Effect of a Nutritional Supplementation on Bone Health in Chilean Elderly Subjects with Femoral Osteoporosis

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Objective: To study the effects of a special nutritional supplement on bone mineral density and bone turnover markers in Chilean elderly subjects with femoral osteoporosis.

Setting: Public primary health care clinics in Chile.

Subjects: Free living elderly subjects with femoral osteoporosis.

Interventions: Subjects were randomized to receive the usual nutritional supplement provided by the Chilean Ministry of Health or a special nutritional supplement providing, among other nutrients, 90 mg isoflavones, 800 mg calcium, 400 IU vitamin D, 60 ug vitamin K and 31 g proteins per day.

Measures of Outcome: At baseline, and after six and twelve months of supplementation, body composition, bone mineral density, serum 25 OH vitamin D, intact parathyroid hormone (iPTH), osteocalcin, decarboxylated osteocalcin, urinary aminoterminal telopeptide of type I collagen (NTX), deoxypyridoline cross links (Dpd) and equol were measured. Every month, urinary daidzein was measured in a morning urine sample.

Results: No differences between treatment groups were observed in body composition or bone mineral density changes. The group receiving the special supplement had a significant increase in serum 25 OH vitamin D and a significant decrease in serum iPTH and decarboxylated osteocalcin. No association between daidzein or equol excretion and changes in bone mineralization was observed.

Conclusions: A special supplement delivered to elderly subjects with osteoporosis improved serum vitamin D and reduced serum iPTH and undercarboxylated osteocalcin levels but did not affect BMD.

INTRODUCTION

Osteoporosis and related fractures are a major public health problem, growing in importance as the population ages. Lifetime risk of fracture exceeds 40% for women and 13% for men. In the elderly, hip fractures are associated with a 20% mortality and great expenses in prostheses and long term nursing home care [1].

Nutritional risk factors are of particular importance to bone health because they are potentially modifiable. It is generally agreed that calcium and vitamin D are important nutrients for bone health, and supplements containing these nutrients are prescribed widely. Low serum concentrations of vitamin K are associated with lower bone mineral density and increased risk of hip fracture. This vitamin promotes gamma-carboxylation of glutamyl residues of osteocalcin, reduces urinary calcium excretion and inhibits the production of bone reabsorbing agents, such as prostaglandin E2 and interleukin 6 [2]. Carboxylated osteocalcin has a higher affinity with hydroxyapatite in bone and enhances bone formation [3]. Supplementation with vitamin K reduces serum concentration of undercarboxylated osteocalcin [4], and reduces bone loss in postmenopausal women [5].

The use of soy phytoestrogens or dietary isoflavones is receiving increasing interest [6]. These weak estrogens can prevent bone loss in ovariectomized rats [7]. Several clinical trials in postmenopausal women showed that a supplement of approximately 90 mg/day of phytoestrogens, improves spine bone mineral density and reduces urinary excretion of bone

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resorption markers [8–13]. However, a recent large double blind randomized trial did not show any effect of 99 mg/day of isoflavones on bone health of postmenopausal women [14]. As expected, phytoestrogens do not have a beneficial effect in pre menopausal women, that maintain adequate endogenous estrogen production [15].

Most studies on bone health have been performed in postmenopausal women and there is less information on the value of nutritional supplements among osteoporotic elderly subjects. Therefore, the aim of this controlled trial was to study the effects of a high protein nutritional supplement enriched with calcium, vitamin D, K and phytoestrogens on bone mineral density and markers of bone metabolism in healthy elderly people, as compared with a standard nutritional supplement provided by the Chilean Ministry of Health.

