Effects of tower climbing exercise on bone mass, strength, and turnover in orchidectomized growing rats

Notomi, Takuya, Yuichi Okazaki, Nobukazu Okimoto, Yuri Tanaka, Toshitaka Nakamura, and Masashige Suzuki. Effects of tower climbing exercise on bone mass, strength, and turnover in orchidectomized growing rats. J Appl Physiol 93: 1152–1158, 2002. First published May 31, 2002; 10.1152/japplphysiol.01221.2001.—To determine the effects of a tower climbing exercise on mass, strength, and local turnover of bone, 70 9-wk-old Sprague-Dawley rats were assigned to seven groups: a baseline control and three groups of sham-operated sedentary, orchidecomized (ORX)-sedentary and ORX-exercise rats. Rats voluntarily climbed a 200-cm tower to drink water from a bottle set at the top. At 4 wk, the periosteal bone formation rate (BFR), moment of inertia, bone mineral content, bone mineral density, and bending load at the midfemur were maintained in ORX-exercise rats, whereas these parameters were reduced in ORX-sedentary rats. At 8 wk, the periosteal mineral apposition rate and BFR in ORX-exercise rats were significantly higher, whereas the parameters in ORX-sedentary rats did not differ compared with sham-sedentary rats. In ORX-exercise rats, the trabecular mineralizing surface, BFR, and bone volume of the lumbar vertebrae were maintained at the same levels as those in the sham-sedentary group, whereas the osteoclast surface decreased compared with the ORX-sedentary group. However, the climbing exercise did not affect bone mineral content, bone mineral density, or the compression load of the lumbar vertebrae. These results show that, in the midfemur, the voluntary climbing exercise maintained cortical bone mass and strength by stimulating periosteal bone formation and partially preventing ORX-induced trabecular bone loss, depressing the elevation of turnover. Interestingly, in ORX rats, the climbing exercise had the opposite effect on bone formation at the periosteal femoral cortical bone, where the exercise increased the bone formation compared with vertebral trabecular bone, where the exercise decreased it.

voluntarily exercise; bone formation; osteoclast; orchidectomy

Bone mass is maintained by both mechanical and hormonal factors. Disuse (15) and sex hormone deficiency (7, 25) cause bone loss. Exercise can increase bone mineral density (BMD) in humans, which suggests that increased physical activity could be useful in the prevention of bone mineral loss (1, 12). Some studies suggested that loading on mature bone was only slightly better for increasing bone mass than normal daily use (9). However, during growth, some evidence supported the notion that exercise strongly influenced the skeleton (2). Other studies suggested increased bone mass in prepubertal boys due to moderate resistance exercise such as a jumping program (8).

In rodents, gonadal hormone deficiency decreases bone mass as it does in humans (29). In men, Stepan et al. (29) reported an increase in bone turnover and a rapid decrease in lumbar spine BMD in response to orchidectomy. In growing (10, 11, 28) and aged or mature ORX rats (5, 24, 27, 31, 32), previous studies have also been suggested that androgen deficiency-induced cancellous bone loss is, at least transiently, associated with increased bone formation and resorption. Bone development was inhibited immediately after orchidectomy in growing rats (27, 28) but not rapidly in mature rats (4). In most studies, orchidectomy in growing rats results in deficits in cortical bone mass within 2–4 wk, but in adult rats, those deficits require a longer period (4, 27).

In growing and mature rats, running (16), jumping (18), and climbing (20) augments lumbar trabecular bone mass and strength, whereas in adult rats, treadmill exercise does not affect lumbar trabecular bone whereas cortical bone formation is found to increase (33). Those positive effects seemed to be greater in young rats than in adult rats. Concerning orchidectomy and exercise in animals, the treadmill exercise was less effective on femoral cortical and tibial trabecular bone (28) and had a beneficial effect on femoral bone mass (10, 11) but not on strength (10). Erect bipedal exercise prevented cortical and trabecular bone loss in the tibia (31) and loss of cortical and trabecular vertebral bone mass in mature ORX rats (32).

