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Cooperative Effects of Exercise Training and Genistein Administration on Bone Mass in Ovariectomized Mice

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ABSTRACT

We reported that genistein, a soybean isoflavone, prevents bone loss caused by estrogen deficiency, without undesirable effects on the uterus. In this study, we examined cooperative effects of genistein administration and running exercise on bone mass in ovariectomized (OVX) mice. Female mice aged 7 weeks were either sham-operated or OVX and divided into six groups: (1) sham; (2) OVX; (3) OVX, treated with genistein at a submaximal dose (0.4 mg/day) subcutaneously (G); (4) OVX, exercised on a treadmill daily for 30 minutes/day at 12 m/minute on a 10° uphill slope (Ex); (5) OVX, given genistein and exercised (ExG); and (6) OVX, treated with 17β-estradiol (0.03 μg/day) in the same manner as genistein (E_2). Four weeks after intervention, bone mass was estimated by dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT). Bone mineral density (BMD) of the whole femur measured by DXA was higher in both the G and the Ex groups than in the OVX group. Furthermore, BMD in the ExG group was significantly higher than that in the groups receiving either intervention alone. Bone area in distal region of the femur was significantly higher in Ex and ExG groups as compared with those in the OVX and G groups. pQCT analysis showed that the cross-sectional areas (CSAs) and periosteum perimeter at midshaft of the femur did not differ in the sham and OVX groups but were significantly higher in Ex and ExG groups. Histomorphometric analysis showed that bone formation rate/bone surface (BFR/BS) was significantly higher in both Ex and ExG groups as compared with that in non-exercised groups. The bone volume (BV/TV) in the distal femoral cancellous bone was lower in the OVX than that in the sham group, and it was restored completely in the ExG group, as in the E_2 group. Thickness of the trabecular bone (Tb.Th) was higher in Ex and ExG groups than that in the OVX and G groups. These results indicate that the combined intervention of moderate exercise and the submaximal dose of genistein administration show a cooperative effect in preventing bone loss in OVX mice. (J Bone Miner Res 2001;16:1829–1836)

Key words: soybean isoflavone, genistein, exercise, estrogen deficiency, osteoporosis

INTRODUCTION

OSTEOPOROSIS IS a major health care problem in the elderly, which is characterized by low bone mass, leading to an increase in risk of fracture. Bone mass is influenced by many factors such as genetics, hormonal status, nutrition, exercise, and lifestyle. Among these factors, nutrition and exercise seem to be important in preventing osteoporosis.

Recent studies have shown that nonsteroidal estrogen-like plant compounds called phytoestrogens are effective in preventing osteoporosis in animal models.[1–2] The major phy-

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toestrogens consumed by humans exist in soybean and are classified as isoflavones. We reported that genistein, one of the soybean isoflavones, prevented bone loss caused by estrogen deficiency without substantial effects on the uterus in ovariectomized (OVX) mice.[1] We also showed that genistein prevented bone loss by normalizing the accumulation of pre–B lymphocytes in bone marrow and suppressing increased bone resorption in OVX mice, as did 17β-estradiol (E_2).[1] Epidemiological studies also suggest that the low incidence of osteoporosis and heart diseases caused by estrogen deficiency in Asian women is attributable to their high intake of isoflavone-rich soy foods.[3–5]

Physical exercise that loads mechanical stress to the bone is effective also in maintaining bone mass in postmenopausal women.[6,7] It has been shown that running exercise partially prevented bone loss induced by estrogen deficiency in animals.[8–10] Furthermore, it has been suggested that combined intervention of exercise and estrogen treatment could prevent bone loss both in postmenopausal women and in animal models with osteoporosis. However, some adverse effects such as uterine bleeding and carcinogenesis accompany estrogen replacement. Thus, we presumed that the combined intervention of running exercise and genistein administration might be effective in preventing bone loss caused by estrogen deficiency without substantial effects on reproductive organs.

In this study, we examined the cooperative effects of moderate intensity exercise and a submaximal dose of genistein on bone mass in OVX mice using dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) and by histomorphometric analyses. Our data indicated that combined intervention exhibited cooperative effects on the prevention of bone loss in OVX mice.

