

Short Communication

Inhibition of markers of bone resorption by consumption of vitamin D and calcium-fortified soft plain cheese by institutionalised elderly women

Jean-Philippe Bonjour^{1*}, Valérie Benoit², Olivier Pourchaire³, Monique Ferry⁴, Brigitte Rousseau² and Jean-Claude Souberbielle⁵

¹Division of Bone Diseases (WHO Collaborating Center for Osteoporosis Prevention), University Hospitals and Faculty of Medicine, Rue Micheli-du-Crest 24, CH – 1211 Geneva 14, Switzerland

²Groupe de Recherche Nutritionnelle, Yoplait, 150 rue Gallieni, 92641 Boulogne, France

³Hôpital Local Intercommunal de Morestel, 539 rue François Perrin, 38510 Morestel, France

⁴Centre Hospitalier Valence, 179 Bvd du Maréchal Juin, 26953 Valence, France

⁵Laboratoire d'Explorations Fonctionnelles, Hôpital Necker-Enfants Malades, Paris, France

(Received 15 July 2008 – Revised 12 January 2009 – Accepted 7 April 2009 – First published online 18 May 2009)

Acceleration of bone remodelling increases the risk of fragility fractures. The objective of the present study was to explore in elderly women whether a vitamin D and Ca-fortified dairy product providing about 17–25% of the recommended intakes in vitamin D, Ca and proteins would reduce secondary hyperparathyroidism and bone remodelling in a way that may attenuate age-related bone loss in the long term. Thirty-seven institutionalised women, aged 84·8 (SD 8·1) years, with low serum 25-hydroxyvitamin D (5·5 (SD 1·7) ng/ml) were enrolled into a multicentre open trial to consume during 1 month two servings of soft plain cheese made of semi-skimmed milk providing daily 686 kJ (164 kcal), 2·5 µg vitamin D, 302 mg Ca and 14·2 g proteins. The primary endpoint was the change in serum carboxy terminal cross-linked telopeptide of type I collagen (CTX), selected as a marker of bone resorption. Thirty-five subjects remained compliant. Mean serum changes were: 25-hydroxyvitamin D, +14·5% ($P=0\cdot0051$); parathyroid hormone (PTH), –12·3% ($P=0\cdot0011$); CTX, –7·5% ($P=0\cdot01$); tartrate-resistant acid phosphatase isoform 5b (TRAP 5b), –9·9% ($P<0\cdot0001$); albumin, +6·2% ($P<0\cdot0001$); insulin-like growth factor-I (IGF-I), +16·9% ($P<0\cdot0001$); osteocalcin, +8·3% ($P=0\cdot0166$); amino-terminal propeptide of type I procollagen (PINP), +19·3% ($P=0\cdot0031$). The present open trial suggests that fortified soft plain cheese consumed by elderly women with vitamin D insufficiency can reduce bone resorption markers by positively influencing Ca and protein economy, as expressed by decreased PTH and increased IGF-I, respectively. The rise in the bone formation marker PINP could be explained by a protein-mediated increase in IGF-I. Thus, such a dietary intervention might uncouple, at least transiently, bone resorption from bone formation and thereby attenuate age-related bone loss.

Secondary hyperparathyroidism: Fortified cheese: Calcium and protein intakes: Bone resorption and formation markers: Insulin-like growth factor-I

In the elderly, insufficient vitamin D supply, from both skin and dietary sources, and low Ca and protein intakes are associated with reduced bone mineral mass and increased risk of fragility fracture^(1–3). Insufficient vitamin D and Ca supply leads to a hyper-production of parathyroid hormone (PTH), which in turn increases bone resorption.

Low protein intake results in a reduction in the circulating level of insulin-like growth factor-I (IGF-I) that can explain the decline in bone formation observed under selective protein undernutrition⁽³⁾. In addition, protein deficiency can also lead to increased bone resorption, even in the presence

of sufficient energy supply⁽³⁾. In keeping with these notions, the circulating level of IGF-I is negatively associated with fragility fracture risk⁽⁴⁾.

