J. Home Econ. Jpn. Vol. 54 No. 8 613～621 (2003)

Preventive Effects of a Fermented Soy Extract Containing Isoflavone Aglycons on Bone Loss in Ovariectomized Rats Fed on a Calcium-Deficient Diet

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A fermented soy extract (FSE) was obtained from soy beans that have been fermented with koji mold. This FSE contained 40% (w/w) isoflavone aglycons such as genistein and daidzein. Its effects on bone loss in ovariectomized (OVX) young (5 w) and old (34 w) rats that were fed on a calcium-deficient diet were tested. FSE was orally administered to both ages of OVX rats for 4 weeks. The femurs of the young rats were significantly reduced in their density and strength (breaking force) in comparison with those of the sham-operated rats. These changes were largely prevented in those rats orally receiving FSE with an isoflavone content of 250, 125 or 62.5 mg/kg/d for 4 weeks and in rats orally receiving genistein (50 mg/kg/d), but not in the daidzein-treated group (50 mg/kg/d). Ovariectomy caused atrophy of the uterus, but the uterus weight of the OVX rats administered with FSE and genistein did not differ significantly from that of the OVX rats. The loss of bone with ovariectomy was prevented by the administration of FSE or genistein, but not of daidzein. We also tested the effect of FSE on the bone loss in aged female rats (34 weeks) whose bones were no longer growing. A decrease in bone density and strength was prevented in aged OVX rats receiving FSE orally for 4 weeks at a dose of 250, 125 or 62.5 mg/kg/d. In conclusion, FSE proved to be effective in preventing bone loss in the old rats. Furthermore, the fact that FSE prevented bone loss in the OVX rats suggested a contribution by genistein but not by daidzein.

(Received August 23, 2002; Accepted in revised form May 30, 2003)

Keywords: fermented soy extract, genistein, daidzein, ovariectomy, bone loss, rat.

INTRODUCTION

Osteoporosis associated with ovarian hormone deficiency after menopause is the most common cause of aged-related bone loss.[1] A sharp decrease in ovarian estrogen production is the predominant cause of the rapid hormone-related bone loss during the first decade after menopause. Phytoestrogens have recently been studied for their potential to prevent postmenopausal osteoporosis caused by a deficiency of estrogen.[2-7]

Soybean is used as a foodstuff and traditional crude drug in the Orient. There are many reports of soy products containing such isoflavones as soymilk, soy powder and soy protein exhibiting a protective effect on the bone loss caused by estrogen deficiency.[8] It has recently been reported that isoflavones increased the bone density in ovariectomized (OVX) rats and postmenopausal women and that the level of deoxypyridinolin in the urine was a marker for bone loss. It is known that soy isoflavones have weak estrogenic activity.[9]

To express their activities, soy isoflavones must be absorbed into the body fluid. It is generally thought that isoflavone glycosides are converted to the corresponding aglycons by gut microflora and gut glucosidase, and then that the aglycons are absorbed from the gut. It has been reported in a recent study that the isoflavone aglycons, genistein and daidzein, were absorbed from the stomach[10] faster and in larger amounts than their glucosides in humans.[11]

Isoflavone glucosides in raw soybean were converted into the corresponding aglycons by a fermentation process using koji mold. We focused on a fermented soybean extract (FSE) containing soy isoflavone aglycons, and investigated the effect of

FSE on the loss of bone mass due to ovarian hormone deficiency in young (5 w) and old (34 w) rats and on whether or not it had any influence on the uterus.

MATERIALS AND METHODS

Materials

A fermented soy extract (FSE) rich in isoflavone (SoyAct™) was provided by Kikkoman Corporation (Chiba, Japan). FSE was obtained by extracting with ethanol/water steamed soybeans that had been fermented with koji mold. The composition of FSE was 429.4 mg/g of isoflavones as their aglycons (192.0 mg/g of genistein, 211.2 mg/g of daidzein and 26.2 mg/g of glycitein), 112.3 mg/g of saponin, 132.4 mg/g of protein containing hydrophobic amino acids and peptides with a molecular weight under 10,000, 126.0 mg/g of carbohydrate, 50.0 mg/g of fat, 29.1 mg/g of moisture, 20.4 mg/g of ash, and 1 mg/g of fiber. FSE did not contain phytic acid, tripsin inhibitors or vitamins.