MATERIALS AND METHODS

Animal and intervention

Seven-week-old female mice of the ddY strain were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and fed an AIN-93G diet with corn oil instead of soybean oil (Funabashi Farm, Chiba, Japan).[11] The mice were housed individually in 24 × 15 × 15 cm^3 cages under a 12/12 h light/dark cycle at 22°C and allowed free access to water and diet. The animals were either sham-operated (sham, $n = 8$) or OVX. The OVX mice were randomly divided into five groups: OVX-control (OVX, $n = 8$); genistein administration (G, $n = 8$); exercise training (Ex, $n = 8$); combined genistein and exercise (ExG, $n = 8$); and E_2 administration (E_2, $n = 8$). Genistein (Fujicco Corp., Kyoto, Japan) was dissolved in 20% dimethylsulfoxide in polyethylenglycol-300 and was administered to mice subcutaneously using a miniosmotic pump (Alza Corp., Palo Alto, CA, USA) at 0.4 mg/day immediately after surgery. E_2 was given 0.03 μg/day the same way as genistein. The Ex regimen consisted of daily running on a treadmill (Natsume Corp., Tokyo, Japan) for 30 minutes/day at 12 m/minute up a 10° slope. The mice were treated with a submaximal dose of genistein and moderate intensity of exercise to assess the cooperation effects of combined intervention. Bone labeling of mice with a subcutaneous injection of calcein (1.6 mg/kg body weight) (Sigma, St. Louis, MO, USA) was performed 6 days and 2 days before death. Four weeks after the start of intervention, the mice were killed and the weight of uterus was measured. Both femora also were removed to analyze bone mineral density (BMD) and structure. All procedures were performed in accordance with the National Institutes of Health and Nutrition Guidelines for the Care and Use Laboratory Animals.

Radiographic analysis

Radiographic analysis of the femora was performed by a soft X-ray system (model SRO-M50; SOFRON, Tokyo, Japan). Bone mineral content (BMC) and BMD of the femur were determined using DXA (model DCS-600R; Aloka, Tokyo, Japan). The BMC of the mouse femora was correlated closely with the ash weight ($r = 0.978$).[12] BMD was calculated by BMC of the measured area. The scanned area of femur was divided equally into three regions (5.3 mm each), proximal femur, midshaft, and distal femur, to assess the regional difference in femur.

pQCT analysis

The femora were scanned with a pQCT system XCT Research SA combined with μ Scope (Norland Stratec Medizintechnik GmbH, Birkenfeld, Germany). The voxel size was 0.08 mm, the slice thickness was 0.5 mm, and the cortical threshold was 464 mg/cm^3. We used the measuring mode for dividing into cortical bone or trabecular bone as Peel mode 20. This mode can detect the inner threshold automatically. One cross-sectional slice from each femora was scanned at midshaft, 8 mm from the distal end, which was determined from the scout view of the pQCT device. The BMC and BMD at midshaft were analyzed for each slice. Total cross-sectional area (CSA) and periosteal perimeter (PERI) were evaluated also by the scanning.

Histomorphometric analysis

Distal femur: Undecalcified 5-μm sections were prepared from femora and stained for tartrate-resistant acid phosphatase (TRAP). Histomorphometry was performed with the semiautomatic image analyzing system (OsteoplanII; Carl Zeiss, Thornwood, NY, USA)[13] linked to a light microscope. Using the sections of distal femora, histomorphometric parameters were quantified in cancellous bone tissue at secondary spongiosa. The region in the trabecular bone within one cortical width from the endosteal surface was excluded from the measurements. Trabecular bone volume/tissue volume (BV/TV), Tb.Th (trabecular thickness), and trabecular separation (Tb.Sp) were calculated.

Femoral midshaft: An undecalcified section was obtained from the site of middiaphysis of the femur. The specimen was embedded in methylmethacrylate (MMA) without staining to yield a 40-μm-thick crosscut ground section. Measurements were made on the semiautomatic image analyzing system mentioned previously. Dynamic parameters such as mineral apposition rate (MAR; interlabel width/day), mineralizing surface/bone surface [MS/BS: (double-

labeled surface + single-labeled surface/2)/BS], and bone formation rate [BFR/BS: MAR × MS/BS/100] on the periosteal surface were measured by calcein double labeling.

Statistical analysis

Data were presented as means ± SEM. The significance of the differences was determined by one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference test. The effect of G administration, running Ex, and interaction of both interventions was analyzed by two-way ANOVA. The significance of the differences was determined by Fisher's protected least significant difference test. Differences were considered significant at the level of $p < 0.05$.

RESULTS

Body weight and uterine weight

The six groups of mice started with similar initial mean body weight (Fig. 1A). The mice in all groups gained weight during a 4-week experimental period. The body weight was significantly higher in the sham group than those in ExG and E_2 groups 3 weeks and 4 weeks after surgery. The uterine weight decreased strikingly in OVX mice, indicating that the mice were estrogen deficient. As reported previously, E_2 restored the decreased uterine weight in OVX mice to the same level as that in the sham mice. In contrast, treatment with genistein for 4 weeks at 0.4 mg/day did not affect the uterine weight in OVX mice (Fig. 1B). The dose of genistein used in this study (0.4 mg/day) has been defined previously as a submaximal dose sufficient for a bone-protective effect in OVX mice.[14] The combination of genistein administration and running exercise did not affect uterine weight either.

