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Original Article

Effect of Soy Protein on Bone Metabolism in Postmenopausal Japanese Women

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Abstract. We conducted a cross-sectional study of the effects of soybean protein intake on bone mineral density and biochemical markers in 85 postmenopausal Japanese women. Nutrients in the diet of postmenopausal Japanese women visiting the osteoporosis unit, including subjects with normal lumbar spine bone mineral density (L2–4 BMD), were investigated by questionnaire, and the calculated daily energy, protein, soy protein and calcium intake were obtained. L2–4 BMD was measured with dual-energy X-ray absorptiometry, and assays done of serum alkaline phosphatase (ALP) and serum intact osteocalcin (IOC) as bone formation markers and urinary pyridinoline (UPYR) and urinary deoxypyridinoline (UDPYR) as bone resorption markers. Soy protein intake was significantly associated with the *Z*-score for L2–4 BMD ($r = 0.23$, $p = 0.038$) and UDPYR ($r = -0.23$, $p = 0.034$). Stepwise multiple regression analyses showed that soy protein intake is significantly associated with the *Z*-score for L2–4 BMD ($\beta = 0.225$, $p = 0.04$) and UDPYR ($\beta = -0.08$, $p = 0.03$) among four nutritional factors. These results suggest that high soy protein intake is associated with a higher bone mineral density and a lower level of bone resorption, but further studies are needed to confirm the causal dynamic mechanisms.

Keywords: Bone resorption; Deoxypyridinoline; Lumbar spine bone mineral density; Postmenopausal Japanese women; Soy protein; *Z*-score for L2–4 BMD

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Introduction

Japanese women ingest more soybean products than their western counterparts. This might help to protect them from atherosclerotic diseases or osteoporosis and extend their life. Soy protein contains many nutrients such as calcium, vitamin K and phytoestrogen. These nutrients protect postmenopausal women against bone loss due to deficiencies in calcium [1–4] and vitamin K [5]. Recently, the effect of the phytoestrogen contained in soybeans on bone loss in oophorectomized rat was reported [6–8]. Although soybeans contain many factors advantageous for bone metabolism, little is known about how soy protein itself protects postmenopausal women from osteoporosis. In order to examine the effect of soy protein intake on bone metabolism in Japanese women, including normal subjects visiting the osteoporosis unit, we measured daily soy protein intake, lumbar spine bone mineral density (L2–4 BMD), bone biochemical markers and calciotrophic hormones. This is the first study to examine the relationship between soy protein intake and bone metabolism in postmenopausal Japanese women.

Subjects and Methods

Subjects

Women who visited our hospital for BMD measurement were enrolled in this study. These women had already undergone primary screening for osteoporosis in an urban health center. Approximately 60% of the women were osteopenic or osteoporotic and the rest were normal in terms of lumbar bone density. Eighty-five women

were examined for BMD, bone biochemical markers and daily nutritional state.

Biochemical Markers and Hormone Measurements

Total urinary pyridinoline (UPYR) and deoxypyridinoline (UDPYR) were measured in morning void urine samples with Pyrilinks and Pyrilinks-D assay kits (Metra Biosystems, CA). Intact osteocalcin (IOC) was measured by ELISA (Kokusai Siyaku, Kobe, Japan). Serum alkaline phosphatase (ALP) was measured with an autoanalyzer. Plasma levels of intact parathyroid hormone (1–84 intact PTH) were measured by an immunoradiometric assay (IRMA; Allegro Intact PTH, Nichols Institute, San Juan Capistrano, CA) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) was measured by radioimmunoassay (RIA; Immunodiagnostic Systems, Bolton, UK). 25-Hydroxyvitamin D (25(OH)D) was measured by a competitive protein binding assay (Mitsubishi Kagaku Bio-Clinical Laboratories, Tokyo, Japan).

Bone Measurement

BMD was measured at the lumbar spine (L2–4 BMD) by dual-energy X-ray absorptiometry (DXA; Lunar DPX-L, Madison, WI). *T*-scores and *Z*-scores for L2–4 BMD were calculated as percentages of the values in young controls and percentage values in age-matched controls, respectively, from the Lunar database of healthy Japanese women. Osteoporosis and osteopenia were defined following the standards of the World Health Organization.

Dietary Survey

Dietary intake was assessed over a period of three consecutive days. The kinds and weights of foods ingested were recorded. The dietician calculated the energy, protein, calcium and soy protein intake of all subjects.

Statistical Analysis

All values are means ± SD unless otherwise indicated. Nutritional intake was analyzed using Pearson's correlation coefficients. The associations between nutritional factors and *Z*-score or bone biochemical markers were evaluated by correlation coefficient and multiple regression analyses by a stepwise method.

