Intake of Fermented Soybeans, *Natto*, Is Associated with Reduced Bone Loss in Postmenopausal Women: Japanese Population-Based Osteoporosis (JPOS) Study[1]

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ABSTRACT Japanese fermented soybeans (*natto* in Japanese), which contain a large amount of menaquinone-7, may help prevent the development of osteoporosis. We assessed the possibility of an association between habitual *natto* intake and bone mineral density (BMD) and BMD change over time in healthy Japanese women who participated in a large representative cohort study (Japanese Population-based Osteoporosis Study: JPOS study). The BMD was measured at the spine, hip, and forearm in 944 women (20–79 y old) at baseline and at a follow-up conducted 3 y later. Dietary *natto* intake was assessed by a FFQ on both occasions. Additional covariates including age, height, weight, lifestyle factors, dietary calcium intake, and the intake of other soybean products, were also measured. The total hip BMD at baseline increased (P for trend = 0.0034) with increasing habitual *natto* intake in the postmenopausal women, although this was not the case at other skeletal sites. There were significant positive associations between *natto* intake and the rates of changes in BMD at the femoral neck ($P < 0.0001$) and at the distal third of the radius ($P = 0.0002$) in the postmenopausal women. The association in the femoral neck persisted even after adjusting for covariates. No significant association was observed between the intake of tofu or other soybean products and the rate of BMD change in the postmenopausal women. *Natto* intake may help prevent postmenopausal bone loss through the effects of menaquinone 7 or bioavailable isoflavones, which are more abundant in *natto* than in other soybean products. J. Nutr. 136: 1323–1328, 2006.

KEY WORDS: • *fermented soybean* • *menaquinone* • *isoflavone* • *bone density* • *Japanese women*

Osteoporosis is one of the most important diseases affecting elderly people worldwide (1). Hip fractures resulting from osteoporosis cause many elderly people to become bedridden, and osteoporosis is a great burden on society today. In the prevention of osteoporosis, nutrition plays a key role in addition to other factors such as genetics, physical activity, cigarette smoking, and alcohol intake (1–4). Many studies have assessed the role of calcium, vitamin D, magnesium, and other micronutrients, as well as macronutrients such as protein, in the prevention of osteoporosis (3–6). There is emerging evidence, however, that vitamin K may also play a protective role against age-related bone loss (7–9).

Vitamin K is a cofactor of γ-carboxylase, which mediates the conversion of undercarboxylated osteocalcin to carboxylated osteocalcin by transforming the glutamyl residues of osteocalcin into carboxyglutamic acid residues; the last-mentioned have a high affinity for calcium ions in hydroxyapatite and regulate the growth of these crystals (4). Vitamin K is classified into 2 groups: phylloquinone, which is the major form and exists in plants, and menaquinone, which is synthesized by bacteria and exists in dairy products.

Natto is a kind of fermented soybean that is consumed very widely and frequently in Japan. This food is rich in menaquinone-7 and contains >100 times more menaquinone-7 than various kinds of cheese (10).

The incidence of hip fracture in Japan is lower than in European countries and the United States. The reason for this difference is not known, but it is thought to be due to the differences in dietary (11), bone-geometric (12), and lifestyle factors (13) between these populations. *Natto* is one of the candidates that may account for this difference in hip fracture rate. Furthermore, there is some evidence suggesting that *natto* is effective in maintaining bone stiffness (10), increasing serum levels of menaquinone-7 and γ-carboxylated osteocalcin (14), and maintaining the bone mineral density (BMD) of middle-aged women (15). However, these studies were conducted

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among subjects with a limited age distribution and selected from limited areas (14,15); the sample sizes were small (14) and the studies were performed cross sectionally (10,14,15).

The purpose of the present study was to assess whether habitual *natto* intake has a positive association with BMD and with change in BMD over time in healthy Japanese women of various ages who participated in a large representative cohort study.

