Combined Intervention of Soy Isoflavone and Moderate Exercise Prevents Body Fat Elevation and Bone Loss in Ovariectomized Mice

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Body fat accumulation and bone loss are both often associated with estrogen deficiency following menopause. In this study, we examined whether soy isoflavone, one of the phytoestrogens, and moderate exercise interventions exhibit cooperative effects on body composition and bone mass in ovariectomized (OVX) mice. Eight-week-old female mice were assigned to 6 groups: (1) sham-operated (sham); (2) OVX; (3) OVX with received a soy isoflavone diet (OVX+ISO); (4) OVX with exercised on a treadmill (OVX+EX); (5) OVX with given both isoflavone and exercise (OVX+ISO&EX); and (6) OVX with treated with 17 β-estradiol subcutaneously (OVX+E2). Body composition and bone mineral density (BMD) were estimated by dual-energy x-ray absorptiometry (DXA). After the 6-week intervention, whole body fat (%) in the OVX group showed significantly higher than that in the sham group. Intervention of exercise and isoflavone alone partially inhibited OVX-induced body fat gain, and the combined intervention as well as E2 treatment completely restored fat mass to the sham level. Lean body mass in the whole body was not different in OVX group compared with that in OVX+ISO, OVX+EX, and OVX+E2 groups, but it was significantly higher in OVX+ISO&EX than in other groups. BMD of the whole body, lumbar spine, or femur showed significantly reduced by OVX, and the bone loss was partially inhibited by intervention of exercise or isoflavone alone. However, the combined intervention completely restored the bone mass to the level of sham, as did E2. Serum total cholesterol was significantly increased by OVX, which was normalized by the combined intervention or E2 treatment. These results demonstrate that combined intervention of soybean isoflavone and exercise prevented body fat accumulation in the whole body with an increase in lean body mass and restoration of bone mass, and reduced high serum cholesterol in OVX mice.

MENOPAUSE is often associated with an increased incidence of several chronic diseases, including obesity, coronary heart disease, and osteoporosis.[1-4] It is well documented that the prevalence of these diseases in the elderly are concurrent with inductions in lipid metabolic abnormalities and changes in body composition that include an increased fat mass and a progressive decline in skeletal muscle mass and bone mineral density (BMD).[2,5]

Hormone-replacement therapy (HRT) has been shown to potentially reduce or prevent hypercholesterolemia and coronary heart disease[6] and is considered to be the most effective therapy to reduce the rate of osteoporosis in postmenopausal women.[7] However, the HRT trial of the Women's Health Initiative (WHI) was stopped (planned follow-up, 8.5 years; actual follow-up, 5.2 years) due to the advice of the Data and Safety Monitoring Board because of an increased risk of breast cancer and an unacceptable rate of unfavorable outcomes (global index).

Recently, nonsteroidal estrogen-like plant compounds called phytoestrogens have been tried as alternatives to HRT for the prevention and treatment of hypercholesterolemia and osteoporosis in postmenopausal women.[8-11] These compounds have been shown to maintain estrogen's positive cardiovascular and bone effects, while minimizing several undesirable side effects of estrogen. We previously found that genistein, one of the phytoestrogens, dose-dependently inhibited bone loss caused by estrogen or androgen deficiency without showing significant adverse effects on reproductive organs in both female and male osteoporotic animal models.[12-14] Exercise has been also reported to prevent bone loss induced by gonadectomy in both sexes.[15-17] In fact, we have reported that the combined intervention of a submaximal dose of genistein and moderate-intensity exercise exhibited cooperative effects on the prevention of bone loss in ovari- (OVX) or orchidectomized (ORX) mice.[18,19]

The association of abnormal fat mass and bone mass in obesity model animals has not been precisely investigated. Mathey et al reported that the percentage fat mass was about 3 times higher but femoral BMD was lower in obese, diabetic, leptin-resistant Zucker rats than in their homozygous lean controls.[20] It has also been reported that OVX in rodents leads to weight gain and fat deposition, whereas estradiol treatment reverses these changes.[21,22] There is considerable evidence indicating that exercise training or intake of soybean isoflavone can suppress body fat deposition in some regions in OVX animals.[23-25] These findings suggest that intervention by exercise or isoflavone could have a beneficial effect by improving both body composition and bone mass in the estrogen-deficient condition.