The effect of nonvoluntary or voluntary resistance exercise, such as jumping or climbing, on bone has been examined in intact rats (18, 20), and the magnitude of bone mass increase seemed to be greater than...
that of treadmill running (19). The tower climbing exercise, as we previously reported (20), has not yet been applied to ORX rats. The purpose of this study was to find out the effects of voluntary resistance exercise on the mass, strength, and local turnover of bone in growing ORX rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats were purchased from Japan CLEA (Tokyo, Japan) and were acclimatized for 1 wk under standard laboratory conditions (22 ± 2°C, 60% humidity). The light-dark cycle was 12 h, with lights on from 0600 to 1800. All rats were housed in metal cages. Drinking water was available at all times. Animals were pair fed, and the amount of food taken was equalized among the groups. All rats were fed commercial rat chow (Japan CLEA; calcium: 1,200 mg/100 g; phosphorus: 1,080 mg/100 g). The body weight of each rat was measured weekly. All rats remained healthy. The protocol was approved by The University of Tsukuba's Institutional Animal Care and Use Committee.

Seventy rats, 9 wk of age, were randomized by body weight to seven groups of 10 animals each. Two rats were housed in each cage. Group C was the baseline control. Forty rats were orchidectomized (ORX), and the other 20 rats were sham operated under anesthesia with nembutal sodium administered intraperitoneally. All the animals recovered well after surgery. The exercise started 1 wk postorchidectomy at 10 wk of age. Groups 4SS and 8SS were sham-operated sedentary control groups killed after 4 and 8 wk of exercise, respectively. Groups 4OS and 8OS were ORX-sedentary groups, and groups 4OE and 8OE were ORX-exercise groups, respectively killed after 4 and 8 wk of exercise. To preclude any difference of food intake induced by orchidectomy (3, 14), the daily consumption of each group was measured, and each was given the mean amount of chow consumed by the ORX groups on the previous day.

At the end of the experiments, rats were killed by exsanguination under ether anesthesia, and blood samples were taken from the abdominal aorta. Soon after the death, the hindlimb muscles (gastrocnemius, plantaris, soleus, tibia, and extensor digitorum longus) and abdominal fat (perigastreatic fat and mesenteric fat) were isolated. The combined hindlimb muscles (gastrocnemius, plantaris, soleus, tibia, and extensor digitorum longus) and abdominal fat (perigastreatic fat and mesenteric fat) were isolated. The combined mid-diaphyseal region (12 mm in length) were obtained.

Bone Mineral Measurement

BMD (mg/cm²) and bone mineral content (BMC; mg) were measured on the left femur with dual-energy X-ray absorptiometry (DCS-3000, Aloka, Tokyo, Japan). The L3 body was prepared by removing the posterior segment, and bone mineral measurements were performed. The mineralization profiles of the specimens were stored with the monitoring images, and BMD and BMC values for the lumbar body and the femur mid-diaphyseal region (12 mm in length) were obtained.

Mechanical Testing

Femur. A three-point bending test was performed as previously described (21) by using a load tester (Tension UT-1T, Orientec, Tokyo, Japan). Each left femur specimen was placed on a holding device with supports located at a distance of 12 mm, with the lesser trochanter proximal to, and in contact with, the proximal transverse bar. The midpoints on the anterior (upper) loading point. A bending force was applied by the crosshead at a speed of 10 mm/min until fracture occurred. The maximum load (N) and Young's modulus (N/mm) were obtained directly from the load-deformation curves that were recorded continually in the computerized monitor linked to the load tester.

Lumbar vertebral body. Each L3 body specimen was fixed with a clamp at the bases of the transverse processes in the holder of a diamond band saw (Exakt, Norderstedt, Germany). By removing the cranial and caudal ends of the specimens, the plano-parallel ends at a height of 3.5 mm were obtained (21). The cylinder samples were placed centrally on the smooth surface of a steel disk attached to the load tester. A cranio-caudal compression force was applied to the specimen via a steel disk at a nominal deformation rate of 2 mm/min. The maximum load (N) and Young's modulus (N/mm) were obtained, as were those of the femur specimens.