MATERIALS AND METHODS

Experimental Subjects

Healthy elderly subjects of both genders aged 70 years or more, living in the community of Santiago, Chile, were screened for the presence of femoral neck osteoporosis, as defined by WHO [16] (either less than 2.5 standard deviations of normal values for young people or less than 1 standard deviation of normal and a history of non traumatic fractures). Subjects with femoral osteoporosis were considered eligible if they were not taking medications that interfere with bone metabolism (glucocorticoids, estrogens, anticonvulsivants, anticoagulants, calcium, vitamin and mineral supplementation), did not suffer a fracture in the preceding six months, had a mini mental state examination (MMSE) [17] score of 19 or more, did not have any chronic or debilitating disease (such as cancer, renal or functional capacity II–III cardiac failure, the presence of chronic infections such as tuberculosis, connective tissue diseases or diabetes mellitus), had a mini nutritional assessment (MNA) [18] over 17 and signed a written informed consent. The study was approved by INTA's ethics committee.

Eligible subjects were randomly assigned to receive a special nutritional supplement (SS) or to receive the standard nutritional supplement (MS), provided by the Chilean Ministry of Health to all elderly subjects aged 70 or more. The composition of both nutritional products is described in Table 1. Subjects were instructed to consume two servings of 58.5 g each, per day.

At baseline, 6 months and 12 months of follow up, a full clinical assessment was done, body composition and bone mineral density were measured using a Lunar Prodigy double beam densitometer (Lunar Corporation, Madison Wisconsin, USA). The equipment was calibrated daily with an anthropometric phantom and all scans were read by the same trained operator to reduce the coefficient of variation. A fasting blood sample was drawn to measure routine blood chemistry (complete blood count, glucose, creatinine, urea nitrogen, total and HDL cholesterol, triglycerides, calcium and phosphorus), plasma intact parathyroid hormone (iPTH), 25 hydroxy vitamin D (Vit D), osteocalcin and percentage of undercarboxylated osteocalcin. Subjects were requested to void a fasting second morning urine sample to measure calcium concentration, deoxypyridoline cross links (Dpd), aminoterminal telopeptide of type I collagen (NTX). On a further analysis, equol was measured also in these urine samples obtained at six and twelve months.

Every month, during one year of follow up, all subjects attended an outpatient clinic, where they were asked about adverse events, falls, adverse effects of the nutritional products and new medication use. A new supply of nutritional products was delivered and subjects returned the leftovers from the previous month. In each visit, subjects were requested to void and collect a second morning urine sample to measure daidzein.

Analytical Methods

1. Clinical biochemistry and complete blood count were analyzed using routine automated methods at Laboratorio Vidaintegra SA.
2. iPTH was measured by an immunoradiometric method using a commercial kit from Diagnostic Products Corporation (DPC, Los Angeles CA USA).
3. Vit D was measured by a double antibody radioimmunoassay ^{125}I RIA Kit DiaSorin (DiaSorin, Saluggia, Italy), following a previous extraction with acetonitrile.
4. Osteocalcin was measured with an immunoradiometric method using a Osteo-RIACT kit from Cis Bio International (Saclay, France).
5. Undercarboxylated osteocalcin was measured using an assay based on the low affinity of decarboxylated osteocalcin for hydroxyapatite [19]. In brief, 250 ul of serum were incubated with 7.5 mg hydroxyapatite in an Eppendorf tube for 1 h under constant agitation at 4°C and centrifuged afterwards at 2000 g at the same temperature. Undercarboxylated osteocalcin was measured with a Osteo-RIACT kit in the supernatant and was expressed as % of total osteocalcin.
6. Total alkaline phosphatases were measured with a commercial kit from Química Clínica Aplicada (Amposta, Spain). This kit uses an optimized enzymatic method recommended by the Deutsche Gesellchaft für Klinische Chemie, DGKC).
7. Urinary creatinine was measured using a commercial kit from Wiener Lab (Rosario, Argentina).
8. Urinary Dpd was measured using a enzyme immunoassay commercial kit (METRA Dpd EIA) from Quidel (San Diego, CA, USA). Results were expressed as mmol Dpd/mmol creatinine.
9. Urinary NTX was measured by an enzyme immunosorbent assay using an OSTEOMARK commercial kit from Ostex International Inc (Seattle, WA, USA).