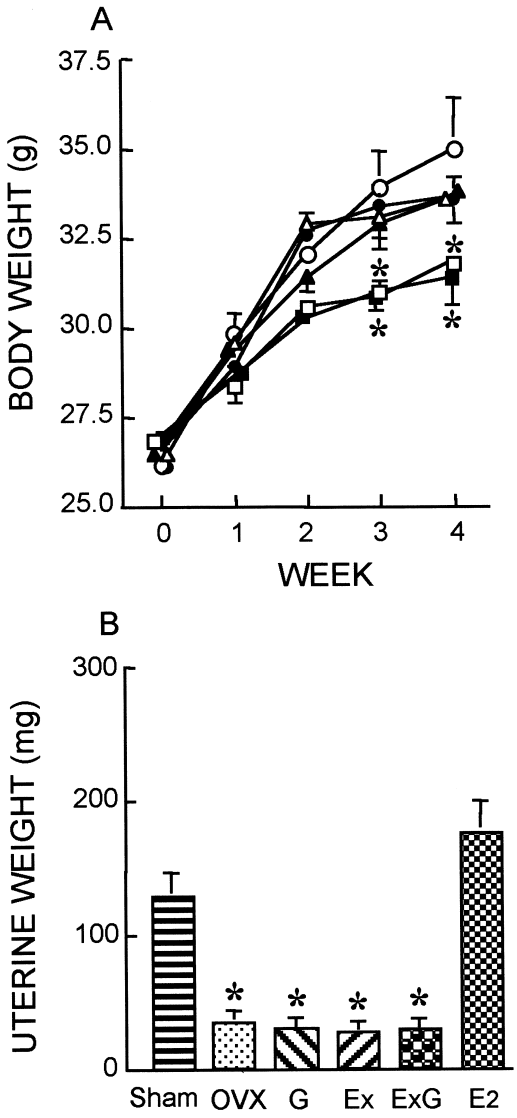

FIG. 1. Body and uterine weight in sham mice, OVX mice, OVX mice treated with genistein or trained to exercise with or without genistein, and OVX mice treated with E_2. (A) Body weight was measured during the 4-week experimental period in sham mice (○), OVX mice (●), OVX mice treated with 0.4 mg/day of genistein (△), trained to exercise with (□) or without genistein (▲), and OVX mice treated with 0.03 μg/day of E_2 (■). (B) Uterine weight was measured 4 weeks after operation in sham mice, OVX mice, OVX mice treated with 0.4 mg/day of genistein (G), OVX mice trained to exercise with (ExG) or without genistein (Ex), and OVX mice treated with 0.03 μg/day of E_2. Data are means ± SEM of 8 mice. *Significantly different from the sham group ($p < 0.05$).

Bone mass and structural properties

Figure 2 shows radiograms of the femora collected from the mice in each group. X-ray analysis revealed that the mineralized cancellous bone mass in OVX mice had significantly decreased, especially in the distal metaphysis of the femur. Combined intervention of genistein administration and running exercise or E_2 administration markedly prevented the bone loss.

On DXA analysis, whole femoral BMD was significantly reduced by OVX, and the decrease in BMD was significantly inhibited by either genistein administration or exercise intervention (Fig. 3A). The combination of exercise and genistein completely prevented the decrease in BMD, and the level was the same as that in sham mice (Fig. 3A). Bone area of the whole femur in the ExG group was significantly larger than that in the other groups (Fig. 3B). To evaluate a site-specific effect of genistein and/or exercise intervention, the femoral BMD and area was analyzed further at proximal, middle, and distal regions of the femur (Table 1). OVX reduced the BMD in proximal, middle, and distal regions of sham mice by 3.8, 12.6, and 15.1%, respectively. In contrast, combined intervention of genistein and exercise completely prevented bone loss at all three regions in OVX mice. The mice in either the Ex or G group exhibited higher BMD compared with those in the OVX group at each region. The bone area was markedly higher in the ExG group as compared with those in the OVX and G groups at all three regions. The area in the distal femur in the Ex group also was significantly higher than those in the OVX and G groups.