The involvement in hospitalised patients with hip fracture of the above-mentioned pathophysiological mechanisms is corroborated by the assessment of biochemical signs of vitamin D insufficiency, secondary hyperparathyroidism and protein undernutrition as expressed by low circulating levels of prealbumin, albumin and IGF-I. Normalising the reduced spontaneous intake of proteins by the consumption of a casein supplement in patients with low trauma fracture

Abbreviations: CTX, carboxy terminal cross-linked telopeptide of type I collagen; IGF-I, insulin-like growth factor-I; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; TRAP 5b, tartrate-resistant acid phosphatase isoform 5b.

* **Corresponding author:** Professor Jean-Philippe Bonjour, fax +41 22 382 99 73, email jean-philippe.bonjour@unige.ch

of the proximal femur increases serum IGF-I, muscle strength and attenuates the rapid bone loss that is observed during the months following the hospitalisation⁽⁵⁾.

A substantial number of hip fractures occur in subjects living in nursing homes or institutions for the elderly⁽⁶⁾. In the management of primary or secondary osteoporosis prevention of this institutionalised population, nutrition should not be neglected, since the risk of hip fracture markedly increases in subjects with a personal history of previous fragility fracture⁽⁷⁾.

In the present open trial, we investigated in institutionalised elderly women the effect of a dairy supplementation, namely in the form of a daily consumption of soft plain cheese moderately fortified in vitamin D and Ca, on several biochemical variables related to bone metabolism. Two servings of this dairy product provided 17–25% of the daily vitamin D, Ca and protein recommended allowances for the elderly⁽⁸⁾.

Materials and methods

Participants

A group of sixty-one women living in six French nursing homes and other institutions for the elderly were screened for eligibility according to the inclusion and exclusion criteria preset for the trial. This initial examination took place between 42 and 21 d before day 0 of the trial, starting on 19 January 2007.

Inclusion criteria were as follows: (1) women aged 65 years or older living in a nursing or elderly people's home having given their informed consent; (2) Ca intake lower than 700 mg/d recorded by frequency questionnaire⁽⁹⁾; (3) sun exposure of uncovered arms limited to less than 20 min/d; serum 25-hydroxyvitamin D (25(OH)D) ≥ 4 ng/ml in order to avoid enrolling subjects with severe vitamin D deficiency requiring appropriate medical management; (4) serum PTH ≥ 46 ng/l and ≤ 150 ng/l; creatinine clearance, as calculated according to the Cockcroft formula, either normal or moderately reduced with value ≥ 30 ml/min; no dislike for dairy products; (5) Mini Nutritional Assessment with a score ≥ 21 ⁽¹⁰⁾.

Exclusion criteria were: (1) consumption of food fortified in vitamin D and/or Ca during the previous 6 months; (2) treatment for osteoporosis or other bone diseases with drugs including calcitonin, bisphosphonates, raloxifene, teriparatide and strontium ranelate; (3) disease with poor prognosis at short term; (4) participation in a clinical trial during the last 3 months preceding entry into the study; (5) osteoporotic fracture during the 12 months preceding the study requiring some specific therapeutic management; (6) confined to bed or taking meals in her room. According to these inclusion and exclusion criteria thirty-seven out of the sixty-one screened subjects were eventually enrolled into the study.

Study design

The intervention consisted of the consumption of soft plain cheese made of semi-skimmed milk which was fortified by both vitamin D₃ (+1.25 μ g/100 g) and milk Ca thus achieving a total Ca content of 151 mg/100 g as compared with 90–120 mg/100 g for standard fresh cheese. Two servings were taken every day during 1 month. They provided daily:

686 kJ (164 kcal); 2.5 μ g vitamin D₃; 302 mg Ca; 233 mg P; 14.2 g proteins. Each subject served as her own control.

The main endpoint was the change in serum carboxy terminal cross-linked telopeptide of type I collagen (CTX), selected as a marker of bone resorption, after 1 month of intervention.