SUBJECTS AND METHODS

Subjects. The present study was conducted as a part of a larger study (Japanese Population-based Osteoporosis Study) (16), and involved 3 cohorts, 1 from the northeastern part of Honshu island (Nishiaizu Town, Fukushima Prefecture), 1 from Shikoku island (Sanuki City, Kagawa Prefecture), and 1 from Miyako island in the subtropical zone (Miyakojima City, Okinawa Prefecture). We recruited healthy women aged 20–79 y, randomly selected from the resident registry of each municipality. Of the 1628 women who participated in the baseline study, 1280 participated in the follow-up study 3 y later. The subjects were interviewed and were excluded if they reported current involvement or history of any disease that might affect bone metabolism, such as amenorrhea, oligomenorrhea, bilateral oophorectomy, diseases of the parathyroid gland, rheumatoid arthritis, or diabetes mellitus. Subjects with abnormal serum calcium, inorganic phosphorus, and parathyroid hormone concentrations in the present study were also excluded. Those who were being administered drugs affecting bone turnover, such as calcium, estrogens, vitamin D, calcitonin, or glucocorticoids, as determined through the interviews conducted in the present study, were also excluded. According to the menstrual information obtained from the interviews, subjects who menstruated regularly at baseline were judged to be premenopausal and those who had entered menopause at least 6 mo before the survey were classified as postmenopausal. Subjects with hysterectomy-induced menopause or whose age at menopause could not be determined were classified as postmenopausal women if they were >57 y old at the time of the survey. In Japan, 97.5% of women ≥57 y old are reported to be postmenopausal (17). The remaining subjects were classified as perimenopausal and were excluded from further analysis. In total, 336 women were excluded from further analysis; the remaining 944 women served as the representative sample of Japanese women free from apparent abnormalities affecting bone mass.

We obtained written informed consent for all study procedures from each subject. The study protocol was approved by the Ethical Committee of the Kinki University School of Medicine.

Bone mass. The BMD was measured at baseline and 3 y thereafter by dual-energy X-ray absorptiometry at the lumber spine (L_{2-4}), the right hip (QDR 4500A, Hologic), and at the distal third of the radius (pDXA, Norland/Stratec). The short-term CV of the BMD measurement in vivo, as calculated on the basis of 5 measurements on different days for each of 5 volunteers, were 1.2, 1.6, 1.2, and 1.2% for the spine, femoral neck, total hip, and distal third of the radius, respectively (16). The BMD of the spine and forearm phantoms were measured every day for quality assurance; they did not drift significantly during the study period.

Body size. The height (cm) and weight (kg) of the subjects were measured with an automatic scale (TK-11868h, Takei Kagaku). The BMI (kg/m^2) was calculated.

Interviews. Detailed interviews were conducted during the survey by trained nurses both at baseline and at follow-up. The interviews were conducted to confirm the information given on a questionnaire that was delivered to the subjects by mail and completed beforehand. The questionnaire included points on menstrual history and on the past history and present involvement of gynecological and other diseases or medications that may affect bone metabolism.

Information on the habitual intake of *natto*, bean curd (tofu), and other soybean products was obtained through an interview conducted by trained dietitians. We determined the portion size and weekly frequency of intake using a FFQ that was developed and validated previously to estimate dietary calcium intake (18). The FFQ was given to the subjects during the baseline and follow-up surveys. *Natto* is sold in a pack that usually contains 40 g of *natto*, a quantity considered to be suitable for 1 meal in Japan. One pack of *natto* contains 35 mg of calcium and 350 μg menaquinone-7 (19). *Natto* intake can be determined quite precisely by counting the number of *natto* packs consumed by the subject over a period of 1 wk. The usual portion size of tofu or other soybean products was determined through the interview by comparison with the portion size of the reference food samples. The total calcium intake, which included calcium intake from mineral supplements, was estimated on the basis of the same FFQ.

Statistical data analysis. The subjects were divided into premenopausal and postmenopausal groups; each of these 2 groups was then classified into 3 subgroups according to their mean habitual intake of *natto* at baseline or the mean intakes at baseline and at follow-up. Because the premenopausal women did not consume large amounts of *natto* compared with the postmenopausal women, we used different intervals to classify the weekly *natto* intake for these 2 groups: no intake, 1 or 2 packs, and <2 packs for the premenopausal women, and no intake, 1–4 packs, and >4 packs for the postmenopausal women.