Results of densitometric evaluation by pQCT are shown in Fig. 4. Both BMD and BMC at femoral midshaft, 8 mm

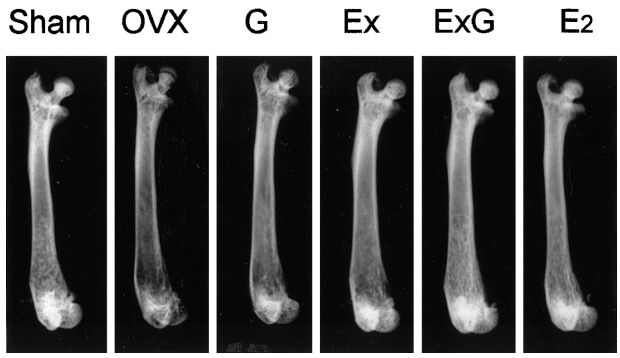

FIG. 2. Radiograms of the femora. Mice were sham-operated (sham) or OVX, and some OVX mice were treated with 0.4 mg/day of genistein or trained to exercise with or without genistein or treated with 0.03 μg/day of E_2. Femora were collected 4 weeks postoperatively and were used for X-ray analysis. Note that marked bone loss occurred in the distal metaphysis of the femoral cancellous bone in OVX mice, and this bone loss was prevented completely by intervention with combined genistein administration and running exercise.

from the distal end, were reduced in OVX mice as compared with those in sham mice. The combined intervention of genistein and exercise restored both femoral midshaft BMD and BMC in OVX mice, as did E_2. Although neither CSA nor PERI at midshaft were affected by OVX, these parameters were increased markedly by either exercise or combined intervention. These results in the analysis by pQCT were well correlated to those evaluated by DXA showing an increase in bone area. These findings suggest that the exercise and combined intervention not only prevent bone loss but also stimulate bone formation at the midshaft of the femur.

Histological analysis

To define the effects of genistein administration and running exercise on trabecular bone, histological sections of distal femoral metaphysis were prepared, and BV/TV, Tb.Th, and Tb.Sp were evaluated (Fig. 5). BV/TV and Tb.Th were decreased markedly by OVX; however, these were significantly recovered by combined intervention. A two-factor ANOVA showed that the effects of exercise and genistein intervention on BV/TV were significant ($p < 0.01$), and the interaction between the two factors also was significant ($p < 0.05$). Individual exercise intervention influenced Tb.Th, but genistein administration alone did not. Tb.Sp was increased dramatically in OVX mice, while combined intervention completely protected the separation, as did E_2.

Figure 6 shows the histological parameters for bone formation in the cortical bone of the femoral diaphysis. The periosteal mineral apposition rate (MAR) was significantly increased by OVX and completely restored by exercise as well as E_2 administration to the level similar to that of sham mice (Fig. 6A). OVX induced a decrease in periosteal bone formation rate/bone surface (BFR/BS) because of reduced mineralizing surface/bone surface (MS/BS) (Figs. 6B and 6C). Exercise significantly increased periosteal BFR/BS

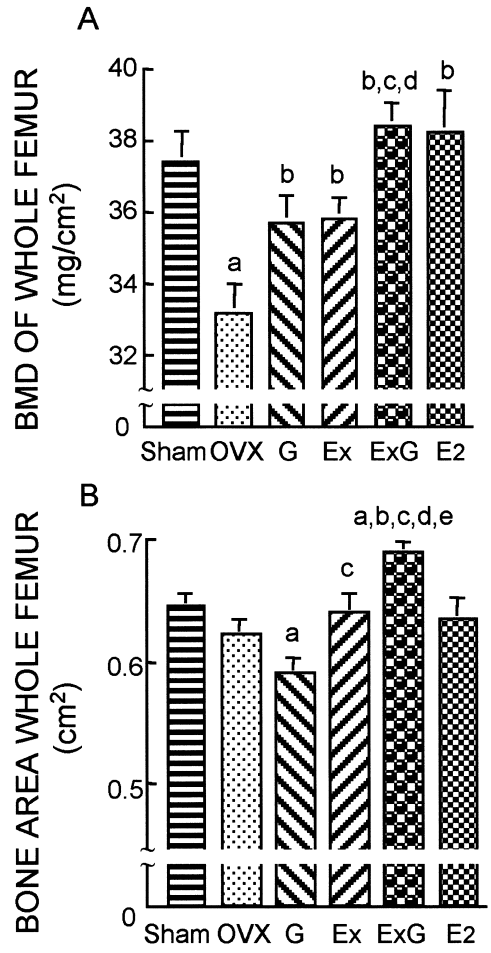

FIG. 3. Effects of genistein, exercise, and combined interventions on (A) BMD and (B) area of whole femur in OVX mice. Mice were sham-operated (sham) or OVX, and some OVX mice were treated with 0.4 mg/day of genistein (G), trained to exercise with (ExG) or without genistein (Ex), or treated with 0.03 μg/day of E_2 (E2). Total femoral BMD in each group was measured by DXA 4 weeks postoperatively. 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 G group; d, significantly different from the Ex group ($p < 0.05$).

through markedly elevated MS/BS (Figs. 6B and 6C). Cooperative effects of exercise and genistein administration on bone formation were not observed.

