Effects of a Fermented Vegetable Product on Fat Deposition and Bone Metabolism in Ovariectomized Rats

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Summary We examined the effects of a fermented vegetable product (FVP), fermented by yeast as well as lactic acid bacteria, on body weight and fat deposition after ovariectomy in 10-wk-old Sprague-Dawley rats. The rats were divided into the following 5 groups: 1) ovariectomized rats fed as usual (OVX); 2) ones receiving FVP at a daily dose of 100 mg/kg (LOW); 3) ones receiving FVP at a daily dose of 1,000 mg/kg (HIGH); 4) ones receiving a calcium-free diet (Ca-FREE); 5) sham-operated rats (SHAM). A slight increase in body weight was observed in the LOW group compared with the OVA group. The rate of increase in total fat content was lowest in the LOW group, but both bone weight and strength were similar to those in the other ovariectomized groups. In conclusion, the low dose of FVP turned out to reduce fat content without affecting bone weight and strength.

Key Words fermented vegetable product, bone metabolism, fat deposition, ovariectomy

A fermented vegetable product (FVP) has been manufactured from more than 50 items of fruits, black sugar, vegetables and seaweeds for periods exceeding 3 y (*1*). FVP contains isoflavones including genistein and daizdzein, carotenoids, antioxidants, vitamins and minerals. In addition, some non-pathogenic bacteria contained in FVP can produce vitamin B_{12}. Among detectable bacteria are predominant *Bacillus subtilis*, *B. licheniformis* and *B. megaterium*, but there are no staphylococci, salmonellae and *Pseudomonas aeruginosa*.

It has been well established that fat metabolism is influenced by sex hormones. In such an estrogen-deficient situation as menopause or ovariectomy, a rise in total cholesterol as well as a reduction in HDL cholesterol has been reported to occur in systemic circulation. Estrogen seems to be effective for the lowering of LDL cholesterol in postmenopausal women with familiar hypercholesterolemia. Dietary soy protein extensively used to improve plasma cholesterol is rich in phytoestrogens. When golden Syrian f(1)B hybrid hamsters were fed soy protein diets with and without isoflavone, there was a close relationship between total plasma isoflavone and LDL+VLDL cholesterol levels in females (*2*).

Kang et al. found that soy isoflavones decreased total cholesterol and LDL cholesterol levels to some extent. The clinical significance of this finding is yet to be determined, but the synthetic isoflavone derivative "ipriflavone" enhanced bone mineral density (BMD) in healthy pre- and postmenopausal women with moderately decreased BMD (*3*).

The aim of the present study was to evaluate the effects of FVP on body weight and fat deposition in ovariectomized rats.

MATERIALS AND METHODS

Preparation of FVP. FVP is a product of Manda Fermentation Co., Ltd. (Hiroshima, Japan), which arises from yeast and bacterial fermentation of fruits, black sugar, vegetables and seaweeds (Table 1). Components of FVP are shown in Table 2.

Animal experiments. Sprague-Dawley rats (SLC Inc., Shizuoka, Japan) were fed a calcium-free diet (AIN-93M; Oriental Yeast Inc., Tokyo, Japan) to which calcium carbonate was added to make a 0.175% calcium diet under conditions of free access to tap water filtered through a filter (OF filter; Organo Inc., Tokyo, Japan). The rats were housed in an air-conditioned facility (temp., 25±1°C; humidity, 55.0±5.0%) with a 12-h light/darkness cycle. Bilateral ovariectomy was performed under ether anesthesia at the age of 10 wk. The postoperative rats were divided into 5 groups; ovariectomized and usually fed rats (OVX), ovariectomized rats receiving FVP at a daily dose of 100 mg/kg (LOW), ovariectomized rats receiving FVP at a daily dose of 1,000 mg/kg (HIGH), ovariectomized rats receiving a calcium-free diet (Ca-FREE), and sham-operated rats (SHAM). FVP was administered through a gastric tube to each animal, while saline was administered at a dose of 2 mL/kg to the Ca-FREE and OVX groups for 70 d

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Table 1. Materials of FVP.

Fruits	akebi, apple, banana, pineapple, persimmon, silvervine, wild vine, etc.	26.1% (weight)
Citrus	orange, citrus hassaku, navel orange, chinese citron, true citron, etc.	14.0%
Root crops	burdock, carrot, garlic, lotus root, etc.	5.3%
Grains	rice, brown rice, glutinous rice, wheat, etc.	8.1%
Pulses	soybean, black sesame, white sesame, etc.	5.2%
Marine algae	kombu, laver, etc.	5.3%
Sugar	black sugar, etc.	33.4%
Others	honey, starch, etc.	2.6%

Table 2. Components of FVP.

