Effects of Moderate Intensity Exercise on Tibial Bone Mass in Mature Ovariectomized Rats: Bone Histomorphometry Study

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Abstract. The effects of moderate intensity exercise of different duration on the cancellous and cortical bone mass of the tibia were investigated in mature ovariectomized rats by bone histomorphometry. A total of twenty 23-week-old female Wistar rats were used in this study. Ovariectomy (OV) was performed on 15 animals, and they were divided into three groups of five animals each: an OV group, an OV + exercise for 30 min a day (EX30) group and an OV + EX60 group. The other 5 animals served as a sham-operated (SH) control group. The exercise consisted of treadmill running at 16 m/min and was performed 5 days a week for 12 weeks after the operation. After 12 weeks of exercise, bone histomorphometry was used to evaluate the cancellous bone (secondary spongiosa) of the proximal tibia and the cortical bone of the tibial shaft. The OV group showed a significant decrease in cancellous bone volume, with increased resorption and formation, compared to the SH group (P<0.01). Although the OV+EX30 group showed a significant increase in cancellous bone volume, with decreased resorption and increased osteoblastic activity, compared to the OV group (P<0.05), cancellous bone volume in the OV+EX30 group remained significantly lower than that in the SH group (P<0.05). There was not a significant difference in cortical bone area between the OV group and the SH group, whereas the OV + EX30 group showed a significant increase in cortical bone area, with decreased resorption on the endosteal surface, compared to the OV group (P<0.05). There were no significant differences in cancellous bone volume or cortical bone area between the OV group and the OV + EX60 group. The data suggest that moderate intensity exercise of appropriate duration may attenuate ovariectomy-induced cancellous bone loss and increase cortical bone mass in mature ovariectomized rats. (Keio J Med 47(3): 162-167, September 1998)

Key words: ovariectomy, treadmill running exercise, bone histomorphometry

Introduction

Senile and postmenopausal osteoporosis are major public health problems, because estrogen deficiency after menopause causes marked bone loss with an increased risk of fractures. To prevent osteoporosis, it is important to prevent or even attenuate the bone loss induced by estrogen deficiency. Since exercise can result in positive bone mass balance, it has been proposed as a strategy to prevent osteoporosis. However, the effects of exercise on bone mass reported in postmenopausal women are inconsistent. Some investigators have found that exercise resulted in no significant effect on bone mass in postmenopausal women,1,2 while others have reported positive results.3,4 Thus, the efficacy of exercise therapy as a method of preventing osteoporosis has not yet been established.

Several experimental studies in intact young rats have demonstrated that exercise stimulates bone growth and bone formation and results in positive bone mass balance.5-8 In adult rats, Yeh and Chen et al.9-11 reported that exercise increased both the cancellous and cortical bone mass of the tibia in aged female rats, but did not result in apparent effects on the femoral

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bone mass in adult ovariectomized rats. Barengolts et al.12 on the other hand, reported that exercise affected tibial trabecular bone mass in intact ovariectomized rats, but not in intact adult rats. One of the reasons for these conflicting results is probably differences in the intensity and duration of exercise, although exercise intensity was not evaluated strictly in these studies. It is hypothesized that the response of bone to exercise differs according to the intensity and duration of exercise and that there is an intensity and duration of exercise that has an optimal effect on bone mass.

Clinically, moderate intensity weight-bearing exercise is usually prescribed as exercise therapy for osteoporosis, because it is thought to be safe and effective in achieving a positive effect on bone mass. We therefore examined the effects of moderate intensity exercise of different duration on cancellous and cortical bone mass and on bone formation and bone resorption by bone histomorphometry in a weight-bearing long bone, the tibia, in mature ovariectomized rats.