Table 1. Nutritional Composition of the Products Delivered to the Elderly

Composition	Special Supplement (SS)		Product provided by Chilean Ministry of Health (MS)	
	Per 100 g	Per serving[§]	Per 100 g	Per serving
Energy (Kj)	1700	995	1674	979
Protein (g)	27	15.7	13	7.6
Total fat	11	6.2	11	6.4
Saturated	2.7	1.6	1.6	0.9
Monounsaturated	4.6	2.7	5.4	3.2
Polyunsaturated	2.4	1.4	4	2.3
Carbohydrates (g)	51.3	30.0	62.3	36.4
Total fiber (g)	5.1	3	6.2	3.6
Vitamin A (IU)	1111	650	240	140
Vitamin D (IU)	342	200	60	35
Vitamin E (IU)	4.1	2.4	4	2
Vitamin K1 (ug)	51.3	30	0	0
Vitamin C (mg)	25.6	15	30	18
Vitamin B1 (mg)	0.2	0.12	0.4	0.2
Vitamin B2 (mg)	0.25	0.15	0.4	0.2
Niacin (mg)	2.6	1.5	4.5	2.6
Vitamin B6 (mg)	1.7	1	1	0.6
Folic acid (μg)	342	200	100	59
Panthotenic acid (mg)	0.85	0.5	0	0
Vitamin B12 (μg)	3.25	1.9	0.5	0.3
Calcium (mg)	684	400	400	234
Magnesium (mg)	179.5	105	150	88
Biotin (μg)			0	0
Iron (mg)	2.7	1.5	2.8	1.6
Copper (mg)	1.28	0.75	0	0
Zinc (mg)	12.8	7.5	3	1.8
Chromium (μg)	7	6	0	0
Fructooligosaccharides (g)[¶]	5.2	3	0	0
Isoflavones[#]	76.9	45	0	0

[§] Each serving of 58.5 g. Two servings per day.

[¶] A mixture of raftilose and raftiline in a ratio of 2:1.

[#] Source: SoyLife (Schouten Products, The Netherlands) containing 3% total isoflavones. Daidzin & daidzein, 15.5 mg/g; genistin & genistein, 4.1 mg/g; glycetin & glycetein, 10.5 mg/g.

10. Urinary daidzein was measured by HPLC and diode array detection, using a modification of the technique described by Nurmy et al [20]. Briefly, samples were hydrolyzed with beta-glucuronidase, isoflavones were extracted with diethylether and injected to a Hypersil BDS C18 3μ column of 53 mm length (Alltech Associates INC, Deerfield Ill, USA).
11. Urine for equol measurements was extracted in the same way as for daidzein. Equol was measured by HPLC and electrochemical detection, using a technique described by Gamache et al, with slight modifications [21].

Statistical Methods

Results are expressed as means ± standard deviations when a normal distribution was found or as median and range for data with non normal distribution. Correlations were calculated using the Pearson correlation coefficient. The effects of the SS on outcomes were evaluated with repeated measures two-way ANOVA, correcting data for covariables such as sex and age (for variables with a normal distribution) or Mann Whitney U test (for variables with a non normal distribution). Calculations were done on Statistica for Windows package version 5, Statsoft, Tulsa OK, USA.

Subjects that were lost to follow up, had experienced serious adverse events that precluded their maintenance in the protocol, had a fracture, took medications that could interfere with bone metabolism (calcium, vitamin D, oral corticosteroids, bisphosphonates, oral anticoagulants) or required a hospital admission for more than seven days during the study period, were excluded from the per protocol analysis of data.

The median value of urine daidzein excretion was calculated for each subject participating in the study.