Although small animals are used extensively in the field of body composition research, the techniques for the measurement are limited. Up to the present, the accepted method for determining body composition has been direct carcass analysis using lipid extraction techniques.[26] Therefore, it has been impossible to examine whether the body composition in the whole body

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changes following estrogen deficiency in alive animals and, thus, it has been somewhat difficult to determine whether the combined intervention of exercise and isoflavone administration further improves body composition. Recently, densitometers were developed to determine body composition in mice in vivo, such as PIXImus (CE Lunar, Madison, WI).[26-28] In this study, we investigated the effects of the combined interventions of soy isoflavone intake and exercise training on body composition, including fat, lean, and bone mass in the whole body using PIXImus, and lipid metabolism in OVX mice fed a high cholesterol diet.

MATERIALS AND METHODS

Animals and Interventions

Eight-week-old female mice of the ddY strain were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan), and were individually housed in 24 × 15 × 15 cm^3 cages under a 12/12-hour light/dark cycle at 22°C. The mice were initially given free access to an AIN-93G[29] diet with corn oil instead of soybean oil (Funabashi Farm, Chiba, Japan) for 2 days before performing the operation. Mice were run on a treadmill for 30 min/d to adapt to running exercise and some mice refusing to run were excluded. The animals were either sham-operated (sham, n = 8) or OVX on the same day. The OVX mice were randomly divided into 5 groups: OVX-control (OVX, n = 8); OVX fed an isoflavone-supplemented diet (OVX+ISO n = 8); OVX exercised with running (OVX+EX n = 8); OVX treated with combined isoflavone and exercise (OVX+ISO&EX n = 8); and OVX given 17 β-estradiol subcutaneously (E2, n = 8). In order to evaluate clearly the effects of interventions on lipid metabolism, all mice were fed a high-cholesterol (HC) diet.

The HC diet contained 3 g cholesterol and 0.5 g sodium cholate per kg diet. The isoflavone-supplemented diet contained 0.4% Fujiflavone P40 (soy isoflavone content: 40%; Fujicco Corp, Kobe, Japan) with the HC diet. Fujiflavone P40 contains isoflavone such as daidzin (20.4%), genistin (4.6%) and glycitin plus glycitein (13%). In this study, a mouse ate approximately 4g diet/d, and consequently ingested approximately 6.4 mg/d of pure isoflavone conjugates (~160 mg/kg body weight/d). E2 was given at 0.03 μg/d using a mini-osmotic pump (Alza Corp, Palo Alto, CA).

It has been reported that OVX resulted in a significant weight gain caused by an increase in food intake, and estrogen replacement reduced body weight through decreasing food intake.[30] To clarify this issue, the mice were pair-fed for 6 weeks, except for the E2 group. In fact, the E2 group (3.1 g/d) demonstrated a lesser food intake than the sham or other OVX groups (4 g/d) in this study. The exercise regimen consisted of running on a treadmill (Natsume Corp, Tokyo, Japan) for 30 min/d, 6 days per week at 12 m/min up a 10-degree slope.

After the 6-week treatment, mice were scanned using a peripheral DXA (Lunar PIXImus densitometer) under anesthetization to measure body composition in whole body and BMD of whole body and lumbar spine. Immediately after DXA scanning, mice were weighed and blood was drawn, and stored at −20°C until assay. The uterus and other organs were removed and wet weights were measured. The right femur was also removed to analyze excised BMD. All procedures were performed in accordance with the National Institute of Health and Nutrition Guidelines for the Care and Use of Laboratory Animals.

DXA Analysis

Body composition and BMD of whole body and lumbar spine were measured with Lunar PIXImus densitometer using software version 1.4x. After anesthetization, the mice were placed on the densitometer using the specimen tray. All mice were placed carefully in the same position, and whole body was settled in the image area. By whole body scanning, body composition including fat mass and lean body mass, and BMD of whole body and L4-L6 vertebrae were analyzed (Fig 1). The in vivo reproducibilities for measuring values were evaluated by measuring coefficient of variation (CV) of 5 times after repositioning the animals. The CV of body composition measurement was 0.2% for lean tissue mass, and 2.8% the fat mass. The CV of BMD measurement was 0.8% for whole body and 0.7% for the L4-L6 vertebrae. The CVs are in agreement with studies examining the precision and accuracy of PIXImus densitometer.[26,31,32]