Component	Content/100 g
Calories	251 kcal
Protein	2.2 g
Fat	0.1 g
Carbohydrate	60.3 g
Food fiber	2.6 g
Ash	1.9 g
Water	32.9 g
Minerals	
Sodium	54 mg
Potassium	660 mg
Calcium	120 mg
Phosphate	40 mg
Magnesium	77 mg
Iron	3.5 mg
Copper	1.6 ppm
Vitamins	
A	32.0 IU
B_1	0.02 mg
B_2	0.05 mg
B_6	0.27 mg
Niacin	0.39 mg
C	0 mg
D	0 mg
E	0.4 mg
K_1	4 µg
K_2	0 mg

from 14 d after ovariectomy (0 d of administration).

Food consumption and body weight were recorded twice a week. Body fat content and BMD of femur were measured every 14 d by dual energy X-ray absorptiometry (DCS-600 EX-III, ALOKA, Tokyo, Japan). Femur strength and toughness were measured with three point loading bone breaking test and torsional loading bone breaking test, respectively, by bone strength measuring apparatus MZ-501D (Maruto Testing Co., Tokyo, Japan) at the finish of the experiment.

Measurement of RatLaps and osteocalcin in sera. The amounts of bone resorption marker (RatLaps) and bone

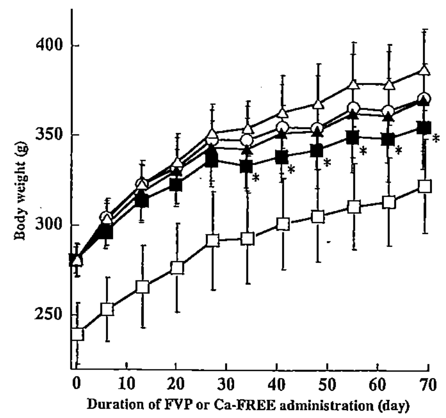

Fig. 1. Changes in body weight of experimental rats. Each point and bar indicates the mean±SD ($n=6$): △, OVX group; ■, LOW group; ▲, HIGH group; ○, Ca-FREE group; □, SHAM group. *Significantly lower at $p<0.05$ in LOW group relative to OVX group.

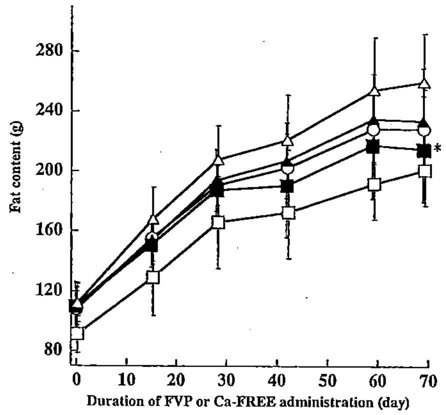

Fig. 2. Changes in body fat content of experimental rats. Each point and bar indicates the mean±SD ($n=6$): △, OVX group; ■, LOW group; ▲, HIGH group; ○, Ca-FREE group; □, SHAM group. *Significantly different at $p<0.05$ between OVX and LOW groups.

formation marker (osteocalcin) in serum were determined by RatLaps™ ELISA kits (OSTEOMETER, Inc., Herlev, Denmark) respectively in accordance with the manufacturer's instructions. The limit of detection was 2 ng/mL for RatLap and 20 ng/mL for osteocalcin.

Statistical analysis. Data were expressed as the means±SD. Stat view was used for analysis of data. Statistical differences were tested by unpaired Student's t-test following one-way ANOVA, which were considered significant at $p<0.05$.

RESULTS

Body weight was generally higher in the OVX, the LOW, the HIGH and the Ca-FREE groups compared with the SHAM group, while the difference between both OVX and LOW groups became significant at each point of measurement from 30 d after administration of FVP (Fig. 1). Food consumption was 16–20 g/d/head and did not differ significantly among the 5 groups. The average volume of drinking water consumed was lowest

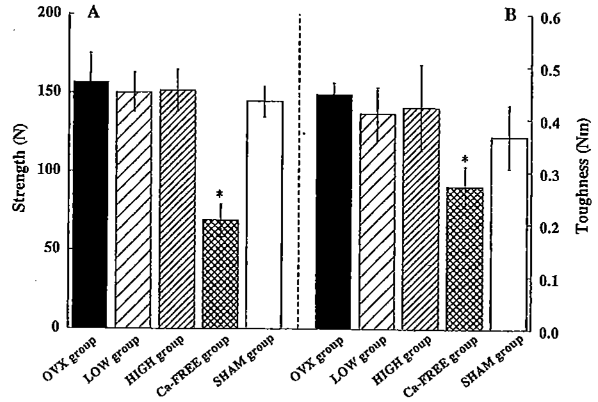

Fig. 3. Effect of FVP on femur strength and toughness. Femur strength (A) and toughness (B) were measured at three points by loading bone breaking test and torsional loading bone breaking test, respectively, at the end of experiment. Each column height and bar represents the mean±SD (n=6). *Significantly different at p<0.05 in Ca-FREE group relative to other OVX, LOW, HIGH and SHAM groups.