Bone Histomorphometry

Lumbar vertebral body. Each L4 specimen was embedded in methyl methacrylate after Villanueva's bone staining. From the middle portion of the specimen, 10-μm-thick undecalcified sagittal sections were cut on a microtome (Reichert Jung Supercut 2050, Heidelberg, Germany). Each L4 specimen was embedded in a mixture of methyl methacrylate, hydroxyglycol methacrylate, and 2-hydroxyethyl acrylate polymerized at 4°C. Six-micrometer-thick specimens of the L3 were obtained as described for those of L4. The L5 sections were then stained for tartrate-resistant acid phosphatase (26). Histomorphometry of L3 and L4 was performed with a semiautomatic image-analyzing system linked to a light microscope (Cosmoe 1S8, Nikon, Tokyo, Japan). For each section, the area of the secondary spongiosa was measured, but the regions within 1.0 mm of the growth plate-metaphy-
scler junction and one cortical shell-width of the endocortical surface were not measured to exclude primary spongiosa.

As structural parameters, the bone volume (BV; \(\mu m^3\)), trabecular tissue volume (TV; \(\mu m^3\)), and cancellous bone surface (BS; \(\mu m\)) were measured. The trabecular thickness (\(\mu m\)), trabecular number (1/mm), and trabecular bone separation (\(\mu m\)) were calculated by a parallel plate model by assuming constant geometry (22, 23). For the bone formation parameters of \(L_4\), the single-labeled surface (\(\mu m\)), double-labeled surface (\(\mu m\)) and trabecular BS (\(\mu m\)) were measured. The mineral apposition rate (MAR; \(\mu m/day\)) was calculated as the distance between double labels divided by the labeling interval and multiplied by \(\pi/4\). The mineralizing surface per BS (MS/BS; \%) was obtained by adding the values of the double-labeled surface/BS and half of the single-labeled surface/BS value. The surface referent bone formation rate (BFR/BS; \(\mu m^2/\mu m^3-day^{-1}\)) was calculated by multiplying the MS/BS value by the MAR (6, 22, 23). For the bone resorption parameters of \(L_4\), the osteoclast surface (Oc.S; \(\mu m\)) and trabecular BS were measured (6, 22, 23). Tartrate-resistant acid phosphatase-positive cells that formed resorption lacunae at the surface of the trabeculae and contained one or more nuclei were identified as osteoclasts (17). Femoral midshaft. An undecalcified section was obtained from the site of the middiaphysis of the right femur. The specimen was embedded in methyl methacrylate without staining to yield a 40-\(\mu m\)-thick crosscut ground section. Measurements were made on a cathode-ray tube monitor with a commercially available statistical package (SPSS version 10.0, SPSS Japan, Tokyo, Japan).

RESULTS

Climbing Distances and Time Periods

The daily climbing distances in the ORX-exercise group after 2, 4, 6, and 8 wk were 135 \(\pm\) 1.8, 148 \(\pm\) 2.6, 140 \(\pm\) 2.3, and 136 \(\pm\) 2.2 m/day, respectively. The respective time periods for the activity were 23.8 \(\pm\) 2.1, 26.5 \(\pm\) 1.0, 24.8 \(\pm\) 0.7, and 23.5 \(\pm\) 1.6 min/day, respectively. No significant differences were found among these values.

Body Weight, Hindlimb Muscles, Abdominal Fat, Biochemical Markers, and Bone Mineral Measurements

After 4 and 8 wk, body weights in both the OE and OS groups were significantly lower than those in the SS groups (Table 1). Hindlimb muscle values did not significantly differ throughout the experimental period. After 8 wk, the abdominal fat value in the OE group was significantly lower than those in both the SS and OS groups. After 4 wk, the serum osteocalcin levels in both the 4OS and 4OE groups were significantly higher than those in the 4SS group. After 8 wk, the serum osteocalcin levels in the 8OS group were significantly higher than those in the 8SS group. However, there was no significant difference in the osteocalcin levels between the 8SS and 8OE groups. BMC and BMD values of the midfemur in the OS groups were significantly lower than those in the SS groups after both 4 and 8 wk. However, the values of OE groups were significantly higher than the values in the OS groups. After 4 or 8 wk, in both the OS and OE groups, the parameters of BMC and BMD in the lumbar vertebrae were significantly smaller than those in the SS group. The values between the OS and OE groups did not significantly differ in either period. The corrected values of U-Dpy in the 8SS, 8OS, and 8OE groups were 87.4 \(\pm\) 3.8, 148.4 \(\pm\) 7.7, and 121.6 \(\pm\) 3.5, respectively. This parameter in the 8OE group was significantly lower than that in the 8OS group and was significantly higher than that in the 8SS group.