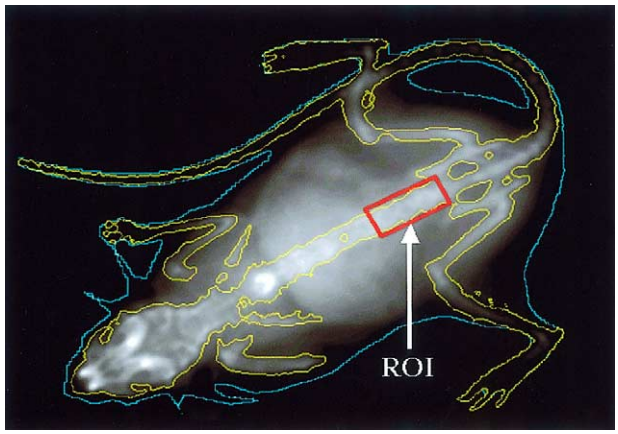

Fig 1. Images of representative scans of total body of the mice measured by Lunar PIXImus. The rectangular region-of-interest (ROI) was placed at the lumbar spine (L4-L6).

The right femur was excised and BMD was measured by DXA using a bone densitometer adapted to small animal research (model DCS-600EX-R; Aloka, Tokyo, Japan). The bone mineral content (BMC) of mouse femora was closely correlated with ash weight ($r = 0.978$).[33] All DXA scanning and analysis were conducted by a same researcher.

Measurements of Serum Lipids

Total cholesterol (TC) and triacylglycerols (TG) in the serum were determined using commercial kits (cholesterol C-test and Triglyceride G-test, Wako Pure Chemical, Osaka, Japan). High-denisty lipoprotein (HDL)-cholesterol in the serum was measured by an enzymatic method (HDL-cholesterol-test, Wako Pure Chemical).

Statistical Analysis

Data are presented as means ± SEM. The significance of differences was determined by 1-way ANOVA followed by Fisher's protected least-significant-difference test. The effects of isoflavone supplement, running exercises and interaction of both interventions were analyzed by 2-way ANOVA. Statistical analyses were performed using the SAS program (SAS Institute, Cary, NC). Differences were considered significant at the level of $P < .05$.

RESULTS

Body and Uterine Weight

Six groups of mice studied started with similar initial mean body weight (Fig 2A). OVX mice had a significantly higher body weight compared to sham and OVX+E2 groups 1 week after the operation. There was no significant difference in body weight among the OVX, OVX+ISO, and OVX+ISO&EX