The ethical committee of Lyon Sud-Est (France) approved the protocol. This approval was ratified by the French 'Direction Générale de la Santé'.

Biochemical measurements

Ca was measured by colorimetry, Na and K by indirect potentiometry with the use of specific electrodes, and creatinine by the Jaffe reaction (Roche Diagnostics, Meylan, France). Serum PTH, osteocalcin, amino-terminal propeptide of type I procollagen (PINP) and CTX (cross-laps) were measured by automated immunochemiluminescence on the Elecsys platform (Roche Diagnostics, Meylan, France) as recently described⁽¹¹⁾. For these four biochemical analyses the within- and between-run CV were lower than 5%, whatever the concentration tested.

Bone alkaline phosphatase (BAP) was measured by automated immunochemiluminescence on the Access II platform (Beckman-Coulter, Chaska, MN, USA). Bone alkaline phosphatase within-run CV was 6.7% at the mean concentration of 13.2 μ g/l and below 5% at concentrations above 25 μ g/l.

Tartrate-resistant acid phosphatase isoform 5b (TRAP 5b), a surrogate marker of osteoclast number, was determined by immunoassay using a monoclonal antibody raised against TRAP 5b purified from human osteoclasts, and recombinant human TRAP as a standard (Bone TRAP[®] Enzyme Assay kit; SBA Sciences, Turku, Finland). Intra- and inter-assay CV were lower than 4.8 and 5.2%, respectively.

The serum level of 25(OH)D was measured by RIA (DiaSorin, Stillwater, MN, USA). 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 as previously described⁽¹¹⁾.

Statistical analysis

The statistical power was estimated from the expected change in serum CTX. A reduction in serum CTX by 15% after 1 month of intervention was assumed. To achieve a power of 80% and a two-sided α of 0.05, such a 15% difference in serum CTX required a sample of fourteen subjects, taking into account a measured intra-individual CV of 10%. Thirty-seven subjects were eventually enrolled in order to secure greater power to the study.

The results were expressed as mean values and standard deviations. Paired Student's *t* tests were employed to assess differences between the values measured before and after 1 month of intervention. For osteocalcin a Wilcoxon rank-sum test was applied, because of its skewed distribution. *P* values ≤ 0.05 were considered as statistically significant. Statistical analysis was made using SAS software (version 8.02; SAS Institute, Inc., Cary, NC, USA).

Results

The baseline characteristics of the thirty-seven subjects were: age 84.8 (SD 8.1) years with standing height of 155.7 (SD 6.4) cm, body weight of 67.5 (SD 12.8) kg and computed BMI of 27.9 (SD 5) kg/m². Ca intake amounted to 549 (SD 134) mg/d. Sixteen (43.2%) out of the thirty-seven women had experienced a non-vertebral fracture after the menopause at the level of either the proximal femur (*n* 10), wrist and forearm (*n* 4) or ribs (*n* 2). At baseline serum creatinine was 80.9 (SD 19.9; normal range 44–80) μmol/l. The calculated renal clearance was 51 (SD 21) ml/min.

The duration of the dietary intervention was 29.8 (SD 2.2, range 20–32) d. Four subjects dropped out of the study for the following reasons: viral gastroenteritis, nausea and vomiting, informed consent withdrawal, hospitalisation for fracture of both tibia and fibula, after 2, 3, 20 and 28 d of product consumption, respectively. Two subjects who were compliant during 20 and 28 d with blood and urine collected by the end of the intervention period were kept in the statistical computation. Results of thirty-five subjects were analysed at baseline and by the end of the intervention. The average number of servings consumed per d was 2.0 (SD 0.1) in the thirty-five subjects.

The absolute values of the serum variables before and by the end of the intervention are presented in Table 1. The serum level of 25(OH)D was markedly inferior to the threshold value (30 ng/ml) below which vitamin D insufficiency is defined⁽¹²⁾. It was associated with an elevation in serum PTH above the upper limit of the normal range.