RESULTS

Eight hundred and eighty subjects were screened and 133 (15%) had a femoral neck osteoporosis. Of these, 100 fulfilled the inclusion criteria and were randomly allocated into one of two supplemented groups of 50 subjects each. The subject flow

is depicted in Fig. 1. The initial demographic and laboratory values are shown in Table 2.

Compliance with the SS, according to the count of leftover sachets, was 82.8 ± 11.7%. Compliance with the MS was 71%, according to subject's report on each clinic visit. During the follow up period, median daidzein excretion among subjects receiving the MS was 0. Among subjects on the SS, 32 subjects did excrete daidzein (median 300.5 ng/ml, range 10.7–2525.4 ng/ml) and 13 did not excrete daidzein. No association between daidzein excretion and compliance with the special supplement was observed.

During the follow up, no changes in body weight, arm, waist or hip circumferences were observed. No change in body composition, measured by DEXA, was observed in any group either. No differences between groups was observed for the evolution of bone mineral density in the spine or femoral neck (Table 3).

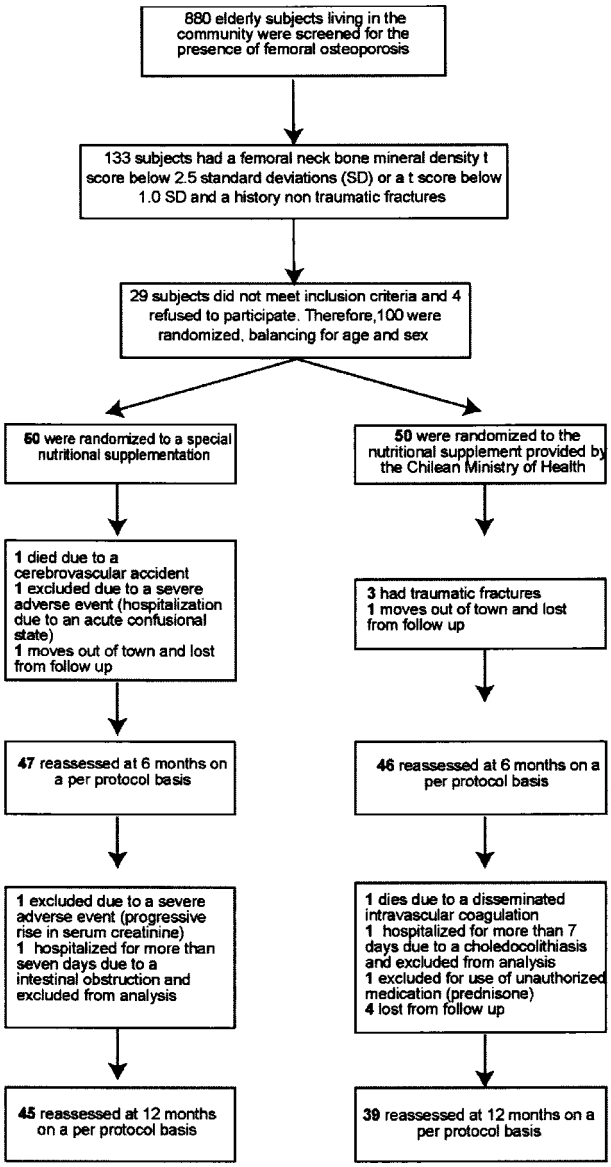

Fig. 1. Patient flow during the follow up period.

The results of bone metabolism markers are shown in Table 4. Forty-eight (24 in each study group) subjects had plasma vit D levels below 16 ng/ml, considered the lower limit of normal. These levels were not influenced by the season in which basal values were obtained. At baseline, there was a significant negative correlation between vit D and iPTH ($r = -0.33$ $p = 0.01$). During follow up, vit D increased significantly in subjects receiving the SS. Among plasma bone turnover markers, total osteocalcin decreased in all groups and the decrease was more marked in subjects with the SS, but without reaching statistical significance. Undercarboxylated osteocalcin decreased significantly in patients receiving the SS, compared with subjects on the standard supplement. Likewise iPTH remained constant in individuals with the SS and increased in subjects with the MS. No significant changes were observed in plasma alkaline phosphatases. There was a wide variation in the urinary markers of bone resorption, and no differences were observed between treatment groups (Table 5).