DISCUSSION

This study clearly shows that the combined intervention of moderate exercise and the submaximal dose of genistein administration exhibit cooperative effects on prevention of bone loss in OVX mice.

Genistein, which is structurally similar to estrogen, has the ability to bind to both estrogen receptors α and β. We previously reported that administration of 0.7 mg/day of genistein prevented bone loss by decreasing osteoclast number without exhibiting adverse effects on the uterus in OVX

TABLE 1. EFFECTS OF GENISTEIN, EXERCISE, AND COMBINED INTERVENTIONS ON BMD AND BONE AREA OF FEMORA IN OVX MICE

	BMD (mg/cm^2)			*Area (cm^2)*		
	Proximal	*Middle*	*Distal*	*Proximal*	*Middle*	*Distal*
Sham	41.8 ± 0.5	34.9 ± 0.9	41.7 ± 1.3	0.200 ± 0.004	0.184 ± 0.003	0.193 ± 0.005
OVX	40.2 ± 0.7	30.5 ± 0.9[a]	35.4 ± 1.1[a]	0.196 ± 0.005	0.167 ± 0.006[a]	0.194 ± 0.005
G	41.8 ± 0.6	33.8 ± 1.0[b]	37.8 ± 1.2[a]	0.191 ± 0.005	0.167 ± 0.005[a]	0.182 ± 0.005
Ex	42.2 ± 0.8[b]	33.4 ± 0.8[b]	38.2 ± 0.6[a]	0.191 ± 0.006	0.175 ± 0.006	0.203 ± 0.005[c]
ExG	43.9 ± 0.8[b]	36.2 ± 0.8[b,d]	41.4 ± 0.8[b,c]	0.210 ± 0.003[b,c,d]	0.191 ± 0.004[b,c,d]	0.217 ± 0.003[a,b,c,d,e]
E_2	44.2 ± 1.2[b]	34.2 ± 1.1[b]	43.6 ± 2.5[b,c,d]	0.198 ± 0.006	0.180 ± 0.006	0.191 ± 0.006

BMD and bone area at the proximal region, midshaft, and distal region of the excised femora were measured by DXA 4 weeks postoperatively. Mice were sham-operated (sham) or OVX, and some OVX mice were treated with 0.4 mg/day of genistein (G), trained to exercise with (ExG) or without genistein (Ex), or treated with 0.03 μg/day of E_2. 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 G group.
[d] Significantly different from the Ex group.
[e] Significantly different from the E_2 group ($p < 0.05$).

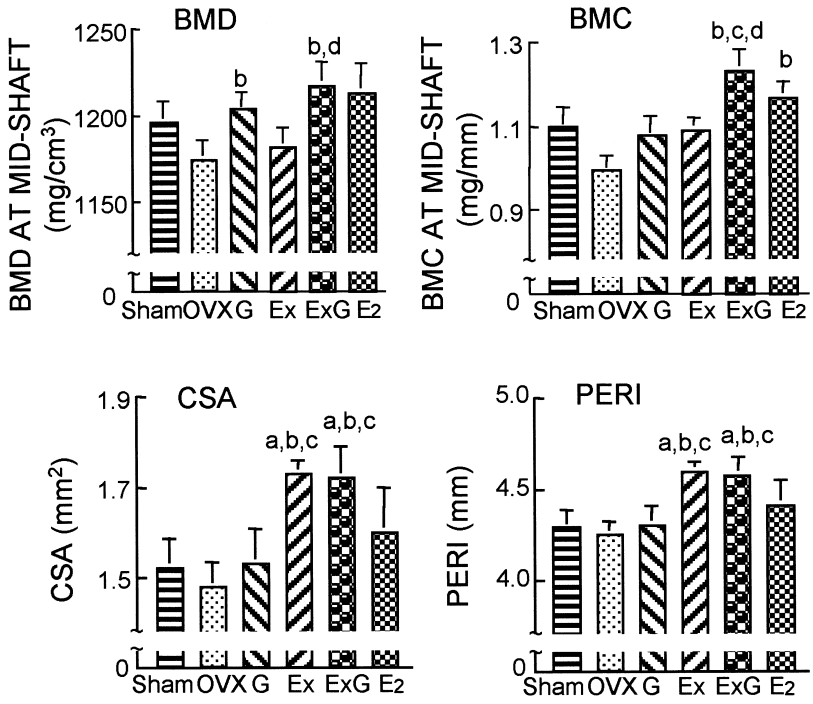

FIG. 4. Effects of genistein, exercise, and combined interventions on BMD, BMC, CSA, and PERI at the midshaft of the femora in the OVX mice. Mice were sham-operated (sham) or OVX, and some OVX mice were treated with 0.4 mg/day of genistein (G), trained to exercise with (ExG) or without genistein (Ex), or treated with 0.03 μg/day of E_2. BMD, BMC, total CSA, and PERI at 8 mm from the distal end in the femur were analyzed by pQCT. 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 G group; d, significantly different from the Ex group ($p < 0.05$).