Mechanical Properties of the Midfemur and Lumbar Vertebrae, and the Geometry of the Femoral Cortical Bone

After 4 wk, the values of the maximum load of the midfemur, the maximum load and Young’s modulus of the lumbar vertebra, total cross-sectional area, and moment of inertia in the OS group were significantly lower than those in the SS group (Table 2). In the OE group, the parameters of the maximum load, cortical bone area, and the moment of inertia were higher than those in the OS group. After 8 wk, the parameters of the maximum load of the midfemur and cross-sectional morphology in the OS group were significantly lower than those in the SS group. In the OE group, the values of the maximum load of the midfemur, the cortical bone area, and moment of inertia were significantly higher than those in the OS group. However, the values of the maximum load and Young’s modulus of lumbar vertebrae were significantly lower than those in the SS group after both 4 and 8 wk. The Young’s modulus values of the midfemur did not significantly differ throughout the experimental period.

Dynamic Parameters of the Periosteal and Endosteal Surfaces

Periosteal surface. After 4 wk, the values of MS/BS and BFR/BS in the OS group were significantly lower
higher than those in the SS group (Table 3). In the OE group, however, the MAR and BFR/BS were significantly higher than those in the SS group. After 8 wk, none of the parameters significantly differed between the SS and OS groups. In the OE group, however, the values of the MAR and BFR/BS were significantly higher than those in both the SS and OS groups.

**Endosteal surface.** After 4 wk, the MS/BS values in both the SS and OE groups were significantly lower than those in the OS group. After 8 wk, however, the MAR value in the OE group was significantly higher than that in both the SS and OS groups. The values of BFR/BS and ES/BS did not significantly differ throughout the experiment period.

### Structural and Dynamic Parameters of the Lumbar Vertebrae

**Structural indexes.** After 4 and 8 wk, the parameters of BV/TV and trabecular number in the OS groups were significantly lower, whereas the trabecular bone separation was significantly higher, than those in the SS groups (Table 4).

### Table 1. Exercise time, body condition, bone metabolic marker, and bone mass of the midfemur and lumbar vertebrae of experimental rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, wk</th>
<th>Body weight, g</th>
<th>Hindlimb Muscles, mg</th>
<th>Abdominal Fat, mg</th>
<th>Serum Osteocalcin, ng/ml</th>
<th>Midfemur BMC, mg</th>
<th>Midfemur BMD, mg/cm²</th>
<th>Lumbar vertebra BMC, mg</th>
<th>Lumbar vertebra BMD, mg/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>247 ± 4</td>
<td>4.60 ± 0.05</td>
<td>3.07 ± 0.16</td>
<td>49.3 ± 2.0</td>
<td>73.6 ± 1.0</td>
<td>113.7 ± 1.2</td>
<td>19.5 ± 0.2</td>
<td>57.8 ± 1.5</td>
</tr>
<tr>
<td>4SS. Sham-Sed-4 wk</td>
<td>14</td>
<td>333 ± 9</td>
<td>6.44 ± 0.13</td>
<td>8.43 ± 0.85</td>
<td>20.7 ± 1.6</td>
<td>101.3 ± 2.8</td>
<td>147.5 ± 2.9</td>
<td>35.0 ± 1.1</td>
<td>91.4 ± 3.9</td>
</tr>
<tr>
<td>4OS. ORX-Sed-4 wk</td>
<td>14</td>
<td>318 ± 8§</td>
<td>6.26 ± 0.13</td>
<td>7.08 ± 0.61</td>
<td>28.0 ± 3.2§</td>
<td>88.0 ± 3.3§</td>
<td>134.8 ± 2.6§</td>
<td>29.8 ± 0.5§</td>
<td>78.4 ± 0.9§</td>
</tr>
<tr>
<td>4OE. ORX-Ex-4 wk</td>
<td>14</td>
<td>316 ± 8§</td>
<td>6.21 ± 0.08</td>
<td>6.89 ± 0.35</td>
<td>26.8 ± 1.8§</td>
<td>103.5 ± 1.6§</td>
<td>148.3 ± 2.6§</td>
<td>28.6 ± 1.4§</td>
<td>76.3 ± 4.5§</td>
</tr>
<tr>
<td>8SS. Sham-Sed-8 wk</td>
<td>18</td>
<td>379 ± 6</td>
<td>7.08 ± 0.20</td>
<td>14.73 ± 0.64</td>
<td>18.3 ± 1.4</td>
<td>116.7 ± 4.3</td>
<td>173.0 ± 4.4</td>
<td>47.3 ± 1.9</td>
<td>104.7 ± 3.8</td>
</tr>
<tr>
<td>8OS. ORX-Sed-8 wk</td>
<td>18</td>
<td>358 ± 4§</td>
<td>6.69 ± 0.15</td>
<td>13.85 ± 0.32</td>
<td>23.8 ± 1.4§</td>
<td>103.6 ± 1.4§</td>
<td>154.2 ± 2.4§</td>
<td>34.4 ± 1.5§</td>
<td>85.0 ± 2.8§</td>
</tr>
<tr>
<td>8OE. ORX-Ex-8 wk</td>
<td>18</td>
<td>353 ± 4§</td>
<td>6.77 ± 0.37</td>
<td>11.02 ± 0.58‡</td>
<td>20.5 ± 1.0</td>
<td>111.9 ± 3.2‡</td>
<td>165.3 ± 2.7‡</td>
<td>35.6 ± 2.0‡</td>
<td>92.2 ± 3.2‡</td>
</tr>
</tbody>
</table>

