Effects of jump training on bone are preserved after detraining, regardless of estrogen secretion state in rats

Yoshihisa Umemura,1 Seigo Nagasawa,1 Naota Sogo,2 and Akiko Honda1

1Laboratory for Exercise Physiology and Biomechanics, School of Health and Sport Sciences, Chukyo University, Toyota; and 2Department of Sports, Kyushu Kyoritsu University, Kitakyushu, Japan

Submitted 4 September 2007; accepted in final form 24 January 2008

Umemura Y, Nagasawa S, Sogo N, Honda A. Effects of jump training on bone are preserved after detraining, regardless of estrogen secretion state in rats. J Appl Physiol 104: 1116–1120, 2008. First published January 24, 2008; doi:10.1152/japplphysiol.00937.2007.—We investigated whether the effects of jump training on bone are preserved after detraining, regardless of estrogen secretion state in rats.

We previously reported that jump training in rats, even of just 10 jumps per day, resulted in a marked osteogenic response and enlarged the cross-sectional area of the tibial diaphysis in an outward direction (17). Moreover, we also reported that jump training has a similar effect on bones in both ovariectomized estrogen-deficient rats and in sham-operated rats (3, 4). Thus we examined three hypotheses in the present study: 1) jump training increases bone mass; 2) the skeletal benefits, in particular the benefits to cortical bone, of jump training are maintained with detraining; and 3) estrogen status does not influence the response of the skeleton to jump training and detraining.

MATERIALS AND METHODS

Animals and experimental design. Initially, 48 female Wistar rats, aged 10 wk, were obtained from Japan SLC, (Hamamatsu, Japan). At the age of 11 wk, they were sham-operated (sham: n = 24) or ovariectomized (Ovx: n = 24), under anesthesia with pentobarbital sodium, via an abdominal approach, and were divided into exercise or sedentary subgroups [sham sedentary (SS), sham exercise (SE), Ovx sedentary (OS), and Ovx exercise (OE)].

The rats in the two exercise groups performed jumping exercise for 8 wk, from 12 to 20 wk of age. They jumped upward from the bottom of the box, which was surrounded by a board, and caught the upper end of the box, 40 cm in height, with their forelimbs. The rats that then

---

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
climbed onto the board surrounding the box were caught by hand and placed at the bottom of the box again for the next jump. They jumped 10 times/day, 5 times/wk. In the early days of training, we used electric stimulation through an electrode plate installed in the bottom of the box. The rats became accustomed to the training within 2 or 3 days, and thus jumped even in the absence of electric stimulation, which we ceased using thereafter. They ceased jump training at 20 wk of age and did not undergo exercise, except for free movement in their cages for the following 24 wk, from 20 to 44 wk of age. The rats in the two sedentary groups did not exercise, except for free movement, for the duration of the experiment. All rats were housed individually in standard cages under constant temperature (23 ± 1°C). The light-dark cycle was 12:12 h with darkness from 6:30 AM to 6:30 PM. They had ad libitum access to food (CE-2, CLEA Japan, Tokyo, Japan) and water. Four rats died during the experiment due to complications with the Ovx or sham surgery or anesthesia; all of their data were omitted from the analyses. Therefore, the number of rats in the SS, SE, OS, and OE groups were 12, 11, 10, and 11, respectively. After the 24-wk detraining period, the rats were anesthetized with diethyl ether, killed by exsanguination, and both tibias dissected from each rat. The experimental protocol was approved by the Animal Subjects Committee at Chukyo University Graduate School of Health and Sport Sciences.

In vivo measurement. The BMC of the tibial and femoral region was determined by dual-energy X-ray absorptiometry (DXA; DCS-600EX; Aloka, Tokyo, Japan) every 4 wk, from 12 to 40 wk of age. The rats were anesthetized using pentobarbital solution and placed on the DXA device in the abdominal position. The rats were fixed such that the vertebrae and femur, and femur and tibia, formed a right angle, judging from the back. The femoral region was assumed to extend from the outside edge of the pelvis to the bottom of the knee joint, and the tibial region was assumed to be the tibia and fibula. The coefficient of variation of the BMC in the tibial and femoral region was 2.6 and 3.0%, respectively, for one rat repositioned for each of eight measurements.