Genistein and daidzein were purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Ipriflavone was extracted from Osten tablets (Takeda Chemical Industries Ltd.).

Animals and administration procedure

Female Sprague-Dawley rats, aged 4 and 8 weeks, were purchased from Japan SLC (Hamamatsu). The animals were acclimatized in an environmentally-controlled animal laboratory and fed on commercial laboratory feed (solid) containing 1.1% Ca and 1.1% P at a room temperature of 25℃, with free access to distilled water, for 1 week or 33 weeks. At 5 weeks of age for the test on young rats, the rats were divided into 7 groups of 6 animals. The animals in group 1 were subjected to a sham operation, while those in groups 2 to 7 underwent a bilateral ovariectomy under halotan anesthesia. In the sham-operated animals, both ovaries were handled, but not removed. All the animals were fed on the commercial feed for 1 week. From 6 weeks of age, all the animals were allowed free access to a commercial calcium (Ca)-deficient diet (powder) containing 0.01% Ca and 0.3% P (Table 1, CLEA Japan, Tokyo) and to de-ionized water for 28 d.

At 33 weeks in the test on the old rats, the rats were divided into 6 groups of 6 animals. The animals in group 1 were subjected to a sham operation, while those in groups 2 to 6 underwent a bilateral ovariectomy. All the animals were fed matched amounts of the commercial feed for 1 week. From 34 weeks of age, all the animals were allowed free access to the commercial calcium-deficient diet and to de-ionized water for 28 d.

FSE, genistein and daidzein were orally administered every day by gavage. The food intake of all rats was measured every 3-4 d. Throughout this period, FSE was given to the young rats in groups 3, 4 and 5 (62.5, 125 and 250 mg/kg of body weight per day, respectively), and 50 mg of genistein and daidzein/kg of body weight per day to those in groups 6 and 7. The aged rats in group 6 were given 50 mg of ipriflavone/kg of body weight per day. Both the normal (sham-operated) and control (ovariectomized) rats received the commercial calcium-deficient diet.

At the age of 14 weeks for the young rats or 42 weeks for the old rats, all animals were killed with an overdose of sodium phenobarbital. The femurs and tibias were removed immediately after bleeding for a bone analysis. The uterus was also removed, and the wet weight, including the intrauterine fluid, was determined.

Table 1. Composition of the diet

Constituent	Ca-deficient diet (%) 0.01% Ca, 0.3% P
Glucose	65.1
Casein (vitamin-free)	18.0
Cotton seed oil	10.0
Cellulose	3.0
Ca- and P-free mineral mixture [a]	2.0
Equimolar mixture of KH_2PO_4 and K_2HPO_4	1.39
$CaCO_3$	0.005
Cystine	0.2
Water-soluble vitamin mixture [b]	0.1
Fat-soluble vitamin mixture	—[c]
Choline Cl	0.2

[a] Ca- and P-free mineral mixture (in %): KCl, 57.7; NaCl, 20.9; $MgSO_4$, 17.9; $FeSO_4 \cdot 7H_2O$, 3.22; $CuSO_4 \cdot 5H_2O$, 0.078; NaF, 0.113; $CoCl_2 \cdot 6H_2O$, 0.004; KI, 0.01; $MnSO_4 \cdot 5H_2O$, 0.06; $ZnSO_4 \cdot 7H_2O$, 0.44; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.05. [b] Water-soluble vitamin mixture consisting of (in %): thiamine, 0.5; reboflavine, 0.5; pyridoxine, 0.5; calcium pantothenate, 2.8; nicotinamide, 2.0; inositol, 20.0; folic acid, 0.02; vitamin B_{12}, 0.002; biotin, 0.01; glucose monohydrate, 73.7. [c] The rats received a supplement of fat-soluble vitamins in cotton seed oil three times a week supplied with 70 μg of beta-carotene, 105 μg of 2-methyl-1,4-naphthoquinone, 875 μg of alpha-tocopherol and 525 IU of vitamin D_3.