**P value, two-way ANOVA**

- Exercise treatment: <0.01 NS <0.01 <0.05 <0.01 <0.01 <0.01 <0.01 <0.01
- Time: <0.01 <0.05 <0.05 <0.05 <0.01 <0.01 <0.05 NS

Values are means ± SE. Exercise time of 4OE group was 25.7 ± 1.4 min/day; exercise time for 8OE group was 23.5 ± 1.6 min/day. Body weight was taken at time of death. ORX, orchidectomy; Sed, sedentary; Ex, exercise; BMC, bone mineral contents; BMD, bone mineral density; NS, not significant. Significantly different from corresponding ORX-Sed group: †P < 0.05; §P < 0.01. Significantly different from corresponding Sham-Sed group: ‡P < 0.01; ‡‡P < 0.05.

### Table 2. Mechanical parameters of the midfemur and lumbar vertebrae and morphology of the midfemur of experimental rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, wk</th>
<th>Maximum load, N</th>
<th>Young's modulus, N/mm</th>
<th>Maximum load, N</th>
<th>Young's modulus, N/mm</th>
<th>Total cross-sectional area, mm²</th>
<th>Cortical bone area, mm²</th>
<th>Bone marrow area, mm²</th>
<th>Moment of inertia, mm⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Control</td>
<td>9</td>
<td>166 ± 4</td>
<td>334 ± 11</td>
<td>201 ± 16</td>
<td>2.838 ± 88</td>
<td>10.4 ± 0.2</td>
<td>4.6 ± 0.1</td>
<td>5.8 ± 0.2</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>4SS. Sham-Sed-4 wk</td>
<td>14</td>
<td>258 ± 9</td>
<td>622 ± 34</td>
<td>302 ± 15</td>
<td>3.912 ± 167</td>
<td>11.8 ± 0.3</td>
<td>5.8 ± 0.1</td>
<td>6.0 ± 0.3</td>
<td>6.3 ± 0.3</td>
</tr>
<tr>
<td>4OS. ORX-Sed-4 wk</td>
<td>14</td>
<td>206 ± 62</td>
<td>568 ± 20</td>
<td>236 ± 72‡</td>
<td>3.126 ± 154‡</td>
<td>10.8 ± 0.1‡</td>
<td>5.6 ± 0.2</td>
<td>5.2 ± 0.1</td>
<td>5.4 ± 0.1‡</td>
</tr>
<tr>
<td>4OE. ORX-Ex-4 wk</td>
<td>14</td>
<td>264 ± 5†</td>
<td>598 ± 36</td>
<td>253 ± 133‡</td>
<td>3.263 ± 131‡</td>
<td>11.3 ± 0.2</td>
<td>5.9 ± 0.1‡</td>
<td>5.4 ± 0.3</td>
<td>6.3 ± 0.2*</td>
</tr>
<tr>
<td>8SS. Sham-Sed-8 wk</td>
<td>18</td>
<td>273 ± 5</td>
<td>768 ± 19</td>
<td>373 ± 25</td>
<td>4.394 ± 312</td>
<td>12.5 ± 0.2</td>
<td>6.4 ± 0.1</td>
<td>6.1 ± 0.3</td>
<td>6.7 ± 0.2</td>
</tr>
<tr>
<td>8OS. ORX-Sed-8 wk</td>
<td>18</td>
<td>239 ± 31</td>
<td>716 ± 25</td>
<td>266 ± 138</td>
<td>3.400 ± 298‡</td>
<td>10.6 ± 0.2§</td>
<td>5.9 ± 0.1‡</td>
<td>4.7 ± 0.2‡</td>
<td>5.4 ± 0.3‡</td>
</tr>
<tr>
<td>8OE. ORX-Ex-8 wk</td>
<td>18</td>
<td>276 ± 9†</td>
<td>773 ± 47</td>
<td>274 ± 178</td>
<td>3.326 ± 205§</td>
<td>11.2 ± 0.4‡</td>
<td>6.3 ± 0.2‡</td>
<td>4.9 ± 0.4‡</td>
<td>6.5 ± 0.4*</td>
</tr>
</tbody>
</table>