Results

Background Characteristics

Table 1 shows the baseline characteristics of the postmenopausal Japanese women participating in our study. The mean L2–4 BMD value of all subjects was 0.806 g/cm^2, which is lower than the mean value in Japanese women in the seventh decade. The *Z*-score and *T*-score for L2–4 BMD were −0.58 and −2.59, respectively. Intact PTH, $1,25(OH)_2D$ and 25(OH)D were 29.1 ± 11.4 pg/ml, 43.6 ± 16.5 pg/ml and 18.0 ± 5.0 ng/ml, respectively. IOC, UPYR and UDPYR were 7.5 ± 3.0 pg/ml, 30.5 ± 11.3 μM/mMCr and 5.5 ± 1.9 μM/mMCr, respectively. Mean values of serum calcium and phosphate, and the ratio of urinary calcium to creatinine, were within the normal range. Calcium intake was 730 mg/day, which is higher than the average calcium intake in Japanese women. Energy, protein and soy protein intake were similar to the average for Japanese women of the same generation.

Table 1. Baseline characteristics of the subjects

Age	66.9 ± 7.4 years (52–83)
Body mass index	21.2 ± 2.5 kg/m^2 (15.4–30.5)
Energy intake	1446 ± 213 kcal/day (1032–2182)
Protein intake	62.5 ± 10.8 g/day (32.3–92.4)
Calcium intake	733 ± 223 mg/day (259–1305)
Soy protein intake	12.6 ± 5.4 g/day (2.8–32.9)
L2–4 BMD	0.806 ± 0.137 g/cm^2 (0.366–1.171)
T-score	−2.59 ± 1.19 (−6.28 to 0.98)
Z-score	−0.58 ± 1.06 (−3.28 to 2.95)
Intact PTH	29.1 ± 11.4 pg/ml (16–59)
$1,25(OH)_2D$	43.6 ± 16.5 pg/ml (14.9–102.0)
25(OH)D	18.0 ± 5.0 ng/ml (7–33)
Serum calcium	9.6 ± 0.4 mg/dl (8.7–10.4)
Serum inorganic phosphate	3.8 ± 0.5 mg/dl (2.7–5.5)
ALP	135 ± 41 IU/l (44–300)
IOC	7.5 ± 3.0 ng/ml (2.3–20.4)
UPYR	30.5 ± 11.3 μM/mMCr (16.3–77.0)
UDPYR	5.5 ± 1.9 μM/mMCr (2.7–5.5)

All values are the mean ± SD; maximal and minimal values are shown in parentheses.
UPYR and 25(OH)D were calculated in only 75 subjects.

Correlation between Nutritional Factors and BMD or Biochemical Markers

The correlations between each nutritional factor and L2–4 BMD, *T*-score and *Z*-score for L2–4 BMD are shown in Table 2. Energy intake showed positive associations with L2–4 BMD, *T*-score for L2–4 BMD and body mass index (BMI) but not with *Z*-score for L2–4 BMD. Protein intake was also associated with the three factors other than *Z*-score for L2–4 BMD. Calcium intake was not associated with any factors. Soy protein was

Table 2. Pearson correlation coefficients between nutrients and BMD or body mass index

	Energy	Protein	Calcium	Soy protein
L2–4 BMD	0.294**	0.223*	–	0.251*
T-score	0.325**	0.225*	–	0.273*
Z-score	–	–	–	0.225*
Body mass index	0.380***	0.259*	–	0.192 (p=0.08)

***p<0.001; **p<0.01; *p<0.05.

Table 3. Pearson correlation coefficients between nutrients and bone biochemical markers

	Energy	Protein	Calcium	Soy protein
ALP	–	–	–	–
IOC	–	–	–	–
UPYR	−0.280*	−0.229*	−0.277 (p=0.05)	–
UDPYR	−0.197 (p=0.07)	–	–	−0.229*

***p<0.001; **p<0.01; *p<0.05.

positively associated with Z-score for L2–4 BMD (p=0.038). For biochemical markers, energy and protein intake were negatively associated with UPYR (p<0.05), but not with UDPYR. Soy protein was significantly associated with only UDPYR (p=0.034), but not with ALP, IOC or UPYR (Table 3).

Stepwise Multiple Regression Analyses

In a multivariate model, soy protein intake is a significant variable as an independent factor contributing to the increase in BMD, whereas energy, protein and calcium intake are not significant in preventing the reduction in BMD (Table 4; β = 0.225, p=0.038). Soy protein intake is the major contributor to the suppression of bone resorption, as shown in Table 5 (β = −0.229, p=0.035).

