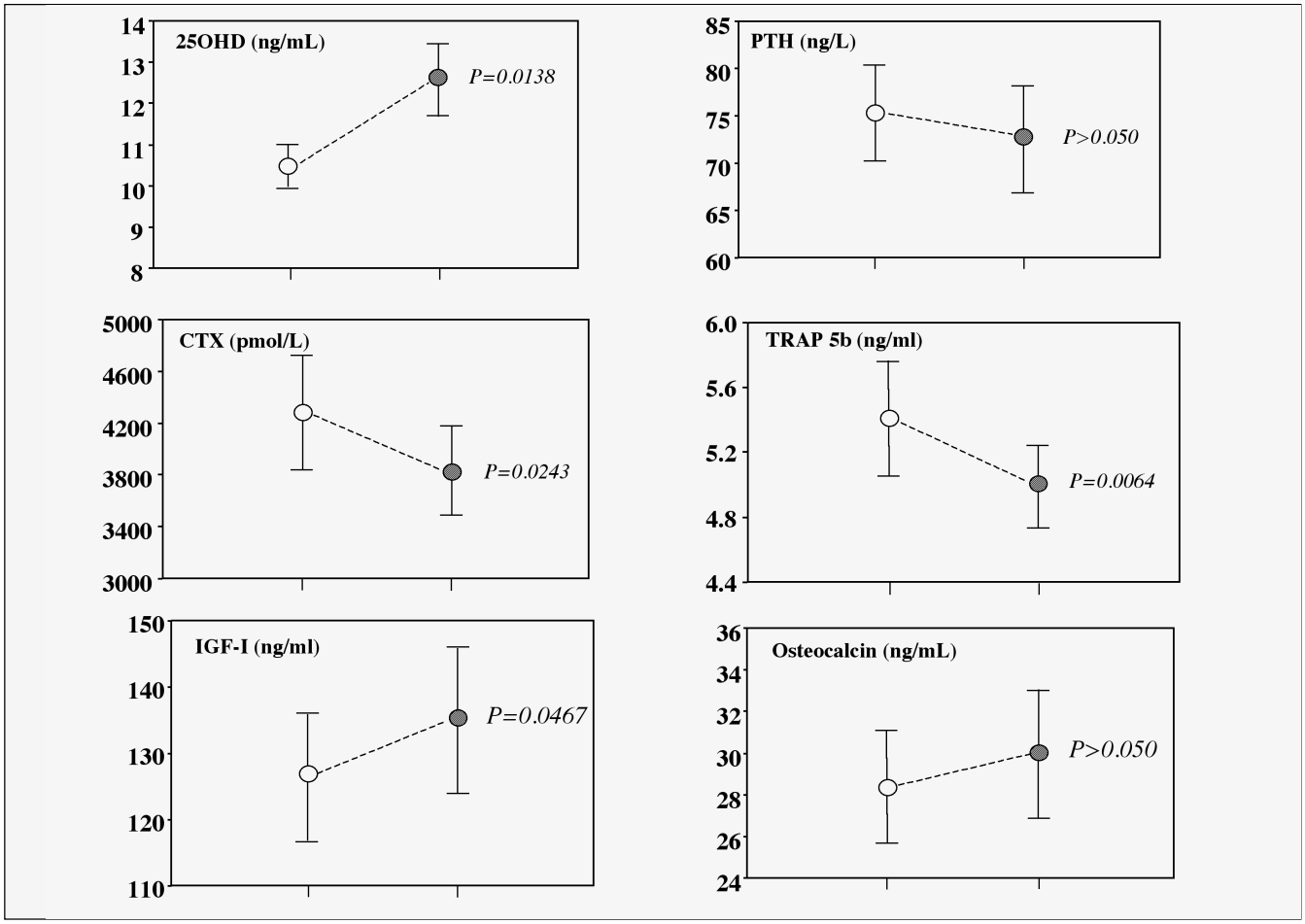
The dietary intervention produced a statistically significant increase in serum levels of both 25OHD and IGF-I, while those of PTH, CTX and TRAP5b were significantly reduced (Figure 1). No significant differences were recorded between the intervention and the control period for serum calcium, phosphate, albumin, prealbumin, BAP and P1NP (data not shown). Serum creatinine, which was slightly above the reference range corresponding to a clearance moderately reduced, but ≥30 ml/min using Cokroft formula. It was not modified by the intervention. MNA scores were not significantly different by the end of the 6-week period with (26.1±2.3) and without (26.3±1.9, mean±SD) soft plain cheese consumption.

The median number of soft plain cheese servings consumed during the 6 intervention weeks was 84.0 (ITT population n=29). The median of consumption duration was 44.0 days (ITT population n=29). The corresponding compliance was 94.8 %. The soft plain cheese acceptability, as evaluated by the end of the 6 weeks consumption was satisfactory with median score on a scale from 0 to 10 (best) for the following criteria: a) tastiness (8.0), b) portion size suited to subjects' appetite (8.5), c) absence of progressing tiredness during the food testing period (8.0).