By the end of the intervention, the mean values of 25(OH)D and PTH were slightly but significantly increased and decreased, respectively. CTX, the marker of bone resorption that was selected as the primary endpoint, was significantly decreased (Table 1). Whereas CTX is considered to reflect osteoclast function, the other serum marker of bone resorption TRAP 5b, a circulating enzyme that would reflect higher

osteoclast number⁽¹³⁾, was also reduced. In contrast, the serum level of two markers of bone formation, namely osteocalcin and amino-terminal propeptide of type I procollagen, were significantly increased, while bone alkaline phosphatase remained unchanged. Associated with a positive change in serum albumin was a significant elevation in IGF-I (Table 1). The percentage changes from baseline of these variables are depicted in Fig. 1.

Discussion

The present study suggests that in institutionalised elderly women with low levels of 25(OH)D, it is possible to significantly reduce serum markers of bone resorption by the daily consumption of a dairy product that provides 17–25% of the recommended allowance for vitamin D (10–15 μg), and 25% for both Ca (1200 mg) and proteins (1.0 g/kg body weight)⁽⁸⁾.

Following the daily consumption of two servings of soft plain cheese during 1 month, the vitamin D supplement of 2.5 μg/d led to a small, although significant, increment in serum 25(OH)D. Such a result could be expected since the amount of vitamin D added to the tested dairy product was quite modest. The estimate of the minimum level of serum 25(OH)D that is optimal for fracture prevention varies between 20 and 32 ng/ml (50 to 80 nmol/l) according to the opinion of several clinical investigators⁽¹²⁾. Despite quite satisfactory adherence to and persistence of the consumption of the soft plain cheese, mean 25(OH)D remained noticeably below (6.3 ng/ml) this minimum level. Therefore, it appears unlikely that the very mild augmentation in 25(OH)D might explain the significant reduction of serum PTH by 12.3% as well as the decrement of the two bone resorption markers CTX and TRAP 5b, by –7.5 and –9.9%, respectively. Other mechanisms were presumably involved. The increased consumption of both Ca and proteins may well have played a major role in the inhibition of PTH and consecutive lowering

Table 1. Serum biochemical variables before and after soft plain cheese consumption in thirty-five institutionalised women (Mean values and standard deviations)

	Reference values	Before		After		Difference	P*
		Mean	SD	Mean	SD		
Ca (mmol/l)	2.20–2.60	2.29	0.09	2.27	0.11	–0.02	NS
Phosphate (mmol/l)	0.85–1.40	1.18	0.12	1.21	0.16	+0.03	NS
Albumin (g/l)	35–50	33.9	2.7	36.0	2.6	+2.1†	<0.0001
Prealbumin (g/l)	0.10–0.40	0.241	0.044	0.237	0.048	–0.004	NS
25(OH)D (ng/ml)	30–80	5.5	1.7	6.3	1.7	+0.8†	0.0051
PTH (ng/l)	10–46‡	74.9	22.6	65.7	23.7	–9.2†	0.0011
CTX (pmol/l)	700–3000‡	4979	2462	4607	2147	–372†	0.01
TRAP 5b (U/l)	1.03–4.15§	4.85	1.46	4.37	1.34	–0.47†	<0.0001
Osteocalcin (ng/ml)	13–32‡	32.6	14.6	35.3	17.5	+2.7†	0.0166
BAP (μg/ml)	4–15‡	16.4	10.1	16.5	13.8	+0.1†	NS
PINP (ng/ml)	19–50‡	82.4	46.5	98.3	76.1	+15.9†	0.0031
IGF-I (ng/ml)	68–247	131.7	54.6	154.0	49.0	+22.2†	<0.0001

25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; CTX, carboxy terminal cross-linked telopeptide of type I collagen; TRAP 5b, tartrate-resistant acid phosphatase isoform 5b; BAP, bone alkaline phosphatase; PINP, amino-terminal propeptide of type I procollagen; IGF-I; insulin-like growth factor-I.

* Level of statistical significance by paired *t* test with the exception of osteocalcin of which the skewed distribution required the use of the Wilcoxon rank-sum test.