Measurement of the extracted tibia. Bone length, anterior-dorsal width, and lateral-medial width at the center of the diaphysis were measured using a sliding caliper on the extracted right tibia. Next, BMC, area bone mineral density (BMD), and bone area were determined by DXA in the whole tibia and in five regions from the proximal to distal tibia. As the X-rays were irradiated through the lateral-medial direction, the measured bone area was an anterior-dorsal projection. In the left tibia, the fracture test was performed using the three-point bending system (TK-252C, Muromachi Kikai, Tokyo, Japan). The test was conducted in the lateral-to-medial direction at the middle of the tibia. The distance between the bottom supports was 20 mm, the crosshead speed was 10 mm/min, and the frequency of data collection was 50 Hz. The maximum load, total energy, and stiffness were determined by the load-deformation curve. Total energy was the integral of the load until the breaking point, and stiffness was the slope of the load-deformation curve between 30 and 70% maximum load.

Statistical analysis. Data are presented as means ± SD and were analyzed by SPSS 12.0 J for Windows. For the in vivo data at each time point, the ex vivo data, and the body weight data, a two-way ANOVA (surgery × exercise) was used to reveal the differences among groups. When the ANOVA revealed a significant interaction, post hoc comparisons (Tukey’s honestly significant difference test) were used to determine the differences between specific means. A significance level of P < 0.05 was used for all statistical tests.

RESULTS

No differences in body weight were observed at the beginning of the experiment. The Ovx significantly increased body weight (P < 0.001); however, exercise did not influence body weight, either at the end of the 8 wk of jump training (SS: 248 ± 12 g, SE: 245 ± 15 g, OS: 289 ± 17 g, OE: 293 ± 23 g), or at the end of the detraining period (SS: 278 ± 18 g, SE: 282 ± 23 g, OS: 336 ± 17 g, OE: 349 ± 31 g). The weight of the extirpated uterine was greater (P < 0.001) in the sham group (0.661 ± 0.242 g) than in the Ovx group (0.075 ± 0.007 g), thus confirming the success of the Ovx operation. In vivo measurement revealed that the jump training increased the BMC of the tibial region compared with the sedentary group (P < 0.001, Fig. 1). These differences were maintained throughout the detraining period (P < 0.001 or P < 0.01). The jump training did not influence the BMC of the femoral region, but the Ovx decreased it (P < 0.01 or P < 0.001, Fig. 2).

After the 24 wk of detraining, the BMC of the whole extracted tibia in the two exercised groups was significantly higher than in the two sedentary groups (P < 0.01, Table 1). In the measurement of the five tibial regions (Table 1), the exercise effect on the BMC was preserved at the diaphysis (P < 0.001; from the center-proximal to the center-distal region). The Ovx significantly decreased the BMC of the proximal region (P < 0.01), whereas the Ovx increased that of the center-proximal region (P < 0.001). The exercise and Ovx significantly increased bone areas from the center to distal region (P < 0.05, P < 0.01, or P < 0.001). ANOVA revealed a significant interaction at the center-proximal region (P < 0.05), and OE had a greater area in this region than OS (P < 0.05).

The maximum load was significantly greater in the exercised groups than in the sedentary groups (P < 0.001), and thus the exercise effect on bone strength was preserved (Fig. 3A). The total energy was also significantly greater in the exercised groups than in the sedentary groups (P < 0.05, Fig. 3B). ANOVA revealed a significant interaction in the stiffness parameter (P < 0.05), which was greater in SE than SS (P < 0.05, Fig. 3C).

The Ovx increased tibial bone length (P < 0.05), but exercise had no influence. The exercise (P < 0.001) and Ovx (P < 0.001) increased the anterior-dorsal width at the center of the tibia. The exercise increased lateral-medial width (P < 0.001), but Ovx did not (Table 2).

Fig. 1. Exercise-induced changes in the bone mineral content of the tibial region. SS, sham-operated sedentary (n = 12); SE, sham-operated exercised (n = 11); OS, ovariectomized sedentary (n = 10); OE, ovariectomized exercised (n = 11). Values are means ± SD. Exercise significantly increased the bone mineral content of the tibial region (from 16 to 24 wk of age: P < 0.001, from 28 to 40 wk of age: P < 0.01). ANOVA did not reveal a surgical main effect or a surgery-exercise interaction at any time.
In the present study, the effects of jump training on tibial bone strength and mass were preserved after 24 wk of detraining and were accompanied by enlargement of the width of the tibial diaphysis. These results were similar in both sham and Ovx rats.