Table 4. Stepwise multiple regression on the effect of four nutrients on Z-score for L2–4 BMD

	Regression coefficients (β)	F-value	p-value
Energy	−0.006	0.003	–
Protein	−0.03	0.092	–
Calcium	−0.145	1.787	–
Soy protein	0.225	4.42	0.038

Independent factor: four nutrients.
Dependent factor: Z-score.
Only soy protein is significantly associated with Z-score for L2–4 BMD.

Table 5. Stepwise multiple regression on the effect of four nutrients on UDPYR

	Regression coefficients (β)	F-value	p-value
Energy	−0.197	3.354	–
Protein	−0.121	1.236	–
Calcium	−0.123	1.284	–
Soy protein	0.229	4.608	0.035

Independent factor: four nutrients.
Dependent factor: UDPYR.
Only soy protein is significantly associated with UDPYR.

Discussion

This is the first evidence that soy protein intake shows a significant negative relationship with bone resorption and the reduction of lumbar spine bone loss in postmenopausal women. Our data indicate that soy protein intake has a more significant effect on BMD and UPYR than energy, protein or calcium intakes. Soy protein might have an estrogen-like effect on bone metabolism from the point of view of reducing UDPYR as a bone resorption marker [9,10]. Soy protein contains many kinds of phytoestrogens. One of these, ipriflavone (7-isopropexy-isoflavone), which is a synthetic flavonoid, inhibits osteoclast recruitment and function, and an intake of 600 mg/day has been shown to prevent bone loss at the distal radius in osteoporotic postmenopausal women [11]. A study was conducted in which postmenopausal women were randomly assigned to receive casein or soy protein containing 1.39 mg total isoflavone/g protein or 2.25 mg total isoflavone/g protein for 6 months. The group which ingested 2.25 mg total isoflavone/g protein demonstrated increased lumbar bone mineral content (BMC) and BMD compared with the control group or with the group that ingested 1.39 mg total isoflavone/g protein [12]. This study suggested that the increase in BMC and BMD is dependent not on soy protein itself, but on the quantity of isoflavone in soy protein. Similarly the isoflavone genistein exhibits an estrogenic action in bone and bone marrow that regulates B-lymphopoiesis and prevents bone loss in mice [13]. Additionally genistein protected from post-ovariectomy loss of trabecular bone volume and BMD, increments in serum osteocalcin and osteoblast number indicating the stimulation of bone formation [14]. Further investigations are needed to examine the effect of all phytoestrogens on bone metabolism via osteoblasts bearing the estrogen receptor or a direct action on osteoclasts in vitro.

Data on the effects of dietary soy protein on bone density are conflicting. A diet containing isolated soybean protein did not prevent the increase in cortical bone turnover in ovariectomized macaques [15]. These discrepancies might be due to the site of measurement, to the animal species used, to the duration of the study or to the soybean protein dose. In our study, the daily soybean intakes were one-tenth to one-hundredth those administered to ovariectomized rats [7,8]. To clarify the effect of soy protein on bone density it is necessary to analyze which components in soy protein and the dose of isoflavone that are most efficient in preventing bone loss.

There is little disagreement concerning the necessity of calcium intake for the prevention of bone loss. Several nutrients in addition to calcium have also been shown to be related to bone loss [16]. The importance of calcium intake in elderly women has been demonstrated at all sites in the hip [17]. The main reason for the negative results concerning calcium intake and L2–4 BMD in our study is that the subjects were biased by their education concerning calcium intake at health centers before participation in this study. Calcium supplementation of

the usual diet of young adults suppresses UPYR and UDPYR, but not the levels of bone-specific IOC or ALP [18]. In our study, calcium supplementation had no influence on bone metabolism in postmenopausal women. In contrast to the negative results for calcium on bone resorption markers, soy protein intake showed an inverse association with UDPYR. This result is compatible with the effect of estrogen, on UDPYR in postmenopausal women [9,10].

High protein intake induces hypercalciuresis [19], while no significant correlations between BMD and protein intake have been found in cross-sectional studies of older women [20]. However, in terms of fracture rates, dietary protein intake and caloric sufficiency have been shown to be important for the prevention of femoral neck fractures [21,22]. Presumably the coexistence of insufficiencies in dietary protein and energy might be an important contributor to bone fracture. Low protein and energy intakes in hospitalized elderly patients are associated with decreased BMD [23]. Additionally, premenopausal women, but not postmenopausal women, show a positive association between protein and energy intake and mineral content in the distal radius and proximal femur [24]. Protein intake might be important for maintaining peak bone mass. In our study, protein intake appears less potent than soy protein for maintaining lumbar BMD in postmenopausal women.

In summary, soy protein intake is significantly correlated with *Z*-score for L2–4 BMD and UDPYR in postmenopausal Japanese women. Further studies are needed to elucidate the factors in soy protein that are the most effective for enhancing bone metabolism.

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