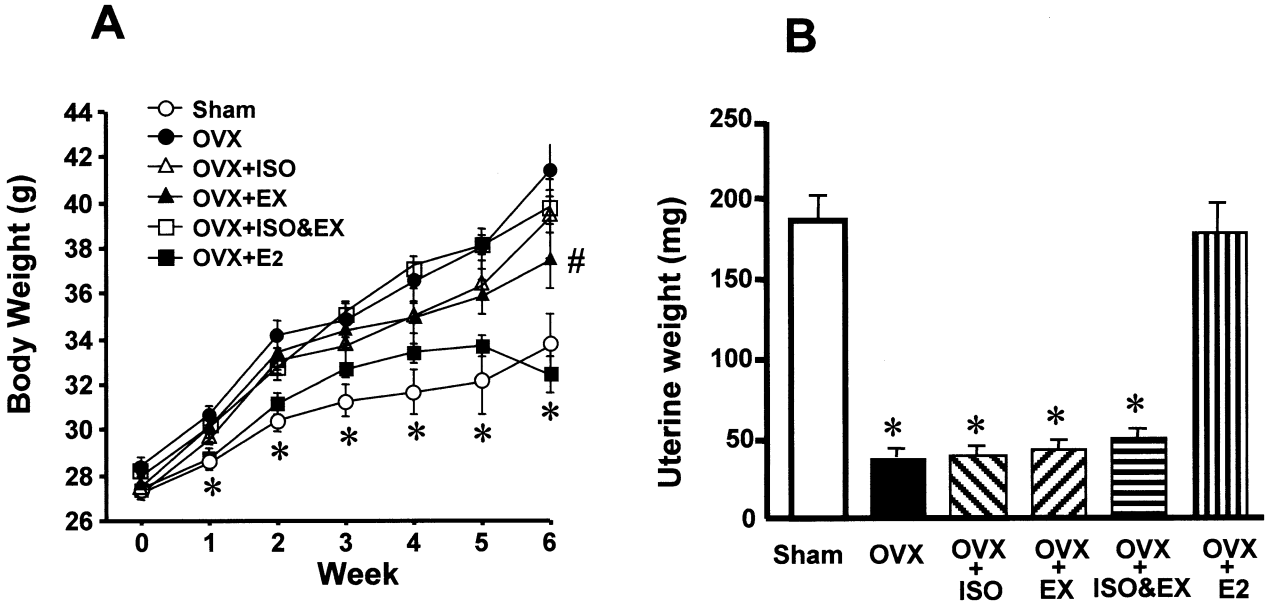

Fig 2. Weight of body and uterus in sham-operated mice, ovariectomized (OVX) mice, OVX mice fed isoflavones or trained to exercise with or without isoflavones, and OVX mice treated with E2. (A) Body weight was measured during the 4-week experimental period in sham-operated mice (○), OVX mice (●), OVX mice treated with isoflavones (△), trained to exercise with (□) or without isoflavones (▲), and OVX mice treated with 0.03 μg/d of E2 (■). (B) Weight of uterus was measured 6 weeks after operation in sham-operated mice (sham), OVX, OVX mice treated with isoflavones (OVX+ISO), OVX mice trained to exercise with (OVX+ISO&EX) or without isoflavones (OVX+Ex), and OVX mice treated with 0.03 μg/day of E2 (OVX+E2). Data are means ± SEM of 8 mice. *Sham and OVX+E2 *v* other groups. #OVX+EX < OVX. *P* < .05.

groups. However, body weight was significantly lower in the OVX+EX group than that in the OVX group at the end of the 6-week experimental period. Uterine weight strikingly decreased in OVX mice, indicating that mice were estrogen-deficient. As reported previously,[13] E2 restored the decreased uterine weight in OVX group mice to a level similar to that in sham-operated mice. In contrast, there was no difference in uterine weight among the other OVX groups after the 6-week treatment (Fig 2B).

Body Composition

OVX mice had a significantly higher whole body fat (%) at the end of the 6-week experimental period than that in sham

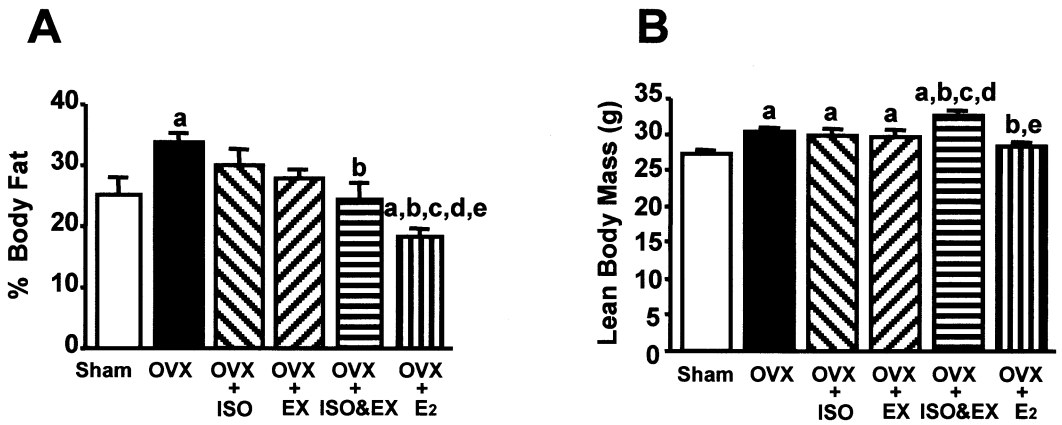

Fig 3. Body composition analyzed by Lunar PIXImus densitometer. Mice were sham-operated (sham) or OVX, and some OVX mice were fed isoflavone (OVX+ISO) or trained to exercise with (OVX+ISO&Ex) or without isoflavone (OVX+Ex) or treated with 0.03 μg/d of E2 (OVX+E2). After a 6-week intervention, the mice were scanned using Lunar PIXImus densitometer, and body fat (%) (A), and lean body mass (B) were determined in each group. Data are means ± SEM of 8 mice. [a]Significantly different from the sham group. [b]Significantly different from the OVX group. [c]Significantly different from the OVX+ISO group. [d]Significantly different from the OVX+Ex group. [e]Significantly different from the OVX+ISO&EX group. *P* < .05.