mice.[1] In the report, we concluded that like estrogen, genistein prevented bone loss by inhibiting accumulation of B lymphocytes in bone marrow, which closely correlated with bone mass. In this study, we used the submaximal dose of genistein, which is sufficient for preventing bone loss in OVX mice, to assess the cooperative effects of the isoflavone with exercise.[14] Administration of 0.4 mg/day of genistein for 4 weeks partially but significantly prevented the decrease in BMD of the femora in OVX mice (Fig. 3; Table 1). However, genistein did not affect either the bone area on DXA analysis (Fig. 3; Table 1) or structural parameters in the midshaft of the femur such as CSA and PERI on pQCT analysis in OVX mice (Fig. 4). These results indicate that genistein at the submaximal dose did not promote bone formation under an estrogen-deficient condition.

Mechanical stimuli are essential for skeletal strength, and immobilization induces rapid bone loss in weight-bearing bone.[15] It has been reported that physical exercise that loads mechanical stress on bone is partially effective in preventing bone loss in postmenopausal women as well as OVX animals.[9–10,16–18]

The effects of running exercise on bone mass depend on several factors such as intensity, duration, and the animal model.[19] Concerning intensity, Peng et al.[9] has reported that exercise with moderate intensity (10 m/minute) was more effective than that with higher intensity (18 m/minute).