Materials and Methods

Animals

Twenty female Wistar rats, aged 8 weeks, were purchased from CLEA Japan Inc. (Tokyo, Japan) and housed in individual cages (25 × 18 × 34 cm²) in a specific-pathogen-free room maintained at a temperature of 23°C ± 2°C and humidity of 55% ± 5%, with a 12-hour lights on/off cycle. The animals were given free access to water and a pelleted chow diet (MM-2; 1.4% calcium diet, Funabashi Farm Co., Ltd., Chiba, Japan). This diet was adopted as the standard diet for rats under specific-pathogen-free conditions on the basis of the instruction of the Research Animal Resource Committee of Kitasato Institute Hospital. After acclimatization to the new environment for 15 weeks, 15 of the rats, aged 23 weeks, were ovariectomized (OV) via a dorsal approach under ether anesthesia and then divided into three groups of five animals each: an OV group, an OV + exercise for 30 min a day (EX30) group and an OV + exercise for 60 min a day (EX60) group. The other 5 animals served as a sham-operated (SH) control group. The exercise consisted of daily running on a flat-bed treadmill (Shinano Instrument Co., Ltd., Tokyo, Japan) at 16 m/min and was performed in the animals in the OV + EX30 group and the OV + EX60 group 5 days a week for 12 weeks after the operation. The animals in the OV group and the SH group were kept sedentary. Exercise intensity (speed) was evaluated on the basis of onset of blood lactate accumulation (OBLA) in the preliminary study using the same models. Briefly, three rats were exercised on the treadmill at 16 m/min for 1 hour, and blood was collected from the eyegrounds before and soon after the exercise to evaluate blood lactate accumulation. Serum lactate levels before and after the exercise were 34.5 ± 2.7 mg/dl and 42.8 ± 2.8 mg/dl (mean ± SEM), respectively. Thus, the intensity (speed) of 16 m/min corresponded to a moderate exercise level with slight blood lactate accumulation when continued for an hour.

Body weight was measured before and after the exercise study. This protocol was approved by the Research Animal Resource Committee of Kitasato Institute Hospital, and the study was performed at Kitasato Institute Hospital.

Bone histomorphometry

All animals were labeled with calcein (10 mg/kg body weight, s.c.) at 7 days and tetracycline (20 mg/kg body weight, s.c.) at 3 days before sacrifice. At the end of the exercise regimen, the animals were sacrificed by exsanguination from the right axillary artery under pentobarbital sodium anesthesia (100 mg/100 g body weight, i.p.) and the right tibia was dissected out. After cutting the bone into three equal parts with a diamond saw, the proximal tibia and the tibial shaft was fixed in 70% ethanol solution for 2 days and then immersed in Villanueva osteochrome bone stain for 5 days. The specimens were dehydrated by sequential changes of ascending concentrations of ethanol (70, 95 and 100%) and then embedded in methyl-methacrylate. Frontal sections of the proximal tibia were cut at 5 µm with a microtome, and cross-sections of the tibial shaft just proximal to tibiofibular junction were cut at 40 µm with a diamond wire. Semi-automatic histomorphometric measurements of the cancellous and cortical bone was performed with a Carl Zeiss Osteoplan II and a Muto ID20BL, respectively. The region measured in the proximal tibia was 980 ~ 2,940 µm distal to the growth plate and 490 µm inner to the endosteal surface (secondary spongiosa). The histomorphometric parameters evaluated for the cancellous bone of the proximal tibia were bone volume (BV/TV, TV: tissue volume), trabecular number (TbN), trabecular thickness (TbTh), osteoblast surface (ObS/BS, BS: bone surface), osteoclast surface (OcS/BS), eroded surface (ES/BS), mineralizing surface (MS/BS), surface based bone formation rate (BFR), and mineral apposition rate (MAR). The parameters evaluated for the cortical bone of the tibial shaft were total tissue area (Tt Ar), marrow cavity area (Ma Ar), cortical bone area (Ct Ar), periosteal mineralizing surface (P-MS/BS), endosteal mineralizing surface (E-MS/BS), and endosteal eroded surface (E-ES/BS). MS was calculated as dLS+sLS/2 (double-labeled surface + single-labeled surface/2). MAR and BFR (surface based) were calculated as IrL Wi/Interval (interlabeled width/interval) and MAR × MS/BS, respectively.
Statistical analysis

All data are expressed as means ± SEM. The Mann-Whitney U test was used to compare differences between two groups. A significance level of P < 0.05 was used for all comparisons.

Results

Body weight

Table 1 shows the initial and final body weight in all groups. There were no significant differences in the initial body weight among the groups. Final body weight in the OV group, the OV + EX30 group and the OV + EX60 group was significantly greater than that in the SH group (P < 0.01, Mann-Whitney U test). Final body weight in the OV + EX30 group was significantly greater than that in the OV group (P < 0.05, Mann-Whitney U test), but the difference in final body weight between the OV + EX60 group and the OV group was not significant.