No association between daidzein excretion and changes in bone mineral density, were observed. At six and twelve months, 16 subjects receiving the SS had an equol excretion of more than 0.5 ug/ml (median 1.78 ug/ml range 0.51–10 ug/ml). No correlation between equol excretion and changes in bone mineral density were observed. Equol was not detected in any subject receiving the MS.

During follow up, subjects reported 72 episodes of falls (39 in subjects receiving the SS and 33 in the group with the MS). These falls resulted in three fractures, as stated in the patient flow (Fig. 1).

DISCUSSION

In this prospective trial, we tested a special nutritional supplement, containing a higher amount of proteins, vitamins involved in bone formation and isoflavones on the bone health of osteoporotic elderly subjects. We observed improvements in several markers of bone formation and resorption.

We did not observe changes in bone mineral density in this study. However it must be borne in mind, that in the elderly, the rate of bone loss is less marked than in middle age subjects, specially among men [22]. Obviously, it is more difficult to observe an effect of an intervention, in subjects with less marked rates of bone mineral density change.

Bone turnover markers are good predictors for long term changes in bone mineral density [23] and risk of fractures [24,25]. Osteocalcin or bone-Gla protein is the most abundant noncollagenous protein of the bone matrix. All subjects in this study experienced a reduction in osteocalcin levels, that was more marked in subjects receiving the SS. This effect could be attributed in part to the isoflavone content of the SS, since other

Table 2. Initial Demographic, Anthropometric and Laboratory Values in the Studied Patients (x ± Standard Deviation)

	Special supplement n = 50	Supplement provided by the Ministry of Health n = 50	*p*
Age and anthropometry			
Age (years)	76.6 ± 5.1	76.4 ± 5.4	0.894
Weight (kg)	55.3 ± 9.6	60.6 ± 9.5	0.007
Height (cm)	146.1 ± 9.0	148.9 ± 7.2	0.093
Waist circumference (cm)	93.8 ± 10.6	97.6 ± 10.7	0.080
Body mass index (kg/m2)	26.1 ± 4.8	27.4 ± 4.7	0.172
Geriatric scores			
Activities of daily living (Katz)	5.4 ± 0.7	5.6 ± 0.5	0.279
Instrumental activities of daily living	15.3 ± 1.5	15.2 ± 1.2	0.823
Depression store (Yessavage)	4.5 ± 2.3	5.3 ± 3.1	0.117
Mini nutritional assessment	26.4 ± 2.4	26.2 ± 2.1	0.688
Mini mental state	24.8 ± 3.1	24.9 ± 3.2	0.975
Routine laboratory			
Red blood cells (M/ul)	4.5 ± 0.4	4.5 ± 0.5	0.739
Packed red cell volume (%)	40.7 ± 3.7	40.6 ± 3.5	0.939
Hemoglobin (g/dl)	13.7 ± 1.2	13.7 ± 1.0	0.839
Creatinine (mg/dl)	0.9 ± 0.2	0.9 ± 0.2	0.881
Total cholesterol (mg/dl)	204.4 ± 36.3	205.8 ± 35.9	0.844
Triacylglycerol (mg/dl)	129.2 ± 78.5	135.9 ± 65.4	0.645
HDL cholesterol (mg/dl)	57.4 ± 15.8	57.4 ± 14.4	0.984
Blood glucose (mg/dl)	95.9 ± 30.4	93.1 ± 11.7	0.548
Serum albumin (g/dl)	4.0 ± 0.3	4.0 ± 0.4	0.911
Serum calcium (mg/dl)	9.2 ± 0.4	9.3 ± 0.5	0.262
Serum phosphorus (mg/dl)	3.2 ± 0.6	3.3 ± 0.6	0.320

