Effect of resistance exercise training on cortical and cancellous bone in mature male rats

KIM C. WESTERLIND,1 JAMES D. FLUCKEY, 2 SCOTT E. GORDON,2 WILLIAM J. KRAEGER,2 PETER A. FARRELL,2 AND RUSSELL T. TURNER2

1Department of Orthopedic Research, Mayo Clinic, Rochester, Minnesota 55905; and 2Noll Physiological Research Center and Center for Sports Medicine, The Pennsylvania State University, University Park, Pennsylvania 16802

Westerlind, Kim C., James D. Fluckey, Scott E. Gordon, William J. Kraemer, Peter A. Farrell, and Russell T. Turner. Effect of resistance exercise training on cortical and cancellous bone in mature male rats. J. Appl. Physiol. 84(2): 459–464, 1998.—The effect of resistance training on tibial cancellous and cortical bone was evaluated in rats by using static histomorphometry and Northern analysis. Five-month-old male Sprague-Dawley rats were randomly assigned to exercise (Ex; n = 8) or control (Con; n = 4) groups. Animals were operantly conditioned to press two levers, facilitating full extension and flexion of the hindlimbs ("squats"), while wearing an unweighted vest. After an 8-wk familiarization period, Ex animals performed 3 sessions/wk for 17–19 sessions with progressively increased amounts of weight applied to the vest. Con rats completed the same exercise protocol without applied resistance. No difference in cross-sectional, medullary, or cortical bone area was observed between Ex and Con rats in the tibial diaphysis. In contrast, the cancellous bone area in the proximal tibial metaphysis was significantly larger in trained rats. Trabecular number, trabecular thickness, and the percentage of cancellous bone covered by osteoid were significantly greater in the Ex animals compared with Con animals. In addition, steady-state mRNA levels for osteocalcin for the Ex group were 456% higher than those in the Con group. The data demonstrate that resistance training increases cancellous bone area in sexually mature male rats and suggest that it does so, in part, by stimulating bone formation.

Cross-sectional data from studies in humans, although inherently affected by selection bias, suggest that resistance-trained athletes have a greater bone mass than endurance-trained athletes or sedentary control subjects (9, 14). The few prospective studies of resistance exercise training programs have yielded conflicting results, with some showing a positive effect (3, 17, 18, 29), no change (13, 22), or a negative effect (26) of resistance training. Sample sizes, training regimens, subject characteristics (gender, age, health), and lack of sensitivity and reproducibility of instruments for measuring changes in bone mass are a few of the factors that preclude a definitive statement on the effects of this type of exercise.

Data from animal studies have generally suggested a beneficial effect of exercise but have predominantly been limited to treadmill (7, 24, 30, 37) or wheel running (19). To date, a resistance exercise training model has not been utilized to assess skeletal effects. The purpose of the present investigation was to utilize resistance exercise training in sexually mature male rats to assess changes in cancellous and cortical bone. Skeletal changes were assessed by histomorphometry and Northern analysis.

METHODS

Twelve male rats (Harlan Sprague Dawley, Madison, WI; mean age = 5 mo) were individually housed in hanging cages at 24°C on a 12:12-h light-dark cycle. Rodent chow (The Richmond Standard Diet, PMI Feeds, St. Louis, MO) and water were supplied ad libitum. The rats were stratified by weight and randomly assigned to the resistance exercise group (Ex; n = 8) or the non-resistance-trained control group (Con; n = 4). All procedures were approved by The Pennsylvania State University’s Institutional Animal Care and Use Committee before initiation of the study.

Resistance Training Program

Familiarization period. All animals underwent an 8-wk familiarization program that was designed to familiarize the animals with the training cage and the resistance training procedures. No resistance was used on the animals at any time during this familiarization period. Operant conditioning was used with shock as an aversive stimulus during the familiarization period and the actual training program. The training protocol was based on modifications of the model of Burgess et al. (6).

The rectangular cage (210 × 350 × 210 mm) consisted of an electric-shock grid in the floor and two levers that would illuminate, one at 35 mm above the floor and the other, on the opposite wall, at 220 mm. Three seconds after a lever would illuminate, mild shock was administered for 5 s. The rats

http://www.jap.org 0161-7567/98 $5.00 Copyright © 1998 the American Physiological Society 459
were trained to push the illuminated lever that simultaneously turned off the light and the shock. As animals learned to successfully accomplish the task, levers would illuminate on an alternate basis.

The animals' responses to alternating lights simulated a whole body standing and squatting movement involving both concentric and eccentric muscle actions. This movement was similar to "squats" in traditional weight room settings. Midway through the 8-wk familiarization program, leather and Velcro unweighted vests were introduced to all animals. The vests were later used to attach resistance to the back of the Ex animals.

Resistance Training Period

The 6-wk resistance training program was initiated after the animals were achieving a 90% success rate. This exercise success rate was reflective of the percentage of time an animal executed a complete repetition (depressing both the upper and lower switches) without receiving aversive stimuli. Training took place in the dark at 1500, the beginning of the animals' dark cycle.

Ex group. Animals performed 17–19 sessions, 3 sessions/wk, for a total of ~6 wk. Each session was separated by a minimum of 48 h of rest. Initial weight, including the vest, was set at 70 g. This represented ~17% of the animal's body weight. The initial weight was established during pilot investigations as a mass that could be lifted during an initial exercise session. Weight was increased at the discretion of the investigators to final resistance of 500 g (~104% of body wt).

Table 1 shows the specific resistance lifted and number of repetitions performed by each animal during each session. Weight was increased at the discretion of the investigators to final resistance of 500 g (~104% of body wt).