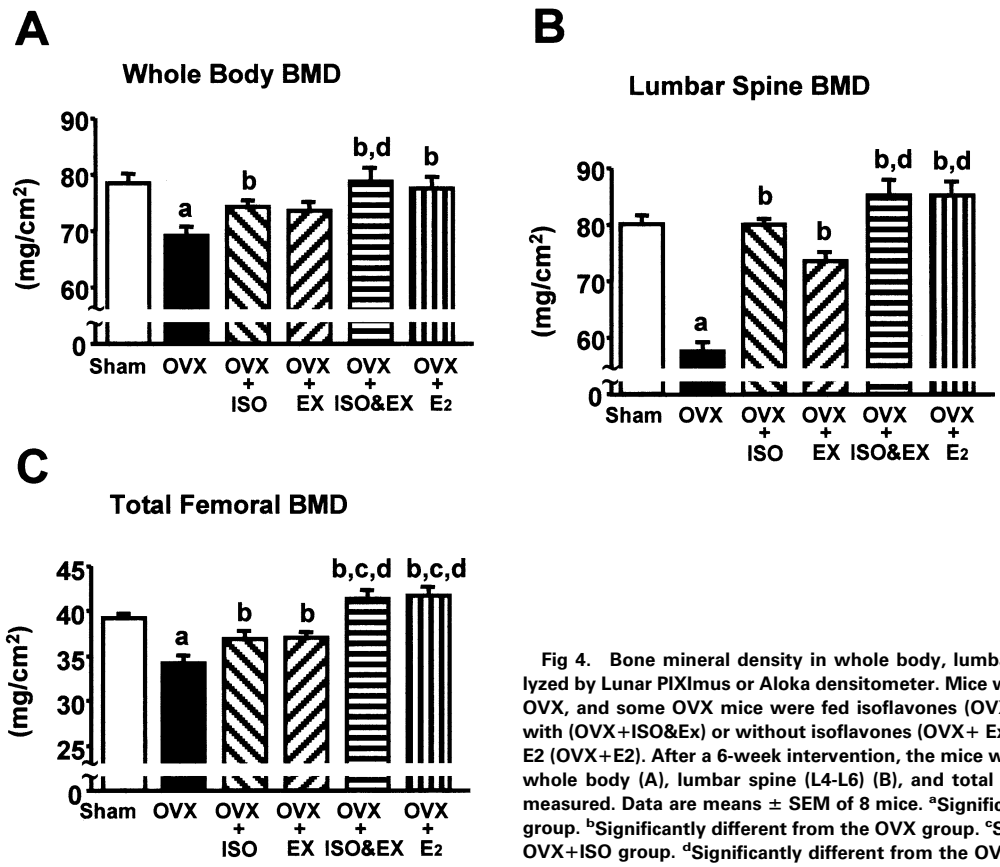

Fig 4. Bone mineral density in whole body, lumbar spine and total femur analyzed by Lunar PIXImus or Aloka densitometer. Mice were sham-operated (sham) or OVX, and some OVX mice were fed isoflavones (OVX+ISO) or trained to exercise with (OVX+ISO&Ex) or without isoflavones (OVX+ Ex) or treated with 0.03 μg/d of E2 (OVX+E2). After a 6-week intervention, the mice were scanned DXA and BMD in whole body (A), lumbar spine (L4-L6) (B), and total femur (C) in each group was measured. Data are means ± SEM of 8 mice. [a]Significantly different from the sham group. [b]Significantly different from the OVX group. [c]Significantly different from the OVX+ISO group. [d]Significantly different from the OVX+Ex group. $P < .05$.

group (Fig 3A). In contrast, the E2 treatment resulted in a reduced percent body fat compared to those of sham and other OVX groups. Intervention of exercise or isoflavone alone partially inhibited OVX-induced body fat gain, and the combined intervention completely restored fat mass to the sham level. Lean body mass in the whole body was not different in the OVX group compared with that in the OVX+ISO, OVX+EX, and OVX+E2 groups. However, it was notable that lean body mass was significantly increased by the combined intervention (Fig 3B).

Bone Mineral Density

Significant decreases in BMD of whole body, lumbar vertebrae, and femur were found in the OVX group compared with sham group (Fig 4A-C). Isoflavone treatment alone (OVX+ISO) completely prevented the decrease in BMD in lumbar vertebrae, and partially inhibited the decrease in BMD in whole body and femur. Exercise treatment alone also significantly inhibited the decrease in BMD in lumbar vertebrae and femur. Furthermore, BMD of whole body, lumbar vertebrae and femur were greatest in OVX+ISO&EX group, and significantly higher than those in OVX and OVX+EX group. Similarly, E2 treatment completely prevented OVX-induced bone loss.

