High-impact exercise strengthens bone in osteopenic ovariectomized rats with the same outcome as Sham rats

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Honda, Akiko, Naota Sogo, Seigo Nagasawa, Takuya Shimizu, and Yoshihisa Umemura. High-impact exercise strengthens bone in osteopenic ovariectomized rats with the same outcome as Sham rats. J Appl Physiol 95: 1032–1037, 2003.—The effect of jump exercise on middle-aged osteopenic rats was investigated. Forty-two 9-mo-old female rats were either sham-operated (Sham) or ovariectomized (OVX). Three months after surgery, the rats were divided into the following groups: Sham sedentary, Sham exercised, OVX sedentary, and OVX exercised. Rats in the exercise groups jumped 10 times/day, 5 days/wk, for 8 wk, with a jumping height of 40 cm. Less than 1 min was required for the jump training. After the experiment, the right tibia and femur were dissected, and blood was obtained from each rat. OVX rats were observed to have increased body weights and decreased bone mass in their tibiae and femurs. Jump-exercised rats, on the other hand, had significantly increased tibial bone mass, strength, and cortical areas. The bone mass and strength of OVX exercised rats increased to approximately the same extent as Sham exercised rats, despite estrogen deficiency or osteopenia. Our data suggest that jump exercise has beneficial effects on lower limb bone mass, strength, bone mineral density, and morphometry in middle-aged osteopenic rats, as well as in Sham rats.

High-impact jump exercise; ovariectomy; osteopenic rats; bone mass; bone strength

It is well known that physical exercise has beneficial effects on bone. Human and animal studies have demonstrated exercise to increase bone mineral density (BMD) (4, 18), as well as bone mass and strength (33, 34). In addition, exercise has been shown to protect against age-related bone loss (13, 18, 19, 36). It is thought that the mechanical stress generated by exercise plays an important role in the osteogenic response (9). Human studies investigating osteogenic responses to exercise have examined many kinds of training programs, including running and walking (8, 12, 19), resistance training (18, 19), aerobic exercise (8, 13, 20), and high-impact or jump exercise (5, 7, 10). Most animal studies have investigated treadmill running (1, 2, 16, 27–29, 37), although other types of training programs have also been examined (11, 25, 26). Among all types of exercise programs, high-impact exercise is thought to be greatly beneficial to bone. The benefit is thought to result from the dynamic nature and high strain rate, as well as the magnitude of mechanical stress, imposed by high-impact exercise on bone (31). In vivo human and animal studies have shown high-impact exercise to produce higher strain and higher strain rate than walking (17, 21). High strain magnitude, high strain rate, or shear strain have been shown to enhance bone formation (17, 21, 22). Extracellular fluid dynamics may be related to the observed osteogenic response (17, 32, 35). In addition, loading protocols consisting of fewer numbers, several sessions rather than repetitive single bout (29), or a longer interval (30 s) rather than shorter interval (3 s) between each loading (35) were more effective for bone mass and strength, because mechanosensitivity in bone may decline and require longer recovery time after loading.

In a previous report, our laboratory reported that jump exercise greatly increases bone mass and strength in rats (33). It is thought that jump exercise generates high-impact loading before the rats leave the ground. Moreover, jump exercise does not require a lot of repetition to generate significant anabolic effects (34), and it has little aerobic or anaerobic effect. Aerobic and anaerobic factors can confuse interpretation of results regarding the osteogenic effects of exercise.

Ovariectomized (OVX) animals have been used to model human menopause. In previous studies, the osteogenic effects of exercise on OVX rats have varied according to the intensity and duration (2, 16, 28), as well as the type of exercise employed (11, 14, 26). One of the reasons why the results of these studies have varied relates to aerobic and anaerobic factors. Previously, our laboratory reported that high-impact, low-repetition, jump exercise could increase bone mass and strength in OVX rats and sham-operated rats to the same extent (14). Our group reported this result after operating on 11-wk-old rats that had just reached sexual maturity and having them exercise from 12 to 20 wk of age. However, the long-term effects of OVX on these rats were not observed, i.e., whether it decreased.
bone mass and strength. The bone responses to exercise might differ with prolonged estrogen deficiency and age.