Table 2. Body weight, food intake and uterine weight at the end of the study on young rats

	Group	Body weight (g)	Food intake (g)	Uterine weight (g)
1	Sham-operated	225.7±9.0	14.49±0.29	0.545±0.109
2	OVX	271.4±15.7**	15.11±0.32**	0.102±0.013**
3	OVX+FSE (250 mg/kg)	264.5±9.3**	15.00±0.50**	0.114±0.017**,†
4	OVX+FSE (125 mg/kg)	276.1±10.0**	15.08±0.36**	0.114±0.020**
5	OVX+FSE (62.5 mg/kg)	261.7±12.8**	15.11±0.30**	0.105±0.013**
6	OVX+genistein (50 mg/kg)	270.0±10.4**	15.02±0.33**	0.127±0.022**
7	OVX+daidzein (50 mg/kg)	265.4±17.1**	14.50±0.50	0.112±0.012**

Each value is the mean±SD (n=6). ** Significantly different from the sham-operated group, $p<0.01$. † Significantly different from the OVX group, $p<0.05$.

Table 3. Body weight, food intake and uterine weight at the end of the study on aged rats

	Group	Body weight (g)	Food intake (g)	Uterine weight (g)
1	Sham-operated	373.7±34.2	13.48±1.04	0.852±0.229††
2	OVX	361.1±37.9	14.29±0.57	0.322±0.184**
3	OVX+FSE (250 mg/kg)	374.8±38.5	13.28±1.84	0.438±0.100**
4	OVX+FSE (125 mg/kg)	371.1±34.5	11.99±2.43	0.359±0.124**
5	OVX+FSE (62.5 mg/kg)	383.9±31.7	11.50±2.79	0.277±0.098**
6	OVX+ipriflavone (100 mg/kg)	375.5±11.4	13.50±1.09	0.267±0.059**

Each value is the mean±SD (n=6). ** Significantly different from the sham-operated group, $p<0.01$. †† Significantly different from the OVX group, $p<0.01$.

Table 4. Concentrations of serum calcium and phosphorus at the end of the study on young rats

	Group	Serum calcium (mg/ml)	Phosphorus (mg/ml)
1	Sham-operated	9.73±0.25*	6.70±1.29
2	OVX	9.36±0.21†	7.01±0.64
3	OVX+FSE (250 mg/kg)	9.61±0.23*	6.74±0.90
4	OVX+FSE (125 mg/kg)	9.50±0.32	6.99±0.81
5	OVX+FSE (62.5 mg/kg)	9.50±0.32	6.53±0.71
6	OVX+genistein (50 mg/kg)	9.63±0.25*	6.53±0.56
7	OVX+daidzein (50 mg/kg)	9.54±0.35	6.10±0.45*

Each value is the mean±SD (n=6). * Significantly different from the OVX group, $p<0.05$. † Significantly different from the sham-operated group, $p<0.05$.

Measurement of the mechanical bone strength

During the dissection, the right and left femoral bones were isolated and the muscles and connective tissues were carefully removed. The mechanical strength of each bone was determined by a breaking test with a DYN-1255 instrument (Lio Co.) as previously reported.[12] The force and energy necessary for a break at the center of the femoral diaphyses were measured under conditions of a 1.0-cm sample space, 100 mm/min plunger speed and 50 kg load range.

Table 5. Concentrations of serum calcium and phosphorus at the end of the study on aged rats

	Group	Serum calcium (mg/ml)	Phosphorus (mg/ml)
1	Sham-operated	10.21±0.37**	6.92±0.51**
2	OVX	9.73±0.31††	6.28±0.47††
3	OVX+FSE (250 mg/kg)	9.94±0.70	6.77±0.59
4	OVX+FSE (125 mg/kg)	9.74±0.46†	6.53±0.56
5	OVX+FSE (62.5 mg/kg)	9.70±0.40††	6.48±0.50
6	OVX+ipriflavone (100 mg/kg)	9.72±0.43†	6.49±0.77

Each value is the mean±SD (n=6). ** Significantly different from the OVX group, $p<0.01$. † Significantly different from the sham-operated group, $p<0.05$. Significantly different from the sham-operated group, $p<0.01$.