The rate of BMD change was calculated as follows:

$$\text{The rate of change} = \{[(\text{BMD at follow up} - \text{BMD at baseline})/\text{BMD at baseline}]/3\} \times 100.$$

The results of the indices are expressed as means and SD or SEM. Student's t test was used to test the significance of the difference in mean BMD and other continuous variables between 2 groups. For comparisons among 3 groups with different habitual *natto* intake or among 3 cohorts, ANOVA was performed and, if significant, pairwise comparisons were done using t tests. The effects of confounding variables, such as age, height, weight, smoking habits, physical activity, years since menopause, and intake of calcium, tofu, and other soybean products, were adjusted using analysis of covariance (ANCOVA) where necessary. The linear regression analysis for BMD or its rate of change was performed after introducing the ordinal variable for *natto* intake, which was coded as 0, 1 or 2 according to the amount of habitual *natto* intake. The significance for the regression coefficient for the ordinal variable for *natto* intake was interpreted as the significance of trend of BMD or its rate of change with the increase in *natto* intake. The χ^2 test was used to compare the prevalence rates of lifestyle factors, such as smoking, exercise, or milk consumption. Differences were considered significant at $P < 0.05$. Scheffé's multiple comparison procedure was applied to adjust the level of statistical significance to 5% when 3 groups were compared. All statistical analyses were performed using the SAS® system for personal computers (release 8.2, SAS Institute).

RESULTS

Characteristics of the subjects at baseline and at follow up. The pre- and postmenopausal women differed in height ($P < 0.0001$) and BMI ($P < 0.0001$) at baseline. Calcium intake and the amount of *natto*, tofu, and other soybean products consumed by the postmenopausal women were higher ($P < 0.0001$) than in the premenopausal women at baseline (**Table 1**). BMD at the spine, total hip, femoral neck, and distal third decreased ($P < 0.0001$) from baseline to follow-up in the postmenopausal women. None of the variables listed in Table 1 differed among the 3 cohorts in either the premenopausal or postmenopausal group.

Habitual* natto *intake and BMD at baseline. The BMD at baseline and the potential confounding variables were compared among the groups classified according to their menopausal status and the habitual *natto* intake at baseline (**Table 2**). Habitual *natto* intake was associated with age in the premenopausal women (P for trend = 0.0019). The calcium intake increased with increasing habitual *natto* intake in both the premenopausal and the postmenopausal women (P for trend < 0.0001). The intakes of other soybean products increased with increasing habitual *natto* intake in the postmenopausal women

TABLE 1

Characteristics of pre- and postmenopausal women at baseline and at the 3-y follow-up[1]

	Premenopausal		Postmenopausal	
	Baseline	Follow-up	Baseline	Follow-up
n	394	394	550	550
Age, *y*	34.0 ± 7.1	37.1 ± 7.1*	64.2 ± 8.4#	67.2 ± 8.4*
Age at menopause, *y*	—	—	49.5 ± 3.3	49.5 ± 3.3
Time since menopause, *y*	—	—	14.7 ± 8.8	17.7 ± 8.8*
Height, *cm*	156.8 ± 5.1	156.9 ± 5.1	148.5 ± 5.3#	148.0 ± 5.6*
Weight, *kg*	53.6 ± 7.7	53.7 ± 8.1	52.9 ± 8.1#	51.7 ± 8.4*
BMI, *kg/m*^{2}	21.8 ± 3.0	21.8 ± 3.1	24.0 ± 3.3	23.6 ± 3.4*
Dietary intakes[2]				
Calcium, *mg/d*	564 ± 211	574 ± 229	685 ± 260#	699 ± 300
Natto, *packs/wk*	1.4 ± 1.8	1.8 ± 2.2*	2.0 ± 2.6#	2.1 ± 2.7
Tofu, *servings/wk*	1.1 ± 1.3	3.6 ± 2.2*	1.5 ± 1.6#	5.1 ± 3.5*
Other soybean products, *servings/wk*	0.8 ± 1.5	1.3 ± 1.7*	2.2 ± 2.5#	2.8 ± 3.3*
Prevalence of smokers, *%*	10.2	6.6*	2.4#	1.8*
Prevalence of exercise, *%*	32.1	32.6*	40.3#	49.1*
Prevalence of milk intake, *%*	71.3	78.7*	77.9	83.6*
BMD, *g/cm*^{2}				
Spine	1.034 ± 0.115	1.033 ± 0.121	0.800 ± 0.138#	0.782 ± 0.136*
Total hip	0.887 ± 0.108	0.885 ± 0.111	0.740 ± 0.117#	0.723 ± 0.117*
Femoral neck	0.798 ± 0.105	0.795 ± 0.106	0.650 ± 0.102#	0.627 ± 0.104*
Distal third of the radius	0.754 ± 0.066	0.755 ± 0.066	0.570 ± 0.110#	0.548 ± 0.106*