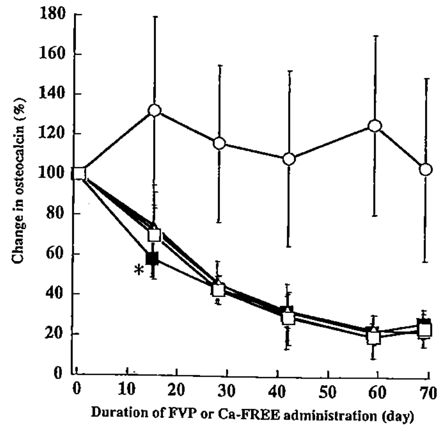

Fig. 5. Changes in osteocalcin concentration as bone formation marker. The osteocalcin concentration was evaluated as bone formation marker. Each symbol and bar represents the mean±SD (n=6): △, OVX group; ■, LOW group; ▲, HIGH group; ○, Ca-FREE group; □, SHAM group. There is a significant difference at p<0.05 between both OVX and LOW groups on day 15.

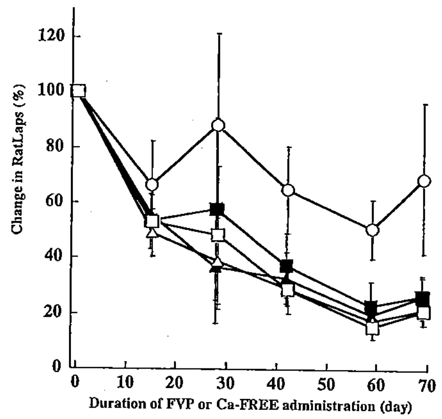

Fig. 4. Decrease rate of bone resorption marker. Bone resorption marker was expressed as RatLap by measurement of degradation products from c-terminal telopeptides of type I collagen. Each symbol and bar represents the mean±SD (n=6): △, OVX group; ■, LOW group; ▲, HIGH group; ○, Ca-FREE group; □, SHAM group. *Significantly different in OVX, LOW, HIGH and SHAM groups in relation to Ca-FREE group in the latter half.

in both LOW and HIGH groups, although the difference was not significant.

Fat content was also lowest in the LOW group except for the SHAM group and the difference was evaluated significant as compared with the OVX group at the finish of the experiment (Fig. 2).

The femur weight in the LOW and HIGH groups was significantly higher than in the OVX group. The ratio of femur weight to body weight was highest in the LOW group, but the difference was not significant compared with the OVX group. The rate of BMD increase was highest in the HIGH group compared with the OVX and LOW groups, but the difference was not significant. The rate of increase in BMD/body weight at the finish of the experiment was significantly higher in the HIGH group compared with the OVX group. Femur length and width did not show significant differences among the ovariectomized and SHAM groups. Bone strength and toughness showed significant increases in the LOW, HIGH and OVX groups compared with the Ca-FREE group (Fig. 3). There were no significant differences among the LOW, HIGH and OVX groups. On the other hand, RatLaps decreased from 15 d in ovariectomized rats and the trend persisted until the end of the experiment, but there were no significant differences among the LOW, HIGH and OVX groups (Fig. 4). There was no difference in the concentration of osteocalcin among all of the ovariectomized groups through out the experimental period with the exception of day 15 (Fig. 5).

DISCUSSION

The present study demonstrated that FVP is effective in reducing the body fat content in ovariectomized rats. Regarding the effect on bone metabolism, bone strength and toughness showed similar values to those in the rats not receiving FVP, but the LOW group showed a more rapid effect on bone turnover as estimated from the osteocalcin data.

FVP is produced by fermentation exceeding 3 y from at least 50 items of plants including typical fruits, black sugar, vegetables and seaweeds. FVP contains isoflavones, carotenoids, polyphenols, vitamins, minerals, etc. It is highly possible that fermentation produces more homogenous and/or hydrophilic preparations besides the intrinsic ingredients.

The effect of FVP on fat metabolism may have been mediated by isoflavone, β-carotene, vitamin C and polyphenols. Adams et al. (4) suggested that an estrogen receptor α-dependent process might be involved in the atheroprotective effect of dietary soy isoflavones. It is not always dependent on isoflavone concentration that substitution of soy protein for animal protein reduces the risk of coronary artery disease because of moderately lowering lipid peroxidation, oxidized LDL cholesterol, homocysteine and blood pressure (*5*). Another report indicates that soy digest serves as a preventive against oxidation of LDL cholesterol and thereby relieves cardiovascular disease (*6*).