In the jump training we used, the lower limb bones were loaded by the ground-reaction force and muscle contraction force at the take-off of jumping, but not at touchdown on the floor, as the rats were placed gently on the floor by the technician. The daily 10-jump program took <1 min and, based on our laboratory’s previous studies, did not increase body weight (3, 4, 16, 17), muscle weight of the lower limb (laboratory data), heart weight (16), or tibial bone length (3, 4, 16, 17). Thus this exercise program had less of an anabolic or aerobic factor, but placed impact loading on the limb bones. In this respect, this exercise program is somewhat similar to artificial bone loading, but has the advantage of excluding the need for anesthesia and associated implications and the disadvantage of not allowing the magnitude of the loading to be estimated.

In our laboratory’s previous studies (3, 4, 16, 17), the daily 10-jump program for 8 wk increased tibial bone mass and strength, as well as the cortical area, periosteal perimeter, and moment of inertia at the midshaft of the tibia, but did not increase the endosteal perimeter at the midshaft. Moreover, the exercise-induced effects on bone mass and strength were similar in both the Ovx and sham rats (3, 4). In the present study, the jumping program used for 8 wk is assumed to have induced the same changes, although we only observed the bones in vivo with DXA. After the exercise training, the tibial BMC increased to 9.6% in SE and 16.4% in OE compared with the corresponding sedentary group. There was no significant surgery-exercise interaction, although the rate of increase was rather greater in OE than in SE. The changes in tibial BMC were preserved in the detraining period, although the ratios fell. In the extracted tibia after the 24-wk detraining period, the exercise effects remained at 7.1% in SE and at 7.1% in OE in the tibial BMC.

The effects of the exercise were primarily seen in the intermediate part of the tibia, where the bone was bent during the jump exercise. A ground-reaction force during jumping might act on the tibia as an axial compression load. Thus the jumping load affected cortical bone at the diaphysis, but little affected cancellous bone at the metaphysis, as seen in the study by Warden et al. (18). Conversely, jump training did not affect femoral BMC, whereas Ovx decreased it. These findings suggest that the jump exercise did not impose a great load at the

---

**DISCUSSION**

In the present study, the effects of jump training on tibial bone strength and mass were preserved after 24 wk of detraining and were accompanied by enlargement of the width of the tibial diaphysis. These results were similar in both sham and Ovx rats.

In the jump training we used, the lower limb bones were loaded by the ground-reaction force and muscle contraction force at the take-off of jumping, but not at touchdown on the floor, as the rats were placed gently on the floor by the technician. The daily 10-jump program took <1 min and, based on our laboratory’s previous studies, did not increase body weight (3, 4, 16, 17), muscle weight of the lower limb (laboratory data), heart weight (16), or tibial bone length (3, 4, 16, 17). Thus this exercise program had less of an anabolic or aerobic factor, but placed impact loading on the limb bones. In this respect, this exercise program is somewhat similar to artificial bone loading, but has the advantage of excluding the need for anesthesia and associated implications and the disadvantage of not allowing the magnitude of the loading to be estimated.

In our laboratory’s previous studies (3, 4, 16, 17), the daily 10-jump program for 8 wk increased tibial bone mass and strength, as well as the cortical area, periosteal perimeter, and moment of inertia at the midshaft of the tibia, but did not increase the endosteal perimeter at the midshaft. Moreover, the exercise-induced effects on bone mass and strength were similar in both the Ovx and sham rats (3, 4). In the present study, the jumping program used for 8 wk is assumed to have induced the same changes, although we only observed the bones in vivo with DXA. After the exercise training, the tibial BMC increased to 9.6% in SE and 16.4% in OE compared with the corresponding sedentary group. There was no significant surgery-exercise interaction, although the rate of increase was rather greater in OE than in SE. The changes in tibial BMC were preserved in the detraining period, although the ratios fell. In the extracted tibia after the 24-wk detraining period, the exercise effects remained at 7.1% in SE and at 7.1% in OE in the tibial BMC.