† Biochemical tests were not carried out in one patient who experienced lower-limb fracture at day 28 (for further details, see the Results section).

‡ Reference range established in healthy premenopausal women aged 35–48 years.

§ Reference range established in healthy premenopausal women aged 22–54 years.

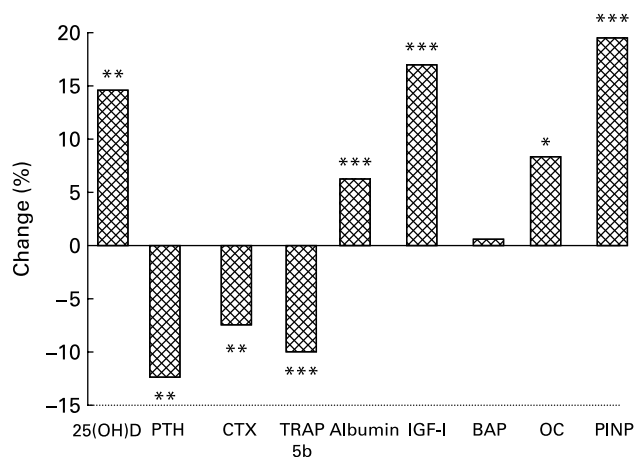


Fig. 1. Percentage changes in serum variables from the onset to the end of the dietary supplement intervention. During about 1 month, thirty-five elderly institutionalised women consumed daily two servings of soft plain cheese providing 302 g Ca, 2.5 µg vitamin D and 14.2 g proteins. The corresponding absolute values before and after the intervention are presented in Table 1. Mean difference was significant: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.02$. The degree of statistical significance P was by paired t test for the mean differences before and after the dietary intervention, with the exception of osteocalcin for which the skewed distribution required the use of the Wilcoxon rank-sum test. 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; CTX, carboxy terminal cross-linked telopeptide of type I collagen; TRAP 5b, tartrate-resistant acid phosphatase isoform 5b; IGF-I, insulin-like growth factor-I; BAP, bone alkaline phosphatase; OC, osteocalcin; PINP, amino-terminal propeptide of type I procollagen.

of the two markers of bone resorption. Besides the increased amount of ingested Ca, its intestinal absorption might have been enhanced by the additional protein supply⁽¹⁴⁾.

The need to use various ways of reducing the age-related acceleration of bone loss is of prime importance in order to reduce the burden of osteoporosis in the future. In patients with established osteoporosis, treatment using efficacious pharmaceutical agents is undoubtedly the best approach for reducing the risk of further fractures. However, even in these subjects nutrition should not be neglected, since inadequate intake of Ca, vitamin D and proteins may limit the efficacy of anti-osteoporotic drugs^(2,15).

In the present study, more than 40% of the enrolled women had already experienced fractures from the time of menopause. Nearly 90% of the recorded fractures were localised at the proximal femur and forearm levels, the two main skeletal sites where non-vertebral osteoporotic fractures do occur. This information indicates that a large proportion of our cohort was at increased risk of experiencing new fragility fractures⁽⁷⁾, underscoring the importance of osteoporosis prevention, whether primary or secondary, in institutionalised elderly women.

The conclusions that can be drawn from the present open trial are obviously limited, since its design did not allow one to compare a period with and without the consumption of the tested fortified soft plain cheese. Nonetheless, the results obtained in the six participating nursing homes were coherent in terms of subject compliance to the tested food as well as of the changes observed in PTH, bone resorption and formation markers, and IGF-I. The biochemical variations measured from baseline to intervention end are also in agreement with previous results obtained in

well-controlled experimental and clinical investigations as designed to test the effects of whole dairy products, or more selectively of either Ca or milk proteins⁽¹⁵⁾. Therefore, it appears unlikely that the statistically significant and biologically coherent changes we recorded in the present open trial conducted in elderly women would be unrelated to the specific dietary intervention.