Table 6. Length, strength and density of the femur at the end of the study on young rats

	Group	Femoral length (mm)	Femoral strength ($\times 10^6$ dyn)	Tibial density (g/cm^3)		
				Proximal	Distal	Diaphysis
1	Sham-operated	29.77±0.63	6.85±1.10††	0.139±0.009	0.128±0.003††	0.121±0.006††
2	OVX	32.13±0.77	5.12±1.11**	0.135±0.006	0.122±0.004**	0.113±0.004**
3	OVX+FSE (250 mg/kg)	31.23±0.87	5.88±0.95††	0.135±0.004	0.130±0.004††	0.120±0.004††
4	OVX+FSE (125 mg/kg)	31.22±0.77	5.55±0.90*	0.137±0.006	0.128±0.006	0.119±0.004†
5	OVX+FSE (62.5 mg/kg)	31.86±0.78	5.60±0.85	0.135±0.004	0.127±0.003	0.120±0.003††
6	OVX+genistein (50 mg/kg)	31.26±0.70	5.78±1.08†	0.137±0.004	0.128±0.005†	0.119±0.007
7	OVX+daidzein (50 mg/kg)	31.45±0.76	5.14±0.70**	0.136±0.004	0.123±0.007	0.114±0.005

Each value is the mean±SD (n=7). * Significantly different from the sham-operated group, $p<0.05$. ** Significantly different from the sham-operated group, $p<0.01$. † Significantly different from the OVX group, $p<0.05$. †† Significantly different from the OVX group, $p<0.01$.

Measurement of the bone mineral density

During the dissection procedure, the lumbar spine and the right and left tibial bones were isolated. All the muscles and connective tissues were carefully removed. Thereafter, the bone mineral density (BMD) of the fourth and fifth lumbar vertebrae (L 4 and L 5) and of the tibial proximal metaphysis and diaphysis were examined by dual X-ray absorptiometry (DXA; Hologic QDR-1000 X-ray bone densitometer) as previously reported.[13] In comparison with the BMD value for a human, BMD of a small animal such as a rat is remarkably low in density. Therefore, all scans were performed in the ultra-high-resolution scan mode (rat mode, Version 2.0 software); *i.e.* a line spacing of 0.0254 cm and point resolution of 0.0127 cm (normally 0.1003 cm and 0.0965 cm). A collimator with a single slit was also attached to the X-ray generator.

Bone ash weight and calcium content of the ash

After measuring the weight of each right femur, the bone was converted to ash by heating for 24 h at 550–600℃. Each ash sample was weighed and then dissolved in 1 N nitric acid. Calcium was measured by atomic absorption spectrophotometry (AA-880, Jarrell Ash, Japan).

Serum calcium and phosphorus

Blood samples were centrifuged within 30 min after their collection. The serum was separated and immediately analyzed. Serum calcium and inorganic phosphate were measured spectrophotometrically by commercial kits (Calcium C-test Wako, Phospha B-test Wako and Creatinine-HR II-test Wako, respectively).

Statistical methods

The significance of differences between values was estimated by Student's t-test and Duncan's multiple-

comparison test; p values of less than 0.05 are considered to indicate statistically significant differences.

RESULTS

Body weight and food intake

At the end of the study, the OVX groups of both young and old rats had a significantly higher mean body weight as well as a significantly higher food intake than the sham-operated groups (Tables 2 and 3).

Uterine weight

The uterus weights are listed in Tables 2 and 3. Ovariectomy caused atrophy of the uterus at both ages. It is known that the administration of estrone to OVX animals causes hypertrophy of the uterus[14]. However, FSE did not produce hypertrophy of the uterus in any of the rats. The uterine weight of the younger rats treated with genistein increased slightly, but not significantly.