Table 3. Evolution of Body Composition and Bone Mineral Density during Follow Up (x ± Standard Deviation)

	Special supplement n = 45		Supplement provided by the Ministry of Health n = 39	ANOVA special sup vs ministry sup
Body composition (kg)				
Fat free mass				
Basal	33.7 ± 5.2		36.1 ± 4.0	0.02
6 months	33.6 ± 5.1		35.6 ± 4.2	0.05
12 months	33.5 ± 5.1		35.6 ± 4.2	0.03
Two way ANOVA¶		0.29		
Fat mass (kg)				
Basal	19.1 ± 8.0		22.6 ± 8.3	0.05
6 months	20.0 ± 8.3		23.2 ± 8.7	0.09
12 months	19.7 ± 8.3		22.3 ± 8.7	0.16
Two way ANOVA¶		0.09		
Bone mineral density (g/kg)				
Femoral neck				
Basal	0.66 ± 0.06		0.66 ± 0.05	0.94
6 months	0.67 ± 0.07		0.66 ± 0.05	0.72
12 months	0.67 ± 0.06		0.66 ± 0.05	0.48
Two way ANOVA¶		0.3		
Spine				
Basal	0.83 ± 0.15		0.88 ± 0.13	0.08
6 months	0.83 ± 0.16		0.89 ± 0.14	0.11
12 months	0.84 ± 0.15		0.89 ± 0.15	0.11
Two way ANOVA¶		0.3		

¶ Two way ANOVA using age and sex as covariates.

studies in rats and humans have shown that these compounds reduce osteocalcin levels [26,27].

Osteocalcin has a vitamin K dependent calcium-binding aminoacid, gamma carboxyglutamic acid at residues 17, 21 and 24. This aminoacid facilitates the binding of osteocalcin to hydroxyapatite in bone [28]. In our subjects, the SS significantly reduced the percentage of undercarboxylated osteocalcin. This effect of the supplement is probably due to its vitamin K content. The product provided 60 μg of vitamin K per day, an amount that has been reported to modify osteocalcin carboxylation [29].

Noteworthy was the high proportion of subjects with low vit D levels in our sample, that was not influenced by the season of the year in which the sample was obtained. As expected, there was a negative correlation between vit D and iPTH, indicating the state of secondary hyperparathyroidism [30].

Table 4. Evolution of Serum Bone Turnover Markers during Follow Up (x ± Standard Deviation)

	Special supplement n = 45		Supplement provided by the Ministry of Health n = 39	ANOVA special sup vs ministry sup
Serum osteocalcin (ng/ml)				
Basal	28.6 ± 9.5		30.0 ± 11.3	0.47
6 months	24.8 ± 10.1		28.6 ± 10.4	0.97
12 months	22.5 ± 8.9		27.4 ± 11.3	0.02
Two way ANOVA		0.12		
Serum decarboxylated osteocalcin (%)				
Basal	21.9 ± 6.8		21.0 ± 7.5	0.85
6 months	18.5 ± 8.6		24.4 ± 9.3	0.01
12 months	19.3 ± 9.7		21.4 ± 7.7	0.5
Two way ANOVA[¶]		0.01		
Serum intact PTH (pg/ml)				
Basal	40.9 ± 17.6		42.8 ± 19.1	0.62
6 months	38.4 ± 18.6		54.3 ± 33.1	0.02
12 months	43.4 ± 23.6		53.0 ± 27.4	0.09
Two way ANOVA[¶]		0.01		
Serum 25 OH vitamin D (ng/ml)				
Basal	16.0 ± 6.7		16.9 ± 10.2	0.64
6 months	24.0 ± 9.4		18.5 ± 8.4	<0.01
12 months	23.2 ± 7.9		13.2 ± 6.6	<0.01
Two way ANOVA		<0.01		
Serum alkaline phosphatases (U/L)				
Basal	181.0 ± 59.4		190.7 ± 55.3	0.44
6 months	174.6 ± 59.7		192.5 ± 61.4	0.42
12 months	194.0 ± 46.9		204.9 ± 50.7	0.31
Two way ANOVA[¶]		0.96		

[¶] Two way ANOVA using age and sex as covariates.