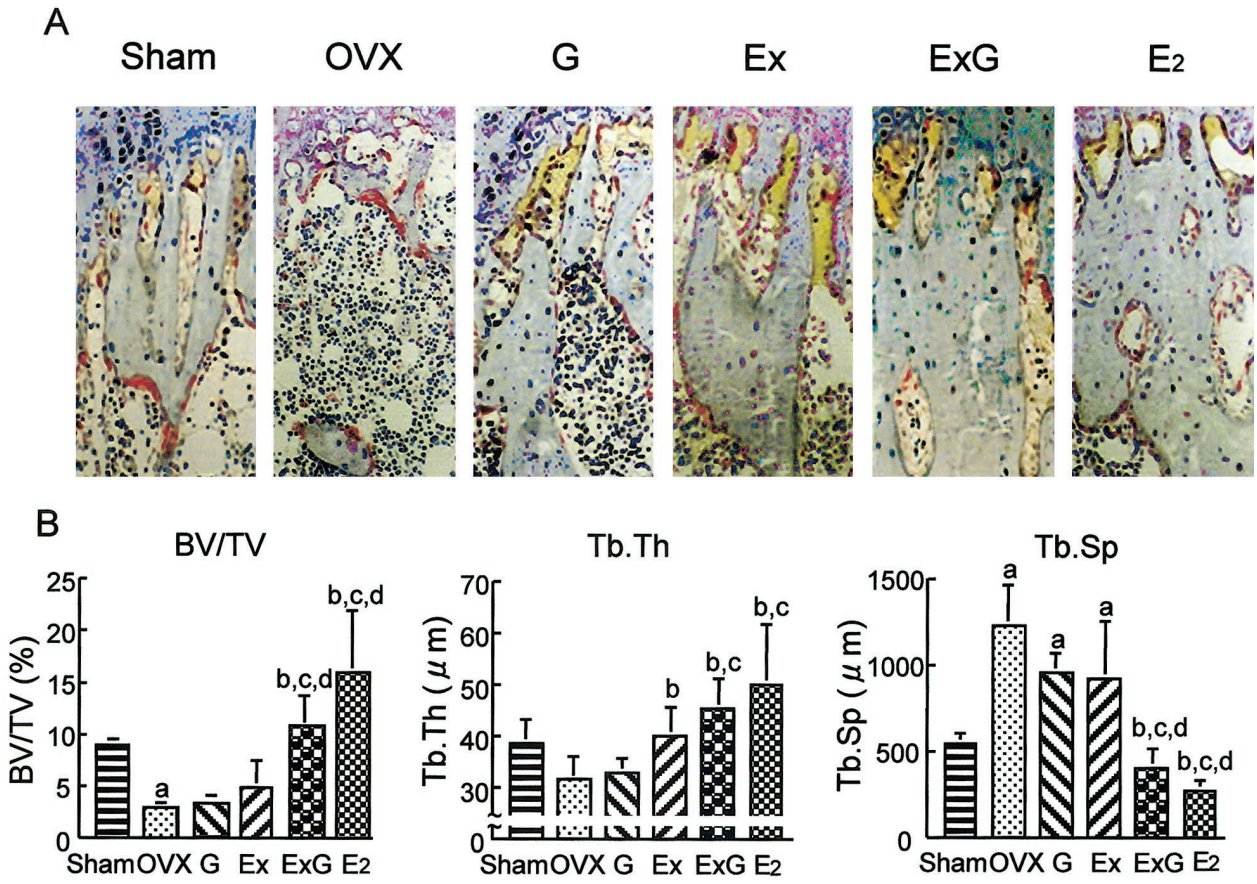

FIG. 5. Histological analysis of trabecular bone collected from sham mice, OVX mice, and OVX mice treated with genistein, OVX mice trained to exercise with or without genistein, and OVX mice treated with E_2. Mice were sham-operated (sham) or OVX, and some OVX mice were treated with 0.4 mg/day of genistein (G), trained to exercise with (ExG) or without genistein (Ex), or treated with 0.03 μg/day of E_2. Femora were collected 4 weeks after the operation, and the sections of distal metaphysis were prepared. (A) Sections of trabecular bone stained for TRAP (×85). (B) Two-dimensional histomorphometric parameters of trabecular bone shown in panel A. Microstructural parameters were determined as described in the Materials and Methods section. 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 G group; d, significantly different from the Ex group ($p < 0.05$).

Iwamoto et al.[10] and Barengolts et al.[20] also observed that training with a relatively low intensity (12 m/minute) was more efficient than that with higher intensity training (18 m/minute or 21 m/minute) for preventing bone loss in OVX rats. Because mice were more active in cages as compared with rats, each mouse was housed in a small individual cage to restrict daily physical activity. We forced mice to run on an inclined treadmill to increase mechanical stress. Together with previous findings and our improvements, OVX mice were trained for 12 m/minute on a 10° uphill treadmill in this study.

In this study, we found that the moderate level of running exercise prevented bone loss and improved structural parameters of the femur in OVX mice. In the analysis using DXA, running exercise significantly prevented bone loss at the proximal region and midshaft of the femur but not at the distal region, although BMD at this region was slightly higher than that in OVX mice (Table 1). This might be because of extension of the bone area at the distal region of the femur in the Ex group, because BMD was calculated as BMC that was divided by scanning area. In fact, Tb.Th at the distal femur in the Ex group was significantly higher than that in the OVX group (Fig. 5). Furthermore, CSA and PERI at diaphysis measured by pQCT was significantly higher than those in the sham and genistein-treated OVX mice as the result of exercise, indicating that exercise promoted bone formation (Fig. 4). In the histomorphometric analysis of the cortical bone at the femoral diaphysis, periosteal MAR significantly increased in OVX mice because of high bone turnover, and this was restored completely by running exercise to the level similar to that in the sham mice (Fig. 6A). OVX induced a decrease in periosteal BFR in mice, which was caused by a reduction in MS/BS (Figs. 6B and 6C). Unlike OVX mice, it has been reported that OVX rats exhibit higher periosteal BFR than that in sham rats.[20] This discrepancy might be because of a higher bone resorption rate in the cortical bone at the femoral midshaft in OVX mice than that in OVX rats. In fact, the rate of decrease in BMD and geometric parameters in the femoral cortical bone in OVX rats was small as compared with that in OVX mice.[21] Running exercise dramatically increased BFR as well as MS/BS up to the levels higher than those in sham mice, indicating that exercise stimulates bone formation. These results are consistent with the evidence that CSA and