Discussion

Elderly residents of age care facilities are at risk of suffering from inadequacies of nutrients such as vitamin D, calcium and

Figure 1
Serum biochemical variables after 6 weeks without (empty circles) and with (hatched circles) soft white cheese consumption in 21 institutionalized women. Vertical bars are SEM. *P*: statistical significance of the differences analyzed by paired t test. The decrease in the two biochemical markers of bone resorption, CTX and TRAP 5b reflecting osteoclast function and number were inhibited by 6.8% and 6% respectively, while osteocalcin was not decreased. See text for further details



proteins. In institutionalized elderly, insufficient supply of these three nutrients, in addition to the lack of sun exposure as far as vitamin D is concerned, amplifies the risk of fractures by increasing both bone loss rate and fall propensity (31-35). Hip fracture incidence is particularly elevated in nursing home residents, as observed in several countries (36-38). We recently reported results of an open trial exploring whether consumption of vitamin D and calcium enriched soft plain cheese may modify bone turnover in a way expected to attenuate the rate of bone loss among institutionalized elderly women (25). The results of this exploratory study suggested that fortified soft plain cheese consumed during one month by elderly women with vitamin D insufficiency can reduce bone resorption markers, namely CTX and TRAP 5b, by positively influencing Ca and protein economy, as expressed by decreased PTH and increased IGF-I, respectively (25). The foregoing randomized cross-over controlled study confirms that dietary intervention with the same fortified soft plain cheese during 6 weeks significantly reduced bone resorption, as expressed by the significant decrease of both specific biochemical markers, CTX and TRAP 5b. As in the initial open study (25), these changes in bone resorption markers were associated with significant increase in serum levels of both 25OHD and IGF-I. Nevertheless, the decrease in serum PTH level was attenuated as compared to the former open trial. Of note, the new study indicated the quite satisfactory adherence and tolerance by this elderly women population of the soft plain cheese consumption, as previously recorded in the open trial (25).

In the former study, 43.2% (16 out of 37) of the enrolled subjects with mean age (±SD) 84.8±8.1 years reported to have experienced a fracture from the onset of menopause. In the present study, the prevalence of fractures was quite similar 44.8% (13 out of 29). Both age and prior fracture are recognized as two main clinical factors for the prediction of fragility fracture risk (39). This underscores the high risk of further fracture occurrence in institutionalized elderly women,

out of which more than 40% have already experienced at least one fracture.

In bone-related diseases, particularly osteoporosis, changes in clinical endpoints, such as bone mineral density and fragility fractures, require months to years, respectively, to manifest. Therefore, bone biochemical markers are extremely useful to predict the long-term responsiveness to treatment after a few weeks. Early changes in bone biochemical markers, particularly those reflecting bone resorption, such as CTX, predict the long-term rate of bone loss as well as the risk of incident fracture (40-49). The magnitude of the reduction in markers of bone resorption observed in the foregoing study is less than that observed following therapeutic doses of strong inhibitors of bone resorption such as bisphosphonates (44, 46, 47). But reduction of lower magnitude was also observed with weaker inhibitors of bone resorption, such as raloxifen (50), low doses of estrogen (51) strontium ranelate (52) and calcium with vitamin D supplementation (53). These compounds have been shown to exert positive effects on bone health in elderly, including reduction in vertebral fracture of similar magnitude to that obtained with most powerful bisphosphonates.

Much attention is now paid to scientific support for claims on foods as they are related to the effects of dietary components on body functions (54), including on bone health (55). In calcium and vitamin D enriched foods, a reduction in biochemical markers of bone resorption such as CTX and TRAP5b in the present study, mimics the effect of pharmaceutical supplementation combining this active agents(53). The concomitant stimulation of IGF-I, after six weeks of soft plain cheese consumption is similar to the early rise recently reported with selective supplementation of milk protein provided to elderly patients (56). Dietary proteins play a key role in the production of IGF-I (15). This physiological relation indicates that the function of proteins as supplied from diet should not be considered as merely that of "brick supplier" to the osteogenic cells, thus conferring on them the capacity to lay down the organic bone matrix. Amino acids from dietary proteins stimulate the hepatic production of IGF-I and, consequently, increase the circulating level of this growth factor that exerts anabolic action on both bone forming and skeletal muscle cells. The dietary protein-IGF-I system also positively influences the calcium-phosphate economy, thus favouring bone anabolism (18). Furthermore, some amino acids can directly stimulate IGF-I production by osteoblastic cells (57) or its expression in skeletal muscle cells (58). These systemic and local actions of both IGF-I and amino acids underscore the importance of adequate protein intake for preventing bone loss and sarcopenia and thus, for reducing the risk of fragility fractures in elderly (21).

Taken together, the biochemical changes observed in the present study are compatible with the combined influence of calcium, vitamin D and proteins, three nutrients contained in the tested dairy product in an amount providing 17-25 % of the daily recommended allowance for the elderly (59).

In conclusion, this randomized cross-over controlled study confirms a previous open trial by showing that, in elderly, regular consumption of a dairy food supplying three nutrients deemed to be essential for bone health exert a positive influence on biochemical markers of bone metabolism.

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