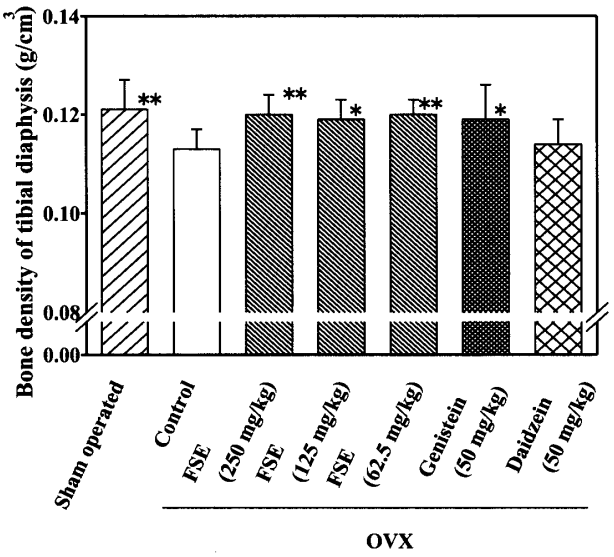

Fig. 1. Bone density of the tibial diaphysis at the end of the experiment on young rats

At the end of week 14, all the young rats were killed with an overdose of sodium phenobarbital. The tibials were immediately removed after bleeding for a bone analysis. The bone mineral density (BMD) of the tibial diaphysis was measured by dual X-ray absorptiometry. $^{*}p<0.05$, $^{**}p<0.01$ (significantly different from the OVX group).

Serum calcium and phosphorus

The serum concentrations of calcium and phosphorus in both the young and old rats are summarized in Tables 4 and 5. The serum calcium concentration was less in the OVX rats than in the sham-operated rats. In the young rats, the administration of FSE (250 mg/kg) and genistein (50 mg/kg) significantly prevented this decrease in the serum calcium content. The administration of FSE and ipriflavone to the old rats tended to prevent the loss of calcium. The phosphorus content was not appreciably different in the ovariectomized rats of any treatment group.

Length, strength, density, ash weight and calcium content of the femur

The results are shown in Tables 6, 7, 8 and 9. None of the treatments influenced the length of the femur in the young or old rats (Tables 6 and 7). However, ovariectomy significantly reduced the other morphological indices that were examined. In both experiments, the tibial density and femoral strength were significantly less in the OVX group than in the sham-operated group (Tables 6 and 7). The results for the young rats are shown in Tables 6 and 8. The femoral mechanical strength of the animals treated with FSE (250 mg/kg) was significantly greater than that of the OVX group. However, the tibial density of the FSE-treated groups was similar to that of the sham-operated group. Notably, the tibial distal and diaphysis of the FSE-treated rats had a higher bone

Table 7. Femoral length, strength and density at the end of the study on aged rats

	Group	Femoral length (mm)	Femoral strength ($\times 10^6$ dyn)	Tibial density (g/cm^3) Proximal	Distal	Diaphysis
1	Sham-operated	34.40±0.114	21.27±1.98†	0.261±0.015††	0.217±0.011	0.216±0.012
2	OVX	34.48±0.060	18.54±2.46*	0.234±0.010**	0.215±0.012	0.214±0.009
3	OVX+FSE (250 mg/kg)	35.20±0.048	21.06±2.36†	0.258±0.005††	0.225±0.009	0.227±0.004††
4	OVX+FSE (125 mg/kg)	35.41±0.102	20.53±2.25	0.238±0.009*	0.221±0.010	0.217±0.010
5	OVX+FSE (62.5 mg/kg)	35.35±0.093	22.26±2.75††	0.242±0.010*	0.222±0.014	0.221±0.011
6	OVX+ipriflavone (100 mg/kg)	34.04±0.077	19.35±2.29	0.236±0.009*	0.219±0.012	0.217±0.009

Each value is the mean±SD ($n=7$). *Significantly different from the sham-operated group, $p<0.05$. **Significantly different from the sham-operated group, $p<0.01$. †Significantly different from the OVX group, $p<0.05$. ††Significantly different from the OVX group, $p<0.01$.

density than those of the OVX group (Fig. 1). On the other hand, feeding genistein (50 mg/kg) significantly increased the femoral strength and distal tibial density when compared with the OVX rats. However, the administration of daidzein didn't result in a recovery of the femoral strength or tibial density (Table 6, Fig. 1). The ash/dry weight ratio of the femur was significantly greater in the FSE- and genistein-treated rats than in the OVX group, but not the sham-operated group (Table 8). In the FSE (250 mg/kg)-treated group, the amount of Ca in the dried femur was significantly higher than that in the OVX group.