There are many compounds in FVP components responsible for lipid metabolism in animals. Gross et al. (*7*) have reported that a concentration adjustment of tocopherols, xanthine plus lutein, and lycopene is effective for regulation of VLDL, HDL and LDL cholesterol. This method is of practical value and can provide a basis for the standardization of carotenoid and tocopherol concentrations. Consumption of a plant sterol-enriched spread favorably lowers plasma total cholesterol, LDL cholesterol, apoprotein B, and remnant-like particle cholesterol (*8*).

Additional compounds affecting lipid metabolism belong to a class of polyphenols. For example, oral administration of pycnogenol obtained from an extract of French maritime pine bark (*Pinus pinaster*), significantly increases the antioxidant activity evaluated by oxygen-radical absorbance capacity, and brings about favorable improvements in the plasma lipid profile (*9*). Many more beneficial compounds are contained in FVP. The HIGH group did not exert any fat-reducing effect in the present study. The possibility cannot be excluded that dietary supplementation with more vitamins and other nutrients causes an increase in body weight.

One of the factors influencing bone fragility is high turnover. The synthetic isoflavone, ipriflavone, has been shown to be effective for increasing BMD in postmenopausal women. Ipriflavone has been approved as a remedy for osteoporosis in Japan. Katase et al. (*10*) showed that there was a 0.3% increase in BMD in a group receiving ipriflavone and a 2.3% decrease in the placebo group in women who had experienced menopause or been ovariectomized at least 3 y earlier. Uesugi et al. (*11*) demonstrated that soy isoflavone orally administered to perimenopausal women significantly decreased urinary bone resorption markers as well as LDL cholesterol. A definite effect of FVP on bone resorption was not demonstrated in the present study, but its effectiveness for bone formation marker was more pronounced than those in other groups.

In conclusion, FVP given at a low dose to ovariectomized rats proved to modify an extra increase in body weight as well as fat content without reducing bone weight and strength. FVP seems to be useful in postmenopausal women for reducing fat content by its oral supplementaion.

REFERENCES

1) Yamamoto H, Kiyomatsu K, Hirose N, Sakai D, Katano M. 1999. Postoperative effect of Manda on NK cell activity in cancer patients. *Biotherapy* **13**: 805–809.
2) Blair RM, Appt SE, Bennetau-Pelissero C, Clarkson TB, Anthony MS, Lamothe V, Potter SM. 2002. Dietary soy and soy isoflavones have gender-specific effects on plasma lipids and isoflavones in golden Syrian f(1)b hybrid hamsters. *J Nutr* **132**: 3585–3591.
3) Kang HJ, Ansbacher R, Hammoud MM. 2002. Use of alternative and complementary medicine in menopause. *Int J Gynecol Obstet* **79**: 195–207.
4) Adams MR, Golden DL, Register TC, Anthony MS, Hodgin JB, Maeda N, Williams JK. 2002. The atheroprotective effect of dietary soy isoflavones in apolipoprotein E−/− mice requires the presence of estrogen receptor-alpha. *Arterioscler Thromb Vasc Biol* **22**: 1859–1864.
5) Jenkins DJ, Kendall CW, Jackson CJ, Connelly PW, Parker T, Faulkner D, Vidgen E, Cunnane SC, Leiter LA, Josse RG. 2002. Effects of high- and low-isoflavone soy foods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *Am J Clin Nutr* **76**: 365–372.
6) Aldercreutz H, Mazur W. 1997. Phyto-estrogens and Western diseases. *Ann Med* **29**: 95–120.
7) Gross M, Yu X, Hannan P, Prouty C, Jacobs DR Jr. 2003. Lipid standardization of serum fat-soluble antioxidant concentrations: the YALTA study. *Am J Clin Nutr* **77**: 458–466.
8) Ntanios FY, Homma Y, Ushiro S. 2002. A spread enriched with plant sterol-esters lowers blood cholesterol and lipoproteins without affecting vitamins A and E in normal and hypercholesterolemic Japanese men and women. *J Nutr* **132**: 3650–3655.
9) Devaraj S, Vega-Lopez S, Kaul N, Schonlau F, Rohdewald P, Jialal I. 2002. Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters the plasma lipoprotein profile. *Lipids* **37**: 931–934.
10) Katase K, Kato T, Hirai Y, Hasumi K, Chen JT. 2001. Effects of ipriflavone on bone loss following a bilateral ovariectomy and menopause: a randomized placebo controlled study. *Calcif Tissue Int* **69**: 73–77.
11) Uesugi T, Fukui Y, Yamori Y. 2002. Beneficial effects of soybean isoflavone supplementation on bone metabolism and serum lipids in postmenopausal Japanese women: A four-week study. *J Am Coll Nutr* **21**: 97–102.