The effects of the exercise were primarily seen in the intermediate part of the tibia, where the bone was bent during the jump exercise. A ground-reaction force during jumping might act on the tibia as an axial compression load. Thus the jumping load affected cortical bone at the diaphysis, but little affected cancellous bone at the metaphysis, as seen in the study by Warden et al. (18). Conversely, jump training did not affect femoral BMC, whereas Ovx decreased it. These findings suggest that the jump exercise did not impose a great load at the

---

**Table 1. BMC, bone area, and BMD in the tibia**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>SE</th>
<th>OS</th>
<th>OE</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC, mg</td>
<td>171±11</td>
<td>183±10</td>
<td>171±16</td>
<td>184±11</td>
<td>Ex&lt;sup&gt;c&lt;/sup&gt;, Surf&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Area, cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.95±0.07</td>
<td>2.02±0.07</td>
<td>2.02±0.11</td>
<td>2.11±0.11</td>
<td>Ex&lt;sup&gt;c&lt;/sup&gt;, Surf&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMD, mg/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>87.8±5.2</td>
<td>90.9±4.1</td>
<td>84.7±4.3</td>
<td>87.2±4.3</td>
<td>Ex&lt;sup<em>e&lt;/sup&gt;, Surf&lt;sup</em>e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Proximal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC, mg</td>
<td>48±3</td>
<td>50±4</td>
<td>44±5</td>
<td>46±4</td>
<td>Surf&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Area, cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.50±0.04</td>
<td>0.53±0.05</td>
<td>0.53±0.03</td>
<td>0.52±0.03</td>
<td>Surf&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMD, mg/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>97.3±11.0</td>
<td>95.9±9.1</td>
<td>82.4±6.8</td>
<td>88.8±8.0</td>
<td>Surf&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Center proximal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC, mg</td>
<td>33±2</td>
<td>36±3</td>
<td>35±3</td>
<td>38±2</td>
<td>Ex&lt;sup&gt;d&lt;/sup&gt;, Surf&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Area, cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.39±0.02</td>
<td>0.39±0.01</td>
<td>0.39±0.02</td>
<td>0.41±0.02&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Int&lt;sup&gt;*&lt;/sup&gt;, Surf&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMD, mg/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>84.3±5.0</td>
<td>92.7±6.4</td>
<td>90.2±4.4</td>
<td>93.4±4.0</td>
<td>Ex&lt;sup&gt;d&lt;/sup&gt;, Surf&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Center</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC, mg</td>
<td>30±3</td>
<td>34±2</td>
<td>31±3</td>
<td>35±2</td>
<td>Ex&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Area, cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.36±0.02</td>
<td>0.37±0.01</td>
<td>0.36±0.02</td>
<td>0.39±0.02</td>
<td>Ex&lt;sup&gt;d&lt;/sup&gt;, Surf&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMD, mg/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>83.3±5.1</td>
<td>91.3±3.9</td>
<td>86.7±5.4</td>
<td>89.8±3.2</td>
<td>Ex&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Center distal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC, mg</td>
<td>31±2</td>
<td>34±2</td>
<td>31±3</td>
<td>34±3</td>
<td>Ex&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Area, cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.35±0.01</td>
<td>0.35±0.01</td>
<td>0.35±0.02</td>
<td>0.38±0.02</td>
<td>Ex&lt;sup&gt;c&lt;/sup&gt;, Surf&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMD, mg/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>90.5±4.5</td>
<td>95.9±3.0</td>
<td>88.6±3.8</td>
<td>91.6±4.0</td>
<td>Ex&lt;sup&gt;c&lt;/sup&gt;, Surf&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Distal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC, mg</td>
<td>29±3</td>
<td>30±3</td>
<td>30±3</td>
<td>30±3</td>
<td>Ex&lt;sup&gt;b&lt;/sup&gt;, Surf&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Area, cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.36±0.03</td>
<td>0.38±0.02</td>
<td>0.39±0.04</td>
<td>0.42±0.06</td>
<td>Ex&lt;sup&gt;b&lt;/sup&gt;, Surf&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMD, mg/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>81.2±9.1</td>
<td>78.1±6.7</td>
<td>74.5±5.5</td>
<td>73.1±7.4</td>
<td>Ex&lt;sup&gt;b&lt;/sup&gt;, Surf&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means±SD. SS, sedentary sham group; SE, sham exercise group; OS, ovariectomized sedentary group; OE, ovariectomized exercise group; BMC, bone mineral content; BMD, bone mineral density. *Significantly different from OS: P < 0.05. Significant exercise (Ex) main effect: *P < 0.05, *P < 0.01, *P < 0.001. Significant surgical (Sur) main effect: *P < 0.05, *P < 0.01, *P < 0.001. Significant surgery-exercise interaction (Int): P < 0.05.
femoral region, defined in this study by DXA measurement, despite muscle contraction. After the 24-wk detraining period, the tibial strengths were higher, at 15.0% in SE and at 8.9% in OE, compared with the corresponding sedentary group, without a significant surgery-exercise interaction. Greater bone strength in the exercised groups can be partially explained by increased BMD or BMC. However, cross-sectional morphological changes may have also contributed to the result. The exercised groups had a greater tibial diaphysis width. Bone with a large diameter is known to be able to withstand greater bending and torsional loads than a thin bone of the same mass and length (12). In the present study, the stronger bone in the detraining groups can be partially explained by their greater widths. Moreover, the increased cortical thickness induced by the exercise might have been preserved and thus strengthened the bone, although this was not measured.