**P value, two-way ANOVA**

- Exercise treatment: <0.01 NS <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01
- Time: <0.01 <0.05 <0.05 <0.05 <0.01 <0.01 <0.05 NS

Values are means ± SE. Significantly different from corresponding ORX-Sed group: *P < 0.05; †P < 0.01. Significantly different from corresponding Sham-Sed group: ‡P < 0.01; ‡‡P < 0.05.
Table 3. Dynamic parameters of the midfemoral periosteal and endocortical surfaces of sedentary and exercised rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, wk</th>
<th>Periosteal</th>
<th>Endocortical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS/BS, %</td>
<td>MAR, μm/day</td>
</tr>
<tr>
<td>C. Control</td>
<td>9</td>
<td>65.5 ± 2.7</td>
<td>3.11 ± 0.13</td>
</tr>
<tr>
<td>4SS. Sham-Sed-4 wk</td>
<td>14</td>
<td>61.4 ± 7.2</td>
<td>2.13 ± 0.09</td>
</tr>
<tr>
<td>4OS. ORX-Sed-4 wk</td>
<td>14</td>
<td>33.1 ± 32.3</td>
<td>1.94 ± 0.09</td>
</tr>
<tr>
<td>4OE. ORX-Ex-4 wk</td>
<td>14</td>
<td>51.6 ± 3.6</td>
<td>2.30 ± 0.06*</td>
</tr>
<tr>
<td>8SS. Sham-Sed-8 wk</td>
<td>18</td>
<td>42.5 ± 4.2</td>
<td>1.53 ± 0.08</td>
</tr>
<tr>
<td>8OS. ORX-Sed-8 wk</td>
<td>18</td>
<td>41.1 ± 3.7</td>
<td>1.39 ± 0.08</td>
</tr>
<tr>
<td>8OE. ORX-Ex-8 wk</td>
<td>18</td>
<td>46.8 ± 1.7</td>
<td>1.90 ± 0.03‡</td>
</tr>
</tbody>
</table>

P value, two-way ANOVA

Exercise treatment <0.05 <0.05 <0.01 <0.05 <0.05 NS NS <0.05

Time <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.05

Values are means ± SE. BS, bone surface; MS, mineralizing surface; MAR, mineral apposition rate; BFR, bone formation rate; ES, eroded surface. Significantly different from corresponding ORX-Sed group: *P < 0.05; †P < 0.01. Significantly different from corresponding Sham-Sed group: ‡P < 0.01; §P < 0.05.