[1] Values are means ± SD or %. *Different from baseline, $P < 0.05$; #different from premenopausal women at that time.
[2] Dietary intake: *natto* (fermented soy beans, 40 g/pack); tofu (bean curd, 75 g/serving); other soy bean products: ground soybeans, 17.5 g/serving; green soybeans, 140 g/serving; boiled soybeans, 45 g/serving; and milk intake (≧800 mL/wk).

(P for trend < 0.0001). In the postmenopausal women, the total hip BMD increased with increasing habitual *natto* intake (P for trend $= 0.0034$), although no association existed for other skeletal sites (Table 2).

Habitual* natto *intake and the rate of BMD change. The premenopausal and postmenopausal women were grouped into 3 subgroups according to the mean habitual *natto* intake at baseline and at follow-up. The postmenopausal women showed a positive trend ($P < 0.0001$) across subgroups in the rate of femoral-neck BMD change (**Table 3**). Even after adjusting for covariates such as age, height, weight, physical activity, smoking habits, and years since menopause, as well as for the intake of calcium, tofu, and other soybean products, these associations persisted (P for trend $= 0.0094$) (**Table 4**). The BMD change at the distal third of the radius in the postmenopausal women showed a positive trend (P for trend $= 0.0002$) across categories of *natto* intake (Table 3). However, the association was not significant after adjusting for the covariates (Table 4). A similar result was obtained for the postmenopausal women grouped by age in 10-y increments. The rate of BMD change was positively associated with increasing *natto* intake at the femoral neck in the subjects in their 60s (P for trend $= 0.0019$)

TABLE 2

Baseline anthropometry, dietary intakes and BMD in pre- and postmenopausal women stratified by habitual natto *intake*[1]

	Premenopausal women				Postmenopausal women			
Natto intake, *packs*	0	1–2/wk	>2/wk	*P* for trend	0	1–4/wk	>4/wk	*P* for trend
n	190	119	85		267	197	85	
Age, *y*	32.7 ± 7.0^b	34.9 ± 7.2^a	35.4 ± 6.3^a	0.0019	64.9 ± 8.5^a	62.9 ± 8.4^b	64.7 ± 8.3^a	0.3039
Height, *cm*	156.9 ± 5.1	156.4 ± 5.0	157.4 ± 5.4	0.6147	148.7 ± 5.3	148.2 ± 5.4	148.5 ± 5.4	0.5221
Weight, *kg*	53.5 ± 7.2	53.2 ± 7.8	54.4 ± 8.6	0.4623	52.6 ± 7.7	53.0 ± 8.4	53.6 ± 8.3	0.3262
BMI, *kg/m*^{2}	21.8 ± 2.9	21.8 ± 3.0	22.0 ± 3.2	0.6428	23.8 ± 3.2	24.1 ± 3.4	24.3 ± 3.5	0.1531
Calcium intake, *mg/d*	509 ± 191^c	570 ± 179^b	679 ± 247^a	<0.0001	594 ± 213^c	721 ± 248^b	892 ± 284^a	<0.0001
Tofu,[2] *servings/wk*	1.1 ± 1.4	1.1 ± 1.3	1.2 ± 1.1	0.4674	1.5 ± 1.7	1.5 ± 1.5	1.7 ± 1.4	0.5155
Other soy bean products,[3] *servings/wk*	0.7 ± 1.3	0.9 ± 1.7	1.0 ± 1.6	0.0731	1.7 ± 2.2^c	2.4 ± 2.2^b	3.5 ± 3.3^a	<0.0001
BMD at baseline, *g/cm*^{2}								
Spine	1.034 ± 0.110	1.031 ± 0.114	1.039 ± 0.126	0.8149	0.795 ± 0.139	0.807 ± 0.139	0.800 ± 0.133	0.5779
Total hip	0.882 ± 0.110	0.893 ± 0.115	0.889 ± 0.095	0.4778	0.724 ± 0.113^b	0.752 ± 0.114^a	0.760 ± 0.134^a	0.0034
Femoral neck	0.796 ± 0.104	0.801 ± 0.112	0.801 ± 0.095	0.6551	0.642 ± 0.100	0.657 ± 0.098	0.655 ± 0.116	0.1481
Distal third of the radius	0.760 ± 0.066	0.750 ± 0.067	0.750 ± 0.064	0.2059	0.567 ± 0.112	0.567 ± 0.111	0.564 ± 0.103	0.7603