Bone histomorphometry

Table 2 shows the results of bone histomorphometric analysis of the cancellous bone of the proximal tibia. Effects of ovariectomy: BV/TV and TbN in the OV group were significantly lower than those in the SH group (P < 0.01, Mann-Whitney U test), but TbTh was not significantly different in these groups. ObS/BS, OcS/BS, ES/BS, MS/BS, and BFR in the OV group were significantly higher than those in the SH group (P < 0.05, Mann-Whitney U test), but MAR was not significantly different in these groups. Finally, ovariectomy decreased cancellous bone mass (BV/TV) by 57.3%. Effects of exercise: BV/TV and TbN in the OV + EX30 group were significantly higher than those in the OV group (P < 0.05, Mann-Whitney U test), but remained significantly lower than those in the SH group.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Initial and Final Body Weight</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>OV</td>
</tr>
<tr>
<td>Initial Body Weight</td>
<td>256.6 ± 1.9</td>
</tr>
<tr>
<td>Final Body Weight</td>
<td>327.6 ± 6.9a</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM.
The Mann-Whitney U test was used to compare differences between the groups.
OV: ovariectomy, OV + EX30: ovariectomy + exercise for 30 min a day, OV + EX60: ovariectomy + exercise for 60 min a day, SH: sham-operated.
a) P < 0.01 versus SH, b) P < 0.05 versus OV.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Bone Histomorphometric Analysis of the Cancellous Bone (Secondary Spongiosa) of the Proximal Tibia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OV</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>10.939 ± 0.688a</td>
</tr>
<tr>
<td>Tbn (1/mm)</td>
<td>2.830 ± 0.320a</td>
</tr>
<tr>
<td>TbTh (µm)</td>
<td>35.115 ± 1.299</td>
</tr>
<tr>
<td>ObS/BS (%)</td>
<td>37.784 ± 3.211bc</td>
</tr>
<tr>
<td>OcS/BS (%)</td>
<td>3.855 ± 0.245b</td>
</tr>
<tr>
<td>ES/BS (%)</td>
<td>14.421 ± 0.441b</td>
</tr>
<tr>
<td>MS/BS (%)</td>
<td>10.353 ± 1.149b</td>
</tr>
<tr>
<td>BFR (µm²/µm²/day)</td>
<td>5.787 ± 0.258b</td>
</tr>
<tr>
<td>MAR (µm/day)</td>
<td>0.559 ± 0.042</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM.
The Mann-Whitney U test was used to compare differences between the groups.
a) P < 0.01 versus SH, b) P < 0.05 versus SH, c) P < 0.05 versus OV.
Table 3  Bone Histomorphometric Analysis of the Cortical Bone of the Tibial Shaft

<table>
<thead>
<tr>
<th></th>
<th>OV</th>
<th>OV + EX30</th>
<th>OV + EX60</th>
<th>SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>TtAr (mm² × 100)</td>
<td>486.0 ± 10.3</td>
<td>511.5 ± 14.6</td>
<td>487.9 ± 20.0</td>
<td>461.6 ± 18.6</td>
</tr>
<tr>
<td>MaAr (mm² × 100)</td>
<td>94.6 ± 5.7</td>
<td>85.2 ± 7.0</td>
<td>89.2 ± 6.8</td>
<td>90.3 ± 7.6</td>
</tr>
<tr>
<td>CtAr (mm² × 100)</td>
<td>391.4 ± 4.9</td>
<td>426.3 ± 6.4b</td>
<td>398.7 ± 9.4</td>
<td>381.3 ± 8.5</td>
</tr>
<tr>
<td>P-MS/BS (%)</td>
<td>23.9 ± 6.2</td>
<td>27.6 ± 9.4</td>
<td>24.2 ± 4.6</td>
<td>18.4 ± 7.4</td>
</tr>
<tr>
<td>E-MS/BS (%)</td>
<td>33.8 ± 7.6a</td>
<td>33.9 ± 8.4b</td>
<td>32.8 ± 8.7a</td>
<td>15.1 ± 5.2</td>
</tr>
<tr>
<td>E-ES/BS (%)</td>
<td>24.7 ± 6.3b</td>
<td>9.6 ± 2.2c</td>
<td>24.7 ± 3.1b</td>
<td>9.4 ± 1.4</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM.
The Mann-Whitney U test was used to compare differences between the groups.
OV: ovariectomy, OV + EX30: ovariectomy + exercise for 30 min a day, OV + EX60: ovariectomy + exercises for 60 min a day, SH: sham-operated.
TtAr: total tissue area, MaAr: marrow cavity area, CtAr: cortical bone area, P-MS/BS: periosteal mineralizing surface, E-MS/BS: endosteal mineralizing surface, E-ES/BS: endosteal eroded surface.
a) P < 0.01 versus SH, b) P < 0.05 versus SH, c) P < 0.05 versus OV.

(P < 0.05, Mann-Whitney U test). TbTh, ObS/BS, and MS/BS in the OV + EX30 group and the OV group did not significantly differ. OcS/BS and ES/BS in the OV + EX30 group were significantly lower than those in the OV group, and BFR and MAR were significantly higher (P < 0.05, Mann-Whitney U test). Finally, cancellous bone mass (BV/TV) in the OV + EX30 group was 28.8% lower than that in the SH group. The exercise in the OV + EX30 group reduced the loss of cancellous bone mass (BV/TV) by 50.3%. However, there were no significant differences in these parameters between the OV + EX60 group and the OV group.