Table 5. Evolution of Urine Bone Turnover Markers during Follow Up. Results Expressed as Median (range)

	Special supplement n = 45	Supplement provided by the Ministry of Health n = 39	Mann Whitney U *p*
Urine aminoterminal telopeptide of type I collagen (mmECO/mmol creatinine)			
Basal	62.0 (19.1–3683.8)	52.6 (17.6–541.2)	0.19
6 months	33.6 (1.0–178.3)	51.5 (6.8–195.2)	0.07
12 months	45.6 (11.0–150.6)	50.8 (15.9–157.1)	0.11
Urine deoxypyridoline cross links (nmol Dpd/mmol creatinine)			
Basal	8.9 (2.0–438.4)	7.0 (2.3–76.1)	0.12
6 months	6.8 (0.7–16.8)	7.0 (2.5–13.5)	0.83
12 months	7.1 (3.6–17.2)	6.8 (2.2–13.8)	0.83
Urine calcium (mmol Ca/mmol creatinine)			
Basal	0.3 (0.0–5.5)	0.2 (0.0–2.2)	0.37
6 months	0.3 (0.0–0.6)	0.2 (0.0–0.8)	0.69
12 months	0.3 (0.0–1.1)	0.3 (0.0–1.1)	0.63

Supplementation increased serum vit D and reduced iPTH. The SS had a higher content of vitamin D and calcium to ensure an adequate calcium retention. Most prospective supplementation studies with vitamin D and calcium show positive effects on bone mineral density and the incidence of fractures [31,32], although some authors have not found an effect on the latter [33]. Vitamin D supplementation may also decrease body sway and therefore reduce the risk of falls in the elderly [34]. However, we did not observe any effect of the supplement on the frequency of falls in our subjects. Vitamin D could also influence osteocalcin synthesis and therefore modify undercarboxylated osteocalcin levels [35,36]. Therefore, part of the effect of the supplement on undercarboxylated osteocalcin levels could be due to its vitamin D content. The great dispersion of NTX and Dpd values observed, similar to other reports [37], precludes a biological interpretation of this finding.

Recently, it has been proposed that responders to isoflavones are those who can convert daidzein to equol, i.e. the

"equol producers". Indeed, in a 2 year clinical trial investigating the effect of soymilk consumption on bone health, equol producers showed the best response [38]. Based on these results, we further analyzed equol excretion on subjects receiving the special supplement. We found equol in urine at six or twelve months of follow up, in 36% of subjects receiving isoflavones. This figure is very similar to that reported by other authors [39]. However, we did not observe an association between equol excretion and changes in bone density or bone turnover markers.

The non specific effect of protein supplementation on bone turnover cannot be disregarded. Several cross sectional analyses show a positive association between protein intake and bone mineral density [40,41]. In a prospective study, performed in elderly subjects recovering from a hip fracture, Schurch reported that a protein supplement attenuated bone loss [42]. We also reported previously that total bone mineral density decreased less in patients receiving the MS, than in non supplemented controls [43]. Therefore, the higher protein content of the SS, could also have a positive effect on bone mineralization.

The SS had also a higher vitamin A content than the MS. Excess vitamin A ingestion is associated with a higher incidence of fractures [44]. However the amount of vitamin A in the SS was below the RDAs and more recent data shows that the association between vitamin A is U shaped. This indicates that both vitamin A deficiency and excess can increase the risk of fractures [45].

In summary, a special nutritional supplement, providing extra amounts of calcium, vitamin D, vitamin K and isoflavones, improved markers of bone turnover in osteoporotic elderly subjects.

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