[1] Values are means ± SD. Within a group, means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] Tofu (bean curd 75 g/serving).
[3] Other soybean products: ground soybeans, 17.5 g/serving; green soybeans, 140 g/serving; boiled soybeans, 45 g/serving.

TABLE 3

The rate of change in BMD in pre- and postmenopausal women stratified by habitual natto *intake at baseline and follow-up*[1]

	Premenopausal women				Postmenopausal women			
	0	1–2/wk	>2/wk	*P* for trend	0	1–4/wk	>4/wk	*P* for trend
n	129	144	73		210	226	81	
		%/y				*%/y*		
Spine	−0.2 ± 1.2	0.0 ± 0.9	−0.1 ± 1.0	0.4914	−0.7 ± 1.5	−0.7 ± 1.7	−0.2 ± 1.3	0.0930
Total hip	−0.1 ± 0.9	0.0 ± 1.1	−0.1 ± 1.1	0.9609	−0.9 ± 1.4	−0.9 ± 1.4	−0.5 ± 1.4	0.0651
Femoral neck	−0.3 ± 1.3	−0.0 ± 1.5	−0.3 ± 1.5	0.8394	−1.6 ± 1.9[c]	−1.2 ± 1.8[b]	−0.5 ± 2.1[a]	<0.0001
Distal third of the radius	−0.1 ± 0.9	0.1 ± 1.1	0.0 ± 1.1	0.3177	−1.4 ± 1.7[b]	−1.4 ± 1.8[b]	−0.3 ± 1.5[a]	0.0002

[1] Values are means ± SD. Within a group, means in a row with superscripts without a common letter differ, $P < 0.05$.

and 70s (*P* for trend = 0.0002) and at the distal third of the radius in the subjects in their 50s (*P* for trend = 0.0069) and 60s (*P* for trend = 0.0002) (**Table 5**).

Natto intake and the rate of BMD change were not associated at any skeletal site in the premenopausal women (Table 3). Furthermore, the results did not change even when the same classification for *natto* intake as that for postmenopausal women was applied.

We also tested whether there was a relation between the intake of tofu or other soybean products and the rate of BMD change after adjusting for the effects of the covariates, but there were no associations (Table 4).

DISCUSSION

The present study showed that habitual *natto* intake was associated with reduced bone loss at the femoral neck and at the distal third of the radius in postmenopausal women. An ordinary 40-g pack of *natto* contains ~350 μg of menaquinone-7, a remarkably high amount for a food. Menaquinone-7 may have mediated the association found in the present study.