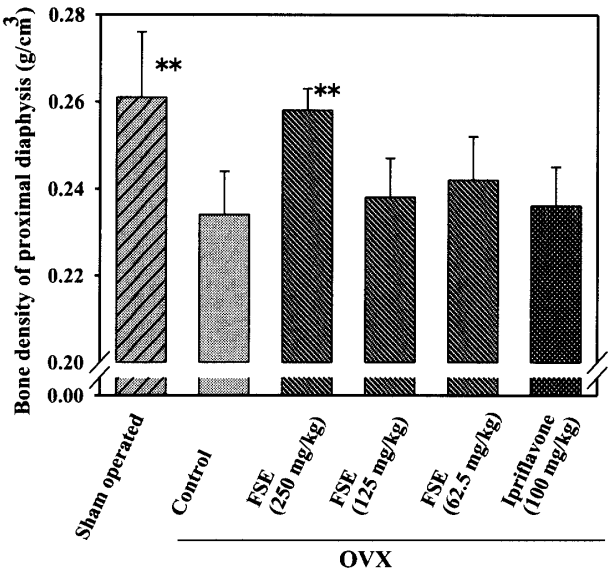

Fig. 2. Bone density of the tibial proximal at the end of the experiment on aged rats

At the end of week 42, all the old rats were killed with an overdose of sodium phenobarbital. The tibials were immediately removed after bleeding for a bone analysis. The bone mineral density (BMD) of the tibial proximal was measured by dual X-ray absorptiometry. $^{**}p<0.01$ (significantly different from the OVX group).

The results for the aged rats are shown in Tables 7 and 9. The administration of FSE appears to have inhibited the decrease in femoral mechanical strength and tibial bone density at doses of 62.5 and 250 mg/kg (Fig. 2). The ratio of dried bone to fresh bone was greater in the FSE-treated rats than in the OVX group. Furthermore, the administration of FSE (250 mg/kg) inhibited the decrease in Ca content of the femoral ash. The Ca content of the FSE-treated group (250 mg/kg) was almost the same as that of the sham-operated group (Table 9). However, ipriflavone (100 mg/kg) didn't have any positive effect on the femoral bone strength and tibial density (Table 7, Fig. 2).

DISCUSSION

This study has examined whether a fermented soy extract (FSE) containing phytoestrogens such as genistein and daidzein from soy beans that had been fermented by koji mold would be effective for preventing bone loss due to ovariectomy in young and old rats (5 weeks and 33 weeks old at the beginning of the test, respectively).

In the young rats, the OVX group had a significantly greater final body weight and food intake than the sham-operated group, as presented in a previous report.[15] The administration of daidzein prevented the ovariectomy-induced increase in food intake (Table 2). However, body weight and food intake in the aged OVX rats were no higher than those in the sham-operated group. The effects of the OVX operation on the body weight and food intake may be at-

Table 8. Ash content of the femur at the end of the study on young rats

	Group	Dry/fresh (%)	Ash/dry (%)	Ca/dry (%)
1	Sham-operated	54.37±3.71†	53.66±2.01††	20.09±0.47††
2	OVX	50.57±2.58*	46.94±1.59**	17.14±0.72**
3	OVX+FSE (250 mg/kg)	51.74±3.32	49.25±1.95**,†	18.11±0.66**,†
4	OVX+FSE (125 mg/kg)	50.73±2.97	49.74±2.44**,†	ND
5	OVX+FSE (62.5 mg/kg)	52.00±2.33	48.43±1.07**	ND
6	OVX+genistein (50 mg/kg)	50.80±4.22	47.89±2.69**	ND
7	OVX+daidzein (50 mg/kg)	51.20±3.40	46.76±3.20**	ND

Each value is the mean±SD ($n=6$). *Significantly different from the sham-operated group, $p<0.05$. **Significantly different from the sham-operated group, $p<0.01$. †Significantly different from the OVX group, $p<0.05$. ††Significantly different from the OVX group, $p<0.01$. ND: Not determined. The mean Ca content in the ash of the sham-operated group was 53.92 mg.