In this study, we used middle-aged osteopenic rats. We attempted to establish osteopenia by withholding hormone replacement therapy for 3 mo after OVX. Osteopenia induced by OVX was achieved after 3 mo. The purpose of this study was to investigate whether jump exercise might have a beneficial effect on bones in middle-aged osteopenic rats.

**MATERIALS AND METHODS**

**Animals.** Forty-two female Wistar rats, aged 10 wk, were obtained from Japan SLC (Hamamatsu, Japan). The rats were housed individually in standard cages under constant temperature (23 ± 1°C). The light-dark cycle was with darkness from 6:30 AM to 6:30 PM. Food (CE-2, Clea Japan, Tokyo, Japan) and water were provided ad libitum. At 9 mo of age, the rats underwent either sham operation (Sham rats; n = 21) or OVX (OVX rats; n = 21) while under anesthesia with pentobarbital sodium via an abdominal approach. Three months after surgery, they were divided into the following four groups: 1) sham-operated sedentary (SS; n = 9), 2) sham-operated exercised (SE; n = 12), 3) OVX sedentary (OS; n = 10), and 4) OVX exercised (OE; n = 11).

At the end of the experiment, the rats were anesthetized with diethyl ether and killed by exsanguination. The right tibia, femur, and uterus were dissected from each rat. Immediately after careful removal of all soft tissue, the right tibia was tested mechanically, and the right femur was fixed with 70% ethanol and stored at 4°C until bone mineral densitometry was performed. The experimental protocol was approved by the Animal Subjects Committee at Chukyo University Graduate School of Health and Sport Sciences.

**Training program.** Rats in the training groups jumped 10 times/day, 5 days/wk, for 8 wk, with a jumping height of 40 cm. This height was chosen for a couple of reasons. For one, the maximum jumping height of the rats was ~45–50 cm. Furthermore, a previous study has shown a jumping height of 40 cm to be more effective for building bone than either 30 or 50 cm. (23). Each exercised rat jumped and grasped the top of a wooden box (40 cm in height) with their forelimbs and pulled themselves up to the edge, after which the rats were carefully returned to the bottom of the box by hand (23, 33, 34). Initially, the rats jumped with electrical stimulation, but, after a few days, they jumped without stimulation. Rats jumped at ~3-s intervals, and <1 min was required for training. The sedentary groups were handled 5 days/wk for 8 wk. Training and handling were begun at 12 mo of age.

**Mechanical testing procedures.** After the length of the right tibia was measured with sliding callipers, the bone was loaded for three-point bending until fracture with a servo-controlled electromechanical testing system (model RX1600, Techno, Tokyo, Japan). The bone was placed on two supports separated by 16 mm, and the test was conducted at the midpoint of the tibia. The maximum sustainable force before breaking was recorded.

**Bone mass and bone morphometry.** After the fracture test, the right tibia was immersed in a solvent (2 vol of chloroform combined with 1 vol of methanol) for 1 wk and then dried at 80°C for 24 h. Then, the fat-free dry weight (FFD) was measured. For cross-sectional analysis, the right tibia was embedded in polyester resin (Rigolac 204, Okinoshijou, Tokyo, Japan) by submersion at room temperature for 7 days after restoration with a bonding agent. The midshaft cross section of each bone was cut near the site of fracture, which was premarked before the fracture test. The cross section was examined at high magnification (×50) under a microscope. A digitizing pad was used to determine the medullary and cortical areas, the endosteal and periosteal perimeters, as well as the moment of inertia. The bending stress was then calculated (34).

**Bone mineral densitometry.** BMD in the right femur was measured with dual-energy X-ray absorptiometry (model DSC-600A, Aloka, Tokyo, Japan). The femur was divided into 10 equal parts, which were grouped as follows: 1) proximal (the first three parts), 2) middle (the fourth to seventh parts), 3) distal (the last 3 parts), and 4) whole femur (all parts combined). The coefficient of variation of BMD was estimated from the results of five rats, examined within 24 h of each other, and a value of 1.3% was obtained.