Table 3 shows the results of bone histomorphometric analysis for the cortical bone of the tibial shaft. Effects of ovariectomy: There were no significant differences in Tt Ar, Ma Ar, Ct Ar, and P-MS/BS between the OV group and the SH group. However, E-MS/BS and E-ES/BS in the OV + EX30 group were significantly higher than those in the SH group (P < 0.05, P < 0.01, respectively, Mann-Whitney U test). Effects of exercise: Tt Ar and Ma Ar in the OV + EX30 group and the OV group did not significantly differ, but Ct Ar in the OV + EX30 group was significantly higher than that in the OV group (P < 0.05, P < 0.01, Mann-Whitney U test). Finally, the exercise in the OV + EX30 group increased cortical bone mass (Ct Ar) by 34.8%. However, there were no significant differences in these parameters between the OV + EX60 group and the OV group.

Discussion

As already demonstrated, ovariectomy results in high bone turnover in rats, with resorption exceeding formation, and induces cancellous bone loss in the proximal tibia. Ovariectomy increases the bone mass of the cortical bone of the tibial shaft in young rats due to increased periosteal and endosteal formation, but in adult rats it decreases bone mass due to increased endosteal resorption. Cortical bone is less susceptible than cancellous bone to the bone loss caused by ovariectomy. Ovariectomy increases the body weight of rats, and this increase is thought to provide an additional stimulus for bone formation and to partially protect against osteopenia in weight-bearing long bones such as the tibia. In our study in young adult mature rats, the OV group showed a significant increase in body weight, a significant decrease in cancellous bone mass (BV/TV) of the proximal tibia, with increased resorption and formation (mainly osteoblastic recruitment, indicated by a high MS/BS), but no significant change in cortical bone mass (Ct Ar) of the tibial shaft, with increased endosteal formation and resorption, compared to the SH group. These findings are in agreement with the results of the previous studies.

Few studies have examined the effect of moderate intensity exercise on cancellous and/or cortical bone mass in mature or adult rats during the early stage after ovariectomy. In our study, moderate intensity exercise of 30-minute duration attenuated the loss of cancellous bone mass (BV/TV) induced by ovariectomy by half and increased cortical bone mass (Ct Ar). In contrast, the 60-minute exercise affected neither cancellous nor cortical bone mass (BV/TV and Ct Ar, respectively). This suggests that there is an effective duration of even moderate intensity exercise that affects cancellous and cortical bone mass. Exercise of moderate intensity of appropriate duration can be useful in achieving positive bone mass balance in the mature ovariectomized rat. However, the exercise in our study failed to prevent the ovariectomy-induced cancellous bone loss completely.
As also demonstrated by Peng et al., moderate intensity exercise during the early stage after ovariectomy in young rats could not totally prevent ovariectomy-induced loss of femoral trabecular bone volume. Thus, it may be difficult to prevent ovariectomy-induced cancellous bone loss completely by means of moderate intensity exercise alone.

The mechanisms that increase bone mass and decreases bone loss have not yet been established. They vary among the experimental models. In young rats exercise stimulates bone growth and bone formation, whereas in adult rats it increases bone formation and decreases bone resorption. Yeh et al. demonstrated that the effect of exercise in adult ovariectomized rats is predominantly mediated by inhibition of bone resorption rather than stimulation of bone formation. Barengolts et al. showed that exercise results in fewer resorption/formation sites but higher activity of individual osteoblasts at locales. The differences between the findings in these studies may be due to differences in the methods used to evaluate bone formation and bone resorption. In our study, the mechanism of attenuation of cancellous bone loss in the OV+ EX30 group was thought to be decreased bone resorption and increased osteoblastic activity (indicated by a high MAR), and the mechanism responsible for increasing cortical bone mass appeared to be a decrease in bone resorption on the endosteal surface. The reason that the exercise did not affect bone mass, and bone formation, or bone resorption in either cancellous or cortical bone in the OV+EX60 group, however, remains to be clarified. We speculate that it was because the exercise of long duration in the mature ovariectomized rats increased bone resorption (remodeling) as a result of increased amounts of bone microdamage, thereby counteracting the increase in bone mass induced by exercise. Further study is needed to specify the mechanism.

In conclusion, the results of our study suggested that the response of bone to moderate intensity exercise differs according to the duration of the exercise, and that moderate intensity exercise of appropriate duration may attenuate ovariectomy-induced cancellous bone loss and increase cortical bone mass in the mature ovariectomized rat.

Acknowledgments: This work was supported by grants from Kitasato Institute Hospital.

References