Table 9. Ash content of the femur at the end of the study on aged rats

	Group	Dry/fresh (%)	Ash/dry (%)	Ca/dry (%)
1	Sham-operated	66.55±1.65	64.87±1.01††	14.78±1.06†
2	OVX	66.13±1.29	60.73±1.45	13.33±1.10*,†
3	OVX+FSE (250 mg/kg)	68.47±2.38*,†	62.50±1.65†	14.42±0.65†
4	OVX+FSE (125 mg/kg)	67.97±1.97†	60.79±1.56	ND
5	OVX+FSE (62.5 mg/kg)	68.22±1.56††	61.97±1.76	ND
6	OVX+ipriflavone (100 mg/kg)	66.85±1.70	61.63±2.94	ND

Mean value is the mean±SD (n=7). * Significantly different from the sham-operated group, $p<0.05$. † Significantly different from the OVX group, $p<0.05$. †† Significantly different from the OVX group, $p<0.01$. ND: Not determined. The mean Ca content in the ash of the sham-operated group was 97.99 mg.

tributed to the difference in basal growth metabolism of growing and grown rats.

In a previous study,[16] the group of OVX rats developed uterine atrophy, which was prevented by the administration of estrone or daidzein at a dose of 50 mg/kg/day, but, interestingly, not by genistein. We found that the uterine atrophy of the OVX rats was not prevented by the administration of FSE or daidzein in the young rats (Table 2), although the uterus weight of the genistein-administered rats increased slightly. Furthermore, the uterine atrophy in the aged OVX rats was increased slightly by the administration of FSE, but not significantly (Table 3). Fanti *et al.* have reported a reduction in uterine atrophy due to the administration of 25 mg/kg of genistein.[17] In our experiments, neither FSE nor isoflavone aglycons influenced the uterine conditions, contrary to their report.[16][17] FSE may have contained a component that inhibited the effects on uterine atrophy caused by ovariectomy. Anderson and Garner have reported that a genistein or genistein-rich safflower seed extract prevented bone loss without exhibiting an estrogenic action on the uterus.[9] Further research is required to clarify the influence of genistein on the uterus. In clinical use, however, the influence on uterine conditions is thought to be a side effect, so isoflavones are effective in clinical use.

The serum calcium and phosphorus levels in the OVX rats were both lower than those in the sham-operated rats (Tables 4 and 5). In the young rats, the decrease in serum calcium level was inhibited by the administration of FSE, and the serum phosphorus level was not changed by ovariectomy (Table 4). The administration of FSE and ipriflavone to the aged OVX rats resulted in a slight increase in the serum calcium concentration. Further study is needed to clarify the effects of FSE and isoflavone on the serum calcium and phosphorus levels.

In respect of the femoral diaphysis of the young rats, the administration of FSE or only genistein for 4 weeks prevented a reduction in the bone breaking strength and calcium content of the bone ash (Tables 6 and 8). Furthermore, the bone density of the tibial distal and diaphysis was improved by the administration of FSE. No dose-response effect on the bone density of the tibial diaphysis in the young rats was apparent (Table 6, Fig. 1). Yamaguchi and Gao have reported that genistein inhibited the decrease in calcium content of femoral-metaphyseal tissues caused by prostaglandin E_2.[18] They have shown that the effect reduced the a plateau level at over 10^{-6} M. The administration of 62.5 mg/kg may have produced a blood concentration high enough to improve the bone density. In the aged rats, the loss of femoral strength and density in the proximal and diaphysis of the tibial bone were prevented by the administration of FSE (Table 7). The administration of ipriflavone to the aged OVX rats had no influence on the bone index (Table 9). These results are consistent with the report by Yamazaki *et al.*[15] that ipriflavone augmented the activity of estrogen to prevent bone loss when a low dose of estrogen was administered simultaneously, but showed a very weak preventive effect itself in OVX rats. In the examination of the OVX rats, ipriflavone may have been unable to fully demonstrate its activity to prevent bone loss.