Acknowledgements

We deeply thank the medical staff of the following nursing homes from cities (Corenc, Morestel, Pont de Beauvoisin, Saint-Martin d'Herès, Vizille, Voiron) all localised in the French Department of Isère. We acknowledge the outstanding contribution of the technical staff of the Laboratoire d'Explorations Fonctionnelles, Hôpital Necker-Enfant Malades, Paris, France. We are also very grateful to Mr Michel Drevon from Optimed Clinical Research (Glières, France) for his substantial involvement in all phases of the study.

The contribution of the authors was: J.-P. B., V. B., B. R., M. F. and J.-C. S. were involved in the study design, data analysis and interpretation; V. B. and O. P. were responsible for organising and supervising all practical aspects of the study; J.-C. S. was in charge of organising and supervising all bone and Ca metabolism-related biochemical determinations; J.-P. B. was responsible for drafting the manuscript; V. B., B. R., O. P., M. F. and J.-C. S. were involved in critically revising the manuscript draft.

This trial was financially supported by Yoplait France.

None of the authors has potential conflict of interest relevant to the study, data analysis and interpretation.

References

1. Dawson-Hughes B & Harris SS (2002) Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. *Am J Clin Nutr* **75**, 773–779.
2. Bell J & Whiting SJ (2002) Elderly women need dietary protein to maintain bone mass. *Nutr Rev* **60**, 337–341.
3. Bonjour JP (2005) Dietary protein: an essential nutrient for bone health. *J Am Coll Nutr* **24**, 526S–536S.
4. Garnero P, Sornay-Rendu E & Delmas PD (2000) Low serum IGF-1 and occurrence of osteoporotic fractures in postmenopausal women. *Lancet* **355**, 898–899.
5. Schurch MA, Rizzoli R, Slosman D, *et al.* (1998) Protein supplements increase serum insulin-like growth factor-I levels and attenuate proximal femur bone loss in patients with recent hip fracture. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* **128**, 801–809.
6. Sugarman JR, Connell FA, Hansen A, *et al.* (2002) Hip fracture incidence in nursing home residents and community-dwelling older people, Washington State, 1993–1995. *J Am Geriatr Soc* **50**, 1638–1643.
7. Lindsay R, Silverman SL, Cooper C, *et al.* (2001) Risk of new vertebral fracture in the year following a fracture. *JAMA* **285**, 320–323.
8. Martin AD (2001) *Apports Nutritionnels Conseillés pour la Population Française (Recommended Nutritional Intakes for the French Population)*, 3rd ed. Paris: Tech & Doc.
9. Fardellone P, Seberty JL, Bouraya M, *et al.* (1991) Evaluation of the calcium content of diet by frequential self-questionnaire (article in French). *Rev Rhum Mal Osteoartic* **58**, 99–103.

10. Guigoz Y, Lauque S & Vellas BJ (2002) Identifying the elderly at risk for malnutrition. The Mini Nutritional Assessment. *Clin Geriatr Med* **18**, 737–757.
11. Gascoïn-Lachambre G, Trivin C, Brauner R, *et al.* (2007) Serum procollagen type 1 amino-terminal propeptide (P1NP) as an early predictor of the growth response to growth hormone treatment: comparison of intrauterine growth retardation and idiopathic short stature. *Growth Horm IGF Res* **17**, 194–200.
12. Dawson-Hughes B, Heaney RP, Holick MF, *et al.* (2005) Estimates of optimal vitamin D status. *Osteoporos Int* **16**, 713–716.
13. Henriksen K, Tanko LB, Qvist P, *et al.* (2007) Assessment of osteoclast number and function: application in the development of new and improved treatment modalities for bone diseases. *Osteoporos Int* **18**, 681–685.
14. Kerstetter JE, O'Brien KO & Insogna KL (2003) Dietary protein, calcium metabolism, and skeletal homeostasis revisited. *Am J Clin Nutr* **78**, 584S–592S.
15. Heaney RP (2001) Constructive interactions among nutrients and bone-active pharmacologic agents with principal emphasis on calcium, phosphorus, vitamin D and protein. *J Am Coll Nutr* **20**, 403S–409S, discussion 417S–420S.