**Biochemical marker.** Blood was centrifuged, and the serum used for osteocalcin determination. The samples were stored at ~80°C until analysis. Serum osteocalcin was measured with a double-antibody radioimmunoassay by using the rat standard (Biomedical Technologies, Stoughton, MA).

**Statistical analysis.** Data are presented as means ± SD. A two-way (training × OVX) ANOVA was used to examine the individual main effect and interaction between these factors with the use of SPSS 9.0J for Windows. If a significant interaction was found, the effects of training and OVX were assessed by post hoc analysis. A significance level of P < 0.05 was used for all statistical tests.

**RESULTS**

Body and uterine weights. Body weights were similar in the Sham (308 ± 23) and OVX (309 ± 25) groups before surgery. Pre- and posttraining body weights of OVX rats were significantly greater than in Sham rats (pre- and posttraining weights were taken at 12 mo and at dissection, respectively). The uterine weights of OVX rats were significantly less than those of Sham rats. We confirmed the success of OVX by the absence of ovarian tissue and atrophy of the uterine horns. Jump training did not affect these parameters (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Training</th>
<th>O VX</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>325.3 ± 28.8</td>
<td>331.7 ± 28.2</td>
<td>366.0 ± 27.8</td>
</tr>
<tr>
<td>SE</td>
<td>328.0 ± 36.8</td>
<td>343.7 ± 34.8</td>
<td>359.9 ± 29.7</td>
</tr>
<tr>
<td>OS</td>
<td>28.2 ± 59.1</td>
<td>558.4 ± 230.5</td>
<td>111.0 ± 14.3</td>
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</tbody>
</table>

Values are means ± SD. OVX, ovariectomy; SS, sham-operated sedentary; SE, sham-operated exercised; OS, ovariectomized sedentary; OE, ovariectomized exercised; NS, not significant.

**Table 1. Body and uterine weights in the Sham or OVX rats**
weight-normalized FDW (FDW/wt) were significantly greater in the exercised groups than in the Sham groups. On the other hand, FDW, FDW/wt, and maximum force were significantly greater in the exercised groups than in the sedentary groups. Moment of inertia and bending stress did not change with OVX or training (Table 2).

Bone morphometry. OVX significantly increased endosteal perimeter and medullary area, which is indicative of bone resorption. Jump training, on the other hand, significantly increased periosteal perimeter and cortical area, indicating bone became larger toward the outside, and thus bone hypertrophy was observed (Table 3).

Bone densitometry. OVX significantly decreased BMD in all segments of the femur. Training significantly increased BMD in parts of the proximal, distal, and whole femur, and it had a tendency to increase BMD in the middle segment of the femur (P = 0.06; Fig. 1).

Biochemical marker. Serum osteocalcin levels in SS, SE, OS, and OE were 21.7 ± 4.5, 19.5 ± 8.7, 29.5 ± 11.0, and 22.0 ± 3.9 ng/ml, respectively. Osteocalcin levels were significantly higher in the OVX groups than in the Sham groups (P = 0.05). Jump training decreased osteocalcin levels in OVX rats but not significantly (P = 0.06).

No significant interactions were observed among all the parameters examined.

**DISCUSSION**

In this study, OVX led to a decrease in bone mass and density, and it increased the level of osteocalcin as well as the endosteal perimeter and medullary area of the midshaft. Thus osteopenia was observed as a long-term effect of OVX. OVX rats were then examined to determine the effect of jump exercise. Jump exercise was found to improve bone mass, density, strength, and morphometry in middle-aged osteopenic rats.