Table 4. Structural and dynamic parameters of the lumbar vertebrae of the experimental rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, wk</th>
<th>BV/TV, %</th>
<th>Tb.Th, μm</th>
<th>Tb.N, 1/mm</th>
<th>Tb.Sp, μm</th>
<th>MS/BS, %</th>
<th>MAR, μm/day</th>
<th>BFR/BS, μm²/μm²·day⁻¹</th>
<th>Oc.S/BS, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Control</td>
<td>9</td>
<td>23.4 ± 0.6</td>
<td>63.5 ± 1.1</td>
<td>3.69 ± 0.04</td>
<td>207.9 ± 3.8</td>
<td>38.8 ± 2.3</td>
<td>1.51 ± 0.08</td>
<td>60.2 ± 6.3</td>
<td>5.90 ± 0.52</td>
</tr>
<tr>
<td>4SS. Sham-Sed-4 wk</td>
<td>14</td>
<td>27.4 ± 0.9</td>
<td>68.6 ± 2.8</td>
<td>4.01 ± 0.07</td>
<td>181.4 ± 3.7</td>
<td>26.9 ± 1.5</td>
<td>1.06 ± 0.02</td>
<td>28.5 ± 1.4</td>
<td>3.55 ± 0.19</td>
</tr>
<tr>
<td>4OS. ORX-Sed-4 wk</td>
<td>14</td>
<td>23.2 ± 0.5‡</td>
<td>67.7 ± 1.8</td>
<td>3.45 ± 0.08‡</td>
<td>224.0 ± 6.0‡</td>
<td>34.0 ± 2.4‡</td>
<td>1.17 ± 0.02‡</td>
<td>39.7 ± 2.7‡</td>
<td>4.39 ± 0.22‡</td>
</tr>
<tr>
<td>4OE. ORX-Ex-4 wk</td>
<td>14</td>
<td>25.6 ± 1.2</td>
<td>67.3 ± 2.7</td>
<td>3.82 ± 0.11‡</td>
<td>197.1 ± 9.0‡</td>
<td>34.4 ± 0.7‡</td>
<td>1.18 ± 0.02‡</td>
<td>40.7 ± 1.2‡</td>
<td>4.20 ± 0.25‡</td>
</tr>
<tr>
<td>8SS. Sham-Sed-8 wk</td>
<td>18</td>
<td>31.3 ± 0.8</td>
<td>75.2 ± 1.1</td>
<td>4.16 ± 0.06</td>
<td>165.7 ± 4.1</td>
<td>22.5 ± 0.9</td>
<td>1.05 ± 0.03</td>
<td>23.9 ± 0.9</td>
<td>2.41 ± 0.11</td>
</tr>
<tr>
<td>8OS. ORX-Sed-8 wk</td>
<td>18</td>
<td>24.1 ± 1.0‡</td>
<td>74.7 ± 2.5</td>
<td>3.22 ± 0.06‡</td>
<td>236.3 ± 6.7‡</td>
<td>33.8 ± 0.8‡</td>
<td>1.22 ± 0.07‡</td>
<td>41.4 ± 2.4‡</td>
<td>3.51 ± 0.20‡</td>
</tr>
<tr>
<td>8OE. ORX-Ex-8 wk</td>
<td>18</td>
<td>31.7 ± 1.3‡</td>
<td>90.0 ± 5.8‡</td>
<td>3.53 ± 0.10‡</td>
<td>194.2 ± 5.2‡</td>
<td>23.7 ± 0.9‡</td>
<td>1.14 ± 0.02‡</td>
<td>26.9 ± 0.9†</td>
<td>3.00 ± 0.04§</td>
</tr>
</tbody>
</table>

P value, two-way ANOVA

Exercise treatment <0.05 <0.05 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01

Time <0.01 <0.01 <0.05 <0.05 <0.05 <0.05 <0.01 <0.05 <0.05

Values are means ± SE. BV, bone volume; TV, tissue volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular bone separation; Oc.S, osteoclast surface. Significantly different from corresponding ORX-Sed group: *P < 0.05; †P < 0.01. Significantly different from corresponding Sham-Sed group: ‡P < 0.01; §P < 0.05.
levels were similar to those of lumbar trabecular bone formation, but not the femoral periosteal surface, in this study. Consistently, reports have shown that the serum osteocalcin was increased by ORX (5, 10, 13). This marker mainly reflects trabecular bone formation in ORX rats. Also, U-Dpy levels were in accordance with the Oc.S/BS at 8 wk. This indicated that both systemic bone formation and resorption were partially reduced by the climbing exercise after 8 wk.