Plasma Lipids Profiles

A significant increase in serum TC was found in the OVX and OVX+ISO groups, compared with that in sham group. Exercise alone partially, and combination of exercise and isoflavone completely inhibited the increase in TC in OVX mice, as did E2 (Fig 5A). By contrast, serum HDL-cholesterol in OVX mice treated with a combination of exercise and isoflavone increased significantly, compared with sham control and either treatment alone (Fig 5B). Serum TG was increased by OVX, and the combined interventions of exercise and isoflavone or E2 inhibited the elevation of TG levels (Fig 5C).

DISCUSSION

The present study is the first to demonstrate that the combined intervention of a diet containing soy isoflavones and moderate exercise prevented fat deposition and bone loss in whole body and restored serum total cholesterol in OVX mice fed a high-cholesterol diet.

It is reported that OVX in rodents leads to weight gain and fat deposition, partially caused by an increase in food intake, and estradiol treatment abolished these effects.[32] In this study, mice were fed equal amounts of diet, except for the E2 group, in which the 17 β-estradiol replacement resulted in a suppressed food intake and weight gain compared to the other OVX mice. Taken together, these results suggest that weight gain in OVX mice was mostly caused by the lack of estrogens.

Dual-energy x-ray absorptiometry (DXA) has been used for determining in vivo body composition in humans, as well as in rats, by using specialized software over the past 10 years.[34,35] Recently, peripheral DXA instruments have been applied for measurement of body composition in mice. Nagy and Clair

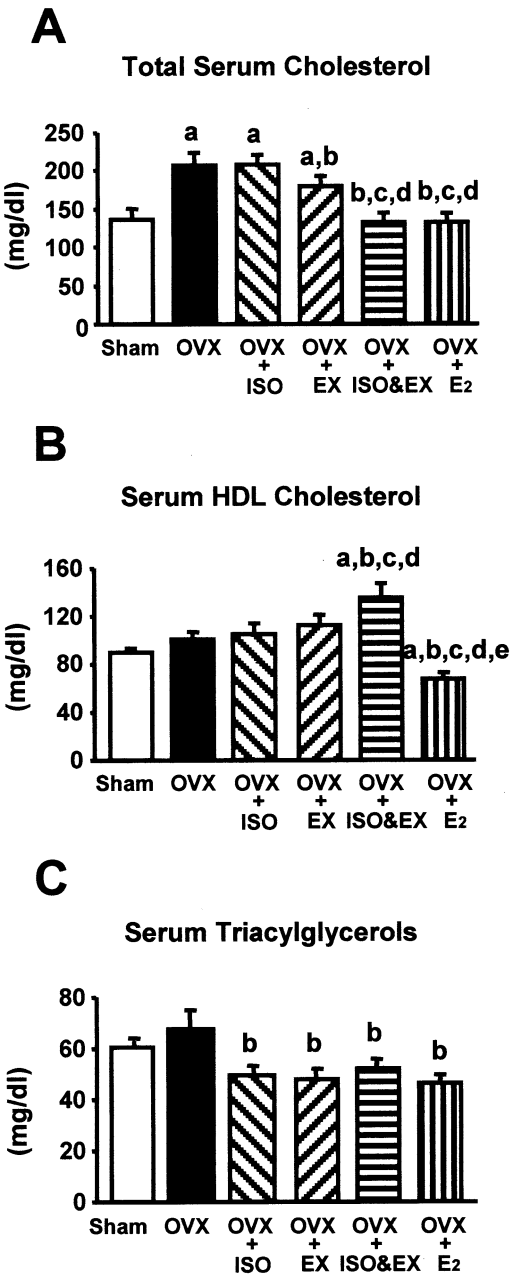

Fig 5. Plasma level of total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglycerides in sham-operated mice, ovariectomized (OVX) mice, OVX mice fed isoflavones (OVX+ISO) or trained to exercise with (OVX+ISO&Ex) or without isoflavones (OVX+Ex), and OVX mice treated with E2 (OVX+E2). Total cholesterol (A), HDL-cholesterol (B), and triglycerides (C) in plasma were measured 6 weeks after operation. Data are means ± SEM of 8 mice. [a]Significantly different from the sham group. [b]Significantly different from the OVX group. [c]Significantly different from the OVX+ISO group. [d]Significantly different from the OVX+Ex group. [e]Significantly different from the OVX+ISO&EX group. $P < .05$.