FDW increased by 6 and 10% in exercised Sham and OVX rats, respectively, compared with their sedentary counterparts. FDW/wt increased by 1 and 9% in these groups, respectively. Maximum force also increased with exercise in Sham and OVX rats, to 17 and 27% above that of their nonexercised counterparts, respectively. Thus FDW and maximum force increased to a greater extent in exercised OVX rats, compared with exercised Sham rats, although, in a previous study, our laboratory observed the same percent increase in each of these parameters in exercised OVX and Sham rats (14). The greater osteogenic effects observed in middle-aged osteopenic rats in the present study might be explained by accommodation to loading. It is thought that mechanosensors within bone respond to bone strain; however, it is thought that bone accommodates loading, after which bone mass and strength do not increase further unless the magnitude of loading is increased. This because an increase in bone mass can diminish the bone strain imposed by a given level of loading (9). This theory is supported by the finding that jump exercise increased bone mass and strength to similar absolute values in SE and OE rats in the present experiment (FDW: 437.8 ± 28.9 and 429 ± 20.0 mg in SE and OE, respectively; maximal force: 103.8 ± 12.0 and 103.9 ± 9.3 N in SE and OE, respectively), regardless of their initial bone mass and strength. Thus greater increases in bone mass and strength were observed among OVX rats with osteopenia than among sham-operated rats. In our laboratory’s previous study, not only the absolute value achieved, but also the rate of increase in these parameters due to exercise, were similar in OVX and Sham rats (14). Combined, these results indicate that a given load might increase bone mass and strength to similar absolute values in OVX and SE rats, regardless of the

**Table 2. Bone parameters of the tibia in the Sham or OVX rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>SS</th>
<th>SE</th>
<th>OS</th>
<th>OE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, mm</td>
<td>39.7 ± 0.6</td>
<td>39.6 ± 0.5</td>
<td>39.9 ± 0.6</td>
<td>39.7 ± 0.6</td>
</tr>
<tr>
<td>FDW, mg</td>
<td>412.0 ± 16.5</td>
<td>437.8 ± 28.9</td>
<td>389.8 ± 19.7</td>
<td>429.6 ± 20.0</td>
</tr>
<tr>
<td>FDW/wt, %</td>
<td>0.127 ± 0.012</td>
<td>0.128 ± 0.008</td>
<td>0.109 ± 0.007</td>
<td>0.119 ± 0.006</td>
</tr>
<tr>
<td>Maximum force, N</td>
<td>88.6 ± 5.1</td>
<td>103.8 ± 12.0</td>
<td>82.0 ± 9.0</td>
<td>103.9 ± 9.3</td>
</tr>
<tr>
<td>Moment of inertia, mm²</td>
<td>216.4 ± 54.6</td>
<td>208.8 ± 35.4</td>
<td>182.2 ± 22.7</td>
<td>217.9 ± 31.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. FDW, fat-free dry weight; FDW/wt, weight-normalized FDW.

**Table 3. Bone morphometry of the tibia in the Sham or OVX rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>SS</th>
<th>SE</th>
<th>OS</th>
<th>OE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosteal perimeter, mm</td>
<td>4.76 ± 0.55</td>
<td>4.63 ± 0.40</td>
<td>5.10 ± 0.32</td>
<td>4.89 ± 0.26</td>
</tr>
<tr>
<td>Periosteal perimeter, mm</td>
<td>8.88 ± 0.66</td>
<td>9.40 ± 0.56</td>
<td>9.17 ± 0.42</td>
<td>9.32 ± 0.43</td>
</tr>
<tr>
<td>Medullary area, mm²</td>
<td>1.58 ± 0.35</td>
<td>1.47 ± 0.22</td>
<td>1.80 ± 0.28</td>
<td>1.67 ± 0.22</td>
</tr>
<tr>
<td>Cortical area, mm²</td>
<td>3.74 ± 0.52</td>
<td>4.27 ± 0.40</td>
<td>3.72 ± 0.31</td>
<td>4.04 ± 0.19</td>
</tr>
</tbody>
</table>

Values are means ± SD.

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presence of estrogen deficiency or osteopenia. However, this needs to be examined further because the OVX rats of the present study were heavier (~10%) than their sham-operated counterparts.