In the femoral cortical bone, both bone formation and eroded endocortical surface did not change after exercise. Thus the effects of climbing exercise on the cortical envelopes were mainly on periosteal bone formation. These findings are consistent with our laboratory’s previous findings that resistance exercise such as jumping and climbing affects the periosteal surface of cortical bone rather than the endosteal surface (18–20). Interestingly, in ORX rats, the climbing exercise increased periosteal cortical bone formation, whereas it decreased cancellous bone formation. The findings that the exercise has the opposite effect on bone formation in cortical and cancellous bone were similar to a previous report (32). In intact rats, both climbing and jumping exercises decrease endocortical bone formation (18–20). However, at 8 wk, the values of endocortical MAR in the exercise group were further increased compared with the values in both the sham and ORX groups in this study. Thus resistance exercise appears to increase osteoblastic function at the endocortical surface in ORX rats long after orchidectomy.

The total cross-sectional area in the OE group was significantly lower compared with the SS group after 8 wk. This suggested that exercise did not fully normalize radial bone growth in this experiment, even though both periosteal bone formation and bending strength were maintained. Because the parameters of cortical bone area and the moment of inertia were prevented by exercise, the maximum load of the femur is mainly due to structural changes in the femoral shaft rather than its cross-sectional area. The directly measured mechanical parameter of femoral maximum load showed a time-dependent change, whereas the indirectly calculated moment of inertia did not. In our laboratory’s previous report, changes in the moment of inertia did not fully correspond to those of bone strength assessed by a bending test (21). A direct mechanical assessment seemed to be more sensitive for detecting the maximum bone strength than an indirect assessment.

BMC, BMD, and compressive load values of the lumbar vertebrae did not appear to greatly depend on the trabecular bone mass and structure in growing ORX rats since the parameters of BV/TV, trabecular thickness, and trabecular number were increased and trabecular bone separation was decreased by exercise compared with the OS group. The reason for the discrepancy between compressive load, bone mass, and trabecular structure is unclear. The main factors responsible for compressive mechanical properties of the lumbar vertebral body include the three-dimensional structure of the cancellous core and surrounding cortical shell (21, 30). Because the trabecular bone tended to be less affected by orchidectomy than cortical bone (24, 27), the trabecular bone structure seems to recover relatively faster than cortical bone with exercise. Unfortunately, our bone mass assessment using dual-energy X-ray absorptiometry could detect only the total mass of cortical and trabecular bone. Thus we may have failed to detect any improvement in the trabecular bone.

The treadmill exercise included daily running of ∼0.3–0.4 km for 8 wk (28) and 1.7–1.8 km for 15 wk (10, 11) in ORX rats. Our voluntary exercise included daily climbing of ∼0.12–0.16 km for 4 and 8 wk. These data suggest that, even in ORX rats, the effect of exercise on bone mass and strength does not depend on the distance or duration of the exercise but may depend on the type of exercise, as in intact rats (16, 18, 29). The body weights were reduced by ORX after 4 and 8 wk, although total food intake was the same between the experimental groups in each period. These data are consistent with previous studies showing that body weight is affected by gonadal hormones rather than by food intake (3, 10, 11). Previously, climbing exercise increased muscle mass and decreased abdominal adipose tissue in intact rats (20). However, in ORX rats, exercise affected only the adipose tissue. Under the condition of gonadal hormone deficiency, it is unlikely that this exercise stimulates muscle mass.

In conclusion, a voluntary climbing exercise maintained cortical bone mass and strength by stimulating periosteal bone formation and partially prevented ORX-induced trabecular bone loss, depressing the elevation of turnover. However, in lumbar vertebrae, bone mass and strength were not affected by exercise. Also, the climbing exercise had the opposite effect on bone formation in periosteal femoral cortical bone compared with vertebral trabecular bone.

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