Two epidemiologic studies derived from the Framingham cohort examined the association between phylloquinone intake and BMD (20,21). In the Framingham Offspring Study (20), greater dietary phylloquinone intake was associated with higher BMD at the hip and spine in women aged 29–83 y (mean age: 58.9 y). However, there was no association between dietary phylloquinone intake and femoral-neck BMD or its change during the 4 y of follow-up in postmenopausal women aged 68–94 y (mean age: 75.3 y) in the Framingham osteoporosis study (21). The authors of these studies suspected that the lack of association in the latter study was due to the relatively small age-related bone loss because the subjects were older. The present findings support this hypothesis because the significant association was present both at the femoral neck and the distal third of the radius in subjects in their 60s but only at the femoral neck in subjects in their 70s. The mean annual BMD change at the femoral neck in the subjects in their 70s, 1.4%, was greater than that of women in their 60s, −1.2%. At the distal third of the radius, however, the mean annual change in BMD in the subjects in their 70s was −0.9%, which was smaller than that of women in their 60s, −1.1%; this change was so much less than the measurement error that the statistical power for detecting a significant association in this age group was reduced. A similar mechanism may have operated in the Framingham osteoporosis study.

For Asian populations, several clinical trials were conducted to investigate the effect of menaquinone in pharmacologic doses on BMD (22,23) and fracture risk (24–26). Shiraki, et al. (24) reported that 45 mg/d of menatetrenone maintained BMD at the spine more effectively than the control regimen (−0.4% vs. −2.6% for 24 mo, respectively) and reduced the incidence of fracture in patients with osteoporosis. That study showed that menaquinone had a preventive effect on bone loss and supports the findings of the present study. The menaquinone-7 content in an ordinary pack of *natto* is <1% of the pharmacologic dose. Therefore, the preventive effects of dietary *natto* intake on bone loss may be small compared with that of menaquinone medication. The preventive effect of phylloquinone or menaquinones on fractures was also previously described (14,21,27). These studies support our speculation that the habitual intake of phylloquinone or menaquinones attenuates bone loss at the femoral neck, and may lead to reduction in the incidence of hip fractures in postmenopausal women. We should follow the present subjects further to assess whether *natto* intake reduces the risk of hip fracture by maintaining the BMD at the femoral neck.

TABLE 4

The rate of change in BMD in postmenopausal women stratified by habitual intakes of natto, tofu, and other soybean products at baseline and at follow-up adjusted for other covariates[1]

Soybean products	Intake classification	Adjusted mean rate of change in BMD	
		Femoral neck	Distal third of radius
		%/y	
Natto[2]	0	−1.5 ± 0.2[b]	−1.2 ± 0.2[ab]
	1–4/wk	−1.3 ± 0.2[b]	−1.5 ± 0.2[b]
	>4/wk	−0.4 ± 0.3[a]	−0.6 ± 0.3[a]
	P for trend	0.0094	0.2261
Tofu[3]	0	−0.6 ± 1.1	−1.5 ± 1.0
	1–4/wk	−1.2 ± 0.1	−1.2 ± 0.1
	>4/wk	−1.5 ± 0.3	−1.4 ± 0.3
	P for trend	0.2990	0.3601
Other soybean products[4]	0	−1.1 ± 0.3	−1.5 ± 0.3
	1–4/wk	−1.4 ± 0.1	−1.2 ± 0.1
	>4/wk	−0.8 ± 0.3	−0.9 ± 0.3
	P for trend	0.4320	0.2283

[1] Values are means ± SEM. Means are adjusted by ANCOVA for the covariates measured at baseline and follow-up, including age, height, weight, calcium intake, exercise, smoke, years since menopause, and intakes of *natto*, tofu, other soybean products. Within a group, means in a column with superscripts without a common letter differ, $P < 0.05$.

[2] *Natto* (fermented soybeans 40 g/pack).

[3] Tofu (bean curd 75 g/serving).

[4] Other soy bean products: ground soybeans, 17.5 g/serving; green soybeans, 140 g/serving; boiled soybeans, 45 g/serving.

TABLE 5

The rate of change in BMD in postmenopausal women stratified by age group and habitual natto intake[1]