Although we could not directly measure parameters of strain in the present experiment, we assumed that higher strain and strain rates were produced during jump training than running, because the ground-reaction force was approximately five times body weight for a 40-cm jump (laboratory data). In a previous report, our group suggested that jump exercise was a more effective form of training than running for bones (33). Because running is an endurance exercise (a form of aerobic training), we would not expect it to generate sufficient mechanical stress to increase bone mass and strength in rats. Moreover, the effects of running vary greatly according to training intensity, running speed, duration and frequency, and estrogen levels. Some researchers have reported strenuous exercise to have no significant, or even adverse effects, on bone mass, as well as on several mechanical and geometric properties (6, 15, 24, 37). Strenuous running might lead to anaerobic metabolism. Other studies have reported treadmill running to have a more pronounced effect on bone in estrogen-deficient rats than in Sham rats (1, 2, 27, 28). Running of low or moderate intensity has been observed to significantly increase bone mass and strength in OVX rats; however, the same level of running was not found to affect these same parameters in Sham rats (2, 28). The mechanical stress generated by running might be under the bone modeling threshold for Sham rats, a concept proposed by Frost (9) in his mechanostat theory. However, the same level of mechanical stress might be adequate to stimulate bone metabolism in estrogen-deficient rats. Alternatively, rats with high bone turnover might be more sensitive to loading compared with normal rats (1, 27, 28). As stated above, results with regard to the effects of running on bone are confusing, whereas jump exercise consistently results in increased bone mass and strength in rats, regardless of estrogen status or age.

In the training groups, BMD was significantly increased in the proximal and distal segments, and a trend toward increased BMD was also observed in the middle segment, when compared with sedentary groups. Our data suggest that jump exercise has a beneficial effect on both cancellous and cortical bone within the femur. The positive effect of jump exercise, especially that observed on cancellous bone, has important implications. This because bones that are primarily cancellous, such as the femoral neck and vertebrae, are more likely to be affected by estrogen deficiency compared with cortical bone, and thus they carry a higher risk of fracture. Studies based on histomorphometric analysis in OVX rats have demonstrated exercise-induced improvements, such as inhibition of loss of trabecular bone, in the metaphysis (3, 27, 28, 30). It can be inferred that microarchitectural changes occurred alongside the observed increase in BMD of cancellous bone in the present experiment. On the other hand, varying effects of exercise on the diaphysis have been observed by a number of researchers (25, 26, 30). Tamaki et al. (30) found running to significantly increase BMD of the femoral diaphysis in OVX rats. In addition, Notomi et al. (25) found resistance exercise to significantly increase BMD of the femoral diaphysis in male rats. However, the same effect was not observed with running in male rats. These results do little to clarify the effect of training on BMD. The differences between the results of our study and other studies may be explained by differences in age, the magnitude of imposed mechanical stress, the amount of training, or the position at which the mechanical stress was loaded, to name a few factors. In this study, we noted significant bone morphometric changes, including increased peristeal perimeter and cortical area, as a result of training, in the tibia. However, the results obtained with regard to increased cortical area contradict those
of a previous report, in which our laboratory reported a nonsignificant increase in cortical area in OVX rats compared with controls (14). In addition, other studies have not observed changes in the cortical area of OVX rats, even when increases in bone mass and strength due to training were observed (1, 2, 27). Although jump exercise enlarged the cortical area in both Sham and OVX rats in this study, the observed increase in this parameter was less in OVX rats than in Sham rats, considering the improvements of FDW and strength or the weight gained in OVX rats. The level of osteocalcin was significantly greater in OVX rats than in Sham rats. Thus high bone turnover induced by OVX might have resulted in bone morphometric changes in OVX rats.

In conclusion, high-impact, low-repetition jump exercise has an osteogenic effect on lower limbs in middle-aged osteopenic rats, as well as sham-operated rats. In addition, greater effects of training were observed in OVX rats, compared with Sham rats. Thus loading activity might enhance bone mass and strength to a given level, depending on the size of the load, regardless of whether estrogen deficiency or osteopenia is present.

REFERENCES