Our findings regarding the preservation effects are supported by the study by Warden et al. (18), in which the effects of the loading on the strength and morphology of the ulnar diaphysis were preserved for 92 wk of detraining in rats. Thus loading effects on the bone diaphysis appear to be preserved for long periods. However, Pajamäki et al. (13) reported that the effects of running on bone strength and total cross-sectional area in the femoral neck disappeared within 42 wk of detraining in rats. In their study (13), running did not influence bone measurements at the femoral midshaft, although running has stimulated radial bone growth in other studies (5, 8). Thus the effects of running on bones are rather complicated, most likely because running involves a combination of factors other than simple loading.

There is some speculation that, once a bone’s diameter has been increased by exercise, the possibility of shrinking after the exercise ceases seems counterintuitive, and that the exercise effect on bone mass and strength is preserved for a long time (12). If one exercises and enlarges bone diameter in childhood or adolescence, when increases in bone diameter are larger in particular, the peak bone mass or bone strength that one experiences in one’s 20’s or 30’s will be greater and may prevent the osteoporosis that often accompanies old and middle age. This speculation is accepted to some extent, and some exercise intervention experiments, using jumping exercise, to increase bone density in prepuberty or puberty have been performed (6, 9, 11). The results of the present study support the idea that the effect of exercise is preserved, although we used an animal model.

In our previous studies, we reported that jump training increased the bone strength of the tibia to the same extent in both sham rats and Ovx rats, and that the periosteal diameter increased in the tibial diaphysis (3, 4). In the present study, the hypothesis that the effects of jump training in estrogen-deficient rats are also preserved after a detraining period was affirmed. However, the degree of the change in the outer width and the bone stiffness in the fracture test differed between the sham groups and Ovx groups. Bone width increased in both the SE and OE groups compared with the corresponding control group, but the increase tended to be higher in the OE group. In addition, a significant interaction was recognized in the bone area of the central-proximal region. The bone area measured by DXA is presumed to be a variable, which provides the bone’s external width, if the length of the bone is the same. These findings may indicate that the increase in the outer width by
exercise was maintained to a greater extent in the Ovx rats than the sham rats. In females, estrogen enhances endocortical contraction but inhibits periosteal bone resorption (14). It is thought that the periosteal diameter of the bone in women does not increase after adolescence compared with men, partially due to estrogen secretion (1, 2, 7). Therefore, in the present study, the estrogen deficiency induced by Ovx might have had the advantage of increasing and maintaining the bone external width.

We also observed a difference in the bone mechanical properties between the sham and Ovx rats, although the maximum load was greater in the exercised group than in the sedentary group, regardless of estrogen secretion state. ANOVA revealed a significant interaction in the stiffness of the fracture test, and post hoc results indicated that the SE rats had greater stiffness in the bone than the SS rats, whereas the OE rats did not have greater stiffness than the OS rats. These results may indicate bone morphological or qualitative differences in the exercise preservation effect in relation to estrogen secretion state.

There are several methodological limitations of this study. First, we used relatively low numbers of rats, too few to adequately power statistical interactions between estrogen status and exercise. Second, rats have lifelong growth of bones and limited ability to remove cortical bone during detraining due to a lack of secondary remodeling. Third, we used a relatively short detraining period in relation to the lifespan of a rat, and thus the lifelong effect of exercise on young animals was not clarified. Fourth, we did not perform qualitative analysis of the nature of the bones or detailed analysis of morphology.

In conclusion, the effects of jump training for 8 wk in female rats on bone mass and strength was preserved after 24 wk of detraining, regardless of estrogen secretion state. These findings suggest that the exercise effect on bone strength is preserved, accompanied by cross-sectional morphological changes, even under estrogen deficiency.

REFERENCES