examined the precision and accuracy of the peripheral DXA (Lunar PIXImus) by measuring total-body mineral density, fat mass and bone-free lean tissue mass in mice.[26] They reported that DXA-derived values were closely related to bone ash content and chemical carcass analysis values. Therefore, the authors suggested that peripheral DXA is a useful tool for the measurement of body composition in mice. In this study, we further examined which part of body composition contributed to weight gain, since body composition in the whole body has not been evaluated in OVX mice using PIXImus. We found that OVX resulted in an increase in percent body fat in the whole body, which clearly indicates that a large part of body weight gain accounts for fat gain after ovariectomy.

It is also suggested that exercise training can reduce weight gain following a decrease in estrogen levels.[24] In the present study, running exercise alone resulted in significantly, but not completely reduced weight gain at the end of the experiment. Furthermore, our data showed that the reduction in weight gain with exercise was due to a decrease in fat deposits in OVX mice by analyzing the body composition. Whereas final body weight and percent body fat were not significantly lower in OVX+ISO group than those in OVX groups, the combination of isoflavones and exercise completely restored percent body fat and increased lean body mass, including high bone mass, in OVX mice. It is known that the skeletal muscle mass were increased by exercise training.[36-38] Changes in skeletal muscle mass would play an important role in the regulation of bone mass, because the largest loads on bones come from muscle contractile force in running exercise.[39] In this study, the weight of organs including heart, liver, kidneys and spleen did not differ among the groups (data not shown). Thus, we presume that the increase in lean body mass with combined intervention in OVX mice was due to the gain in skeletal muscle mass, which might contribute to the increase in bone mass. These results suggest that combined intervention of isoflavone and exercise might be a useful regimen for maintenance of body composition in postmenopausal woman.

Exercise has been reported to inhibit bone loss after ovariectomy in rats.[15,16] In the present study, we found that bone loss was not only significantly inhibited in the femur, but also in the lumbar spine in OVX mice. However, the lumbar vertebrae were likely to receive less mechanical stress than the femur during treadmill running, because the BMD values of the lumbar vertebrae were not completely restored after the 6-week intervention (Fig 4B). These findings are in agreement with those of other studies where the effect of running exercise on the inhibition of bone loss was site-specific.[40,41]

In this study, we showed isoflavone treatment alone completely inhibited the decrease in BMD in lumbar vertebrae but not in other regions such as the femur and whole body in OVX mice. Thus, the combination of isoflavone administration and exercise was required for complete prevention of bone loss in whole body under an estrogen-deficient condition. Yeh et al. reported that the combined intervention of estrogen and running exercise was more effective than with either intervention used alone for the inhibition of bone loss in the specific sites such as appendicular and vertebrae bone.[42] However, to our knowledge, no study has evaluated bone loss in the whole body induced by OVX in mice. In this study, another important new finding is that the combined intervention increases bone mass in whole body along with femur and lumbar spine in OVX mice by using the Lunar PIXImus densitometer.

In addition to body composition and bone action, our results

showed that the combined intervention also has a beneficial effect on serum cholesterol in OVX mice fed a HC diet. It is reported that isoflavone supplementation effectively lowered the serum cholesterol in OVX rats, as well as in postmenopausal women.[25,43,44] However, it was also shown that isoflavones were beneficial to bone mass, but not to blood lipids.[45] In this study, exercise partially inhibited the increase in TC in OVX mice, but the combined intervention of exercise and isoflavone not only restored TC to the sham level, but also increased HDL cholesterol in OVX mice. Therefore, it is likely that phytoestrogens such as soy isoflavones can enhance the effect of exercise on lipid metabolism, as well as body composition in OVX animals.

In conclusion, soy isoflavone intake and moderate exercise exerted cooperative effects not only in preventing bone loss, but also in restoring abnormal body composition and lipid metabolism under estrogen-deficient conditions. The results suggest that a combined intervention of isoflavone supplementation and moderate exercise may offer an effective regimen for the prevention of life-style–related health problems such as obesity, osteoporosis, and hypercholesterolemia in postmenopausal women.

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