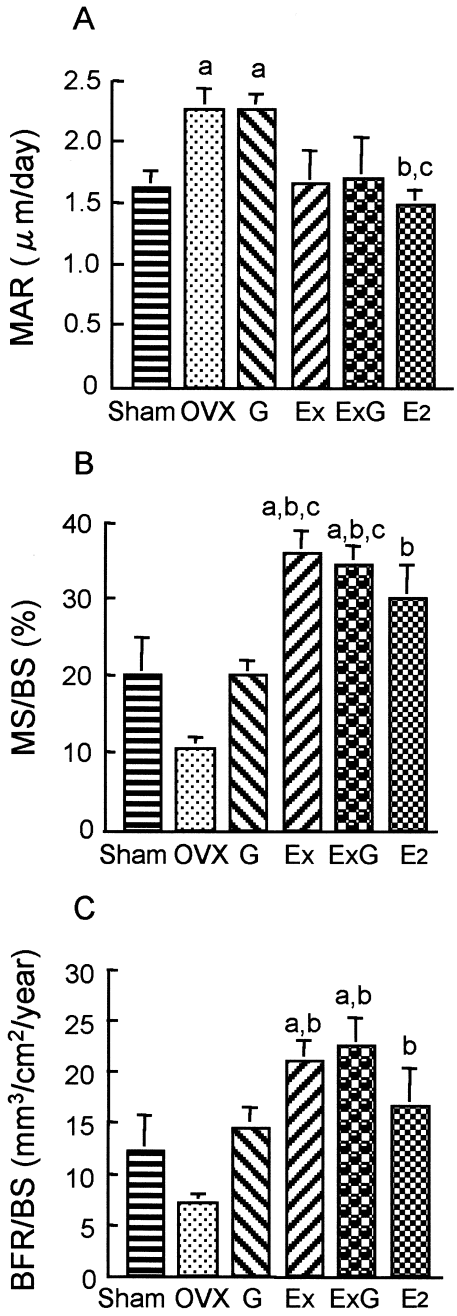

FIG. 6. Histological analysis of cortical bone at the midshaft collected from sham mice, OVX mice, and OVX mice treated with genistein, OVX mice trained to exercise with or without genistein, and OVX mice treated with E_2. Mice were sham-operated (sham) or OVX, and some OVX mice were treated with 0.4 mg/day of genistein (G), trained to exercise with (ExG) or without genistein (Ex), or treated with 0.03 μg/day of E_2. Femora were collected 4 weeks after the operation, and the sections of diaphysis were prepared. (A) MAR (interlabel width/day), (B) MS/BS (double-labeled surface + single-labeled surface/2)/BS), and (C) BFR/BS (MAR $\times$ MS/BS/100) were measured by calcein double-labeling on the periosteal surface. Data are means $\pm$ SEM of 8 mice. a, Significantly different from the sham group; b, significantly different from the OVX group; c, significantly different from the G group ($p < 0.05$).

PERI at the femoral midshaft were significantly higher in Ex and ExG groups than those in non-exercised groups on pQCT analysis. These findings are consistent with evidence reported by Newhall et al.[22] that shows running exercise significantly increased BMD in the proximal and middle femur but not in the distal femur of young male rats. It also has been shown that those structural parameters are significantly decreased by immobilization in the mouse model.[23] These results suggest that exercise training not only prevents bone loss but also strengthens structural properties in the specific regions of femur under estrogen-deficient conditions.

Based on the efficacy of each genistein administration and exercise training, we hypothesized that combined intervention would result in maximizing the potential for preventing bone loss in OVX mice. As we expected, a pronounced and additive effect of the two interventions was observed. This combined intervention not only completely restored bone mass to the sham level at all three femoral regions, but also increased total bone area, CSA, and PERI at the diaphysis of the femur on analyses using DXA and pQCT (Table 1; Fig. 4). The increase in PERI at the diaphysis might be because of bone formation, because BMD and CSA significantly increased at the region (Fig. 4). Further marked interaction between exercise and genistein administration on bone mass was found in the histomorphometric analysis at the distal cancellous bone of the femur (Fig. 5). Although, the exercise or genistein administration had no significant effects on low BV/TV and high Tb.Sp in the distal femoral metaphysis in OVX mice, the combined intervention completely restored these parameters to the levels in the sham group. It has been reported that combined treatment with exercise and estrogen increased the spine and total body BMD, although estrogen therapy alone did not affect BMD in menopausal women.[24] In OVX rats, the bone mass in the appendicular and vertebrae bone was higher in the group loaded with running exercise and estrogen intervention than in those receiving either intervention alone.[18] These results strongly support our finding that combined intervention of exercise and genistein exhibit additive effects on prevention of bone loss in OVX mice.

Frost[25] showed that estrogen deficiency increased the "set point" for the skeleton to respond to loading, causing the skeleton to be less sensitive to mechanical force and decreasing its bone mass. In this regard, it is likely that phytoestrogens such as genistein can influence the set point of the mechanical loading that affects bone mass. In this study, the serum concentrations of genistein did not significantly differ in the groups that were treated with genistein alone and that combined with exercise (data not shown). Therefore, it is unlikely that exercise affects the metabolism of genistein in OVX mice. Further studies are necessary to define the mechanism of interaction between exercise and genistein administration in bone metabolism under estrogen-deficient conditions. Furthermore, it is important to examine whether the combined intervention affects the bone mass in postmenopausal women. If so, it may be useful to establish a clinical treatment regimen for the prevention of osteoporosis.

In conclusion, moderate exercise and a submaximal dose of genistein administration exhibited cooperative effects in preventing bone loss, especially that of trabecular bone at the distal metaphysis under estrogen-deficient conditions. Exercise training strengthens structural properties by promoting bone formation as well as preventing bone loss. It can be speculated that genistein influences the set point of the mechanical load to affect bone mass. Therefore, it may be possible that moderate exercise combined with appropriate intake of soybean products is useful for preventing osteoporosis in postmenopausal women.

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