We found that genistein was more effective in preventing bone loss than daidzein (Table 6). The prevention of bone loss by the administration of genistein has also been reported by Fanti *et al.*[17] It has recently been shown that the administration of genistein with exercise training was useful for increasing the bone

mass of OVX rodents.[19)20)] Furthermore, genistein has been shown to have an estrogenic action on the bone,[9)] to inhibit osteoclastic activity [21)-23)] and to stimulate osteoprotegerin production.[24)] Therefore, genistein was more efficient than daidzein in preventing bone loss, although it has been reported that daidzein was the key isoflavone, and not genistein, in preventing bone loss.[16)25)26)] Further study is needed to clarify the difference in activity between genistein and daidzein.

We have shown in this study the positive effect of FSE and genistein on bone loss in young and old OVX rats. This preventive action is attributed to the weak estrogenic activity of isoflavones. Studies *in vivo* and *in vitro* have shown that genistein and daidzein exerted a weak estrogenic effect,[9)27)] approximately 1×10^{-3} to 1×10^{-5} that of estradiol. However, genistein, daidzein and FSE did not affect atrophy of the uterus in the OVX rats. The clinical use of isoflavones for treating bone loss after a climacteric disturbance may be effective due to the absence of such side effects as metrorrhagia associated with hormone replacement therapy. Studies are continuing to clarify the potential clinical value.

The authors express their thanks to Dr. S. Ishii, Manager of the Research & Development Division of Kikkoman Co. for discussions and encouragement. They are also indebted to the members of the Pharmacology & Product Safety group for technical assistance.

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低カルシウム食で飼育した卵巣摘出ラットの骨密度低下に対するイソフラボンアグリコンを含有する大豆発酵抽出物の予防効果

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原稿受付平成14年8月23日；原稿受理平成15年5月30日

麹菌によって発酵した大豆から抽出した大豆発酵抽出物（FSE: Fermented Soy Extract）はゲニステインとダイゼインのようなイソフラボンアグリコンを40%（w/w）含んでいる．今回我々は，低カルシウム食で飼育した卵巣摘出（OVX）若年ラット（5 w）および加齢ラット（34 w）の骨密度および骨強度の低下などに対するFSEの影響を試験した．FSEは，4週間にわたって両方のOVXラットに経口的に投与された．飼育期間終了後，若年OVXラットの大腿骨は，偽手術されたラットの大腿骨と比較して著しく骨密度および強度が減少した．しかしながら，FSEを250，125および62.5 mg/kg/d（イソフラボンとして100，50および25 mg/kg/d）を投与した各群とゲニステイン（50 mg/kg/d）を投与した群において，これらの減少は，観察されなかった．また，ダイゼインによる骨密度の減少の予防効果は，ゲニステイン投与群と比較して弱かった．卵巣を摘出されたラットのすべてにおいて，子宮の萎縮が観察されたが，FSEまたはゲニステインを投与された群において，子宮重量の増加は認められなかった．さらに，骨の代謝が若年ラット（5 w）より活発でない加齢ラット（34 w）を用いて，OVXによる骨塩量減少に対するFSEの影響を試験した．加齢ラットにおいても，OVXによる骨密度および強度の減少は，FSEとして62.5 mg/kg/dを摂取させることにより予防できた．以上の結果より，FSEは卵巣を摘出した若年ラットおよび加齢ラットにおいて，骨密度および強度の維持改善に有効であることが示された．また，その効果は，イソフラボンであるゲニステインよることが示唆された．

キーワード：大豆発酵抽出物，ゲニステイン，ダイゼイン，骨密度低下，卵巣摘出ラット．