Age	*Natto* intake classification	*n*	Spine	Total hip	Femoral neck	Distal third of the radius
y					*%/y*	
50–59	0		−1.0 ± 1.4	−0.8 ± 1.2	−1.6 ± 1.6	−1.9 ± 1.7[b]
	1–4/wk	53	−1.3 ± 1.4	−0.7 ± 1.1	−1.2 ± 1.9	−1.8 ± 1.7[b]
	>4/wk	77	−0.8 ± 1.2	−0.4 ± 0.9	−1.0 ± 1.4	−0.7 ± 1.5[a]
	P for trend	26	0.9093	0.0842	0.0779	0.0069
60–69	0		−0.6 ± 1.3[b]	−0.6 ± 0.9	−1.4 ± 1.6[b]	−1.4 ± 1.8[b]
	1–4/wk	71	−0.4 ± 1.7	−0.7 ± 1.3	−1.1 ± 1.6	−1.1 ± 1.5[b]
	>4/wk	86	0.5 ± 1.2[a]	−0.4 ± 1.2	−0.2 ± 1.9[a]	0.1 ± 1.3[a]
	P for trend	28	0.0043	0.6486	0.0019	0.0002
70–79	0		−0.4 ± 1.6	−1.1 ± 1.8	−1.8 ± 2.3[b]	−1.0 ± 1.7
	1–4/wk	80	0.1 ± 1.5	−1.0 ± 1.8	−1.2 ± 1.9	−1.0 ± 2.2
	>4/wk	48	−0.3 ± 1.2	−0.8 ± 2.1	−0.5 ± 2.9[a]	−0.4 ± 1.5
	P for trend	25	0.4216	0.4268	0.0076	0.2081

[1] Values are means ± SD. Within a group, means in a column with superscripts without a common letter differ, $P < 0.05$.

Isoflavones also may be effective components of *natto*. *Natto* contains large amounts of isoflavones, which were reported to reduce bone resorption through estrogenic mechanisms (28–30). One portion of tofu also contains an amount of isoflavones similar to that in 1 pack of *natto*. However, tofu intake was not associated with bone loss prevention in our subjects. This may be due to the difference in the types of isoflavones present in *natto* and tofu. Isoflavone aglycones are absorbed faster in humans and are more bioavailable than isoflavone glycosides (31). Fermented soy products contain larger amounts of aglycones than other soy products (32–34). Morabito et al. (35) conducted a randomized placebo-controlled trial to evaluate the effect of genistein, one of the aglycones, on BMD in women aged 47–57 y. The administration of 54 mg/d of genistein significantly increased BMD at the femoral neck (genistein: 3.6 ± 3.0% vs. placebo: −0.7 ± 0.1%) after 1 y of treatment. In the present study, only *natto* decreased bone loss. We assume, therefore, that this may be due to the greater amount of isoflavone aglycone contained in *natto* in addition to menaquinone-7.

The present study had several advantages over previous studies with respect to study design. It used a larger representative sample of the general population, the ages of the subjects varied widely, and the follow-up rate was acceptable. However, its limitations should be addressed. First, our FFQ listed only calcium-rich foods. Therefore, we could not adjust the results for potential confounding due to energy intake and protein intake. Subjects who consumed a large amount of *natto* may also have consumed large amounts of energy and protein, which may have resulted in part in the protective effect on bone loss in the present study. This possible confounding could be controlled for through the adjustment for height and weight in the present analysis, but may still have been present. Second, *natto* contains large amounts of menaquinone-7, but we did not examine the effects of any other food containing menaquinone-7. Therefore, the results should be interpreted carefully and the conclusion should be restricted to the effect of *natto*. Third, the information on *natto* intake was obtained through interviews based on a questionnaire, and there may have been individual misclassifications of *natto* intake. Fourth, we did not observe any association between tofu or other soybean products and BMD in this study. One reason for this is that *natto* intake could be determined easily and precisely through the FFQ (because *natto* is sold in packs containing a similar amount of *natto*). This information may yield a more accurate estimate of the consumption of this soybean product compared with any others. Fifth, the 3 study areas were not randomly selected from all municipalities in Japan; thus, the subjects might not be representative of the Japanese female population. However, there were no differences in body size or the BMD at the skeletal sites between the present subjects and the Japanese female population. It is not likely, therefore, that the present findings originated from a sampling bias.

The present representative cohort study suggests that *natto* intake may decrease the loss of bone mass at the femoral neck and possibly at the distal third of the radius in postmenopausal women. Further studies on this topic should be designed to allow for possible confounding effects due to protein and energy intakes and to address whether the effect of menaquinone-7 or of the isoflavones is the primary mechanism.

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