Histomorphometry

Histomorphometric measurements were performed by one investigator, blinded to group assignment, with use of the SM1-Microcomp-PM semiautomatic image-analysis system (Southern Micro Instruments, Atlanta, GA), which consists of a computer (Compaq 285, Compaq Computer, Houston, TX) coupled to a photomicroscope and image-analysis system. In this system, a high-resolution color video camera records the image of the specimen through the microscope (Olympus BH-2, New Hyde Park, NY) and displays the image on a video monitor that registers the movement of a digitizing pen on a graphics tablet. As the pen is moved along the graphics tablet, a tracing appears superimposed on the image of the specimen displayed on the video screen. The region of interest is traced, and the line lengths and area bound by the lines are calculated.

Cortical bone measurements. Ground transverse sections were used for histomorphometric analysis of cortical bone. Cross sections 150 µm thick were cut at a site just proximal to

Table 2. Repetitions by Con animals

<table>
<thead>
<tr>
<th>Session No.</th>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Rat 3</th>
<th>Rat 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>70</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>107</td>
<td>107</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>30</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>25</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>24</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>30</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>25</td>
<td>33</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>28</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>25</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td>11</td>
<td>28</td>
<td>25</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>31</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>13</td>
<td>24</td>
<td>28</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>14</td>
<td>31</td>
<td>28</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>15</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>16</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>17</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>

Con, control.
the tibia-fibula synostosis with a low-speed saw (Isomet, Buehler, Lake Bluff, IL) equipped with a diamond wafer blade. The sections were ground to a thickness of 15–20 μm on a roughened glass plate, and the following measurements were made: 1) cross-sectional area, defined as the area of bone and marrow cavity bounded by the periosteal surface of the specimen; 2) medullary area, defined as the area delineated by the endocortical surface of the specimen; and 3) cortical bone area, calculated as the difference between cross-sectional area and medullary area.

Cancellous bone measurements. The proximal tibial metaphysis was dehydrated in a series of increasing concentrations of ethanol, embedded without demineralization in a mixture of methylmethacrylate-2-hydroxyethyl-methacrylate (12:5:1) and sectioned at a thickness of 5 μm. A standard sampling site was established in the secondary spongiosa of the metaphysis at a distance 1 mm distal to the growth plate. A total metaphyseal area of 4.45 mm² was sampled for each section. Measurements made in unstained sections included 1) cancellous bone area, defined as the area of total cancellous bone per square millimeter metaphyseal area within the sampling site and expressed as a percentage; 2) cancellous bone perimeter, defined as the perimeter of cancellous bone per square millimeter of metaphyseal area; 3) trabecular thickness, calculated as 2/(bone perimeter/bone area), expressed in micrometers; and 4) trabecular number, calculated as the cancellous bone area divided by the trabecular thickness, expressed without units (20).

Metaphyseal sections were additionally stained with toluidine blue to visualize and measure the percentage of cancellous bone covered with osteoid. Osteoid is unmineralized bone laid down by osteoblasts. Percent osteoid provides an indication of bone formation.

RNA Analysis

Total RNA isolation. Total RNA was isolated from the proximal metaphysis of the right tibia. Individual samples, frozen in liquid nitrogen, were homogenized in 2 ml of guanidine isothiocyanate by using a Spex Freezer Mill (Edison, NJ). Total cellular RNA was extracted and isolated by using a modified organic solvent method (8), and the yields of the amounts of RNA loaded and transferred were assessed by ethidium bromide staining of the gels and hybridization of the RNA with a 32P-labeled cDNA for the 18S rRNA (5).

Table 3. Effect of resistance exercise training on body weight and tibia length

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ex</th>
<th>Con</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>401.9 ± 5.0</td>
<td>404.9 ± 14.1</td>
<td>0.80</td>
</tr>
<tr>
<td>Pre</td>
<td>479.3 ± 6.5</td>
<td>514.0 ± 21.6</td>
<td>0.08</td>
</tr>
<tr>
<td>Post</td>
<td>775.2 ± 2.3</td>
<td>1090.0 ± 25.8</td>
<td>0.10</td>
</tr>
<tr>
<td>Tibia length, cm</td>
<td>4.14 ± 0.05</td>
<td>4.22 ± 0.08</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 Ex and 4 Con rats. Pre and Post, before and after study.

Statistical Analysis

Comparisons between Ex and Con groups of body weight, bone measurements, and levels of expression for osteocalcin were assessed by Student’s t-test for unpaired observations. A P value < 0.05 is considered statistically significant. All values are expressed as means ± SE.

RESULTS

No significant difference in body weights was observed between the Ex and Con animals before or at the conclusion of the study (Table 3), although a trend toward greater weight gain was observed in the non-weight-trained controls (P = 0.10). Tibia length was not significantly different between the two groups (P = 0.33).

The effects of resistance exercise training on cortical and cancellous bone measurements are shown in Table 4. Six weeks of resistance training resulted in no significant change in cross-sectional, medullary, or cortical area. In contrast, the metaphysis of Ex animals had significantly greater cancellous bone area and cancellous bone perimeter compared with the Con group. In addition, Ex animals had thicker trabeculae than did the Con group. The percentage of cancellous bone perimeter covered by osteoid was significantly greater in the Ex animals compared with the Con group.

mRNA levels for 18S rRNA did not differ between the resistance- and non-resistance-trained animals (data not shown) and were utilized to normalize message

Table 4. Effect of resistance exercise training on cortical and cancellous bone measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ex</th>
<th>Con</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional area, mm²</td>
<td>8.05 ± 0.20</td>
<td>7.69 ± 0.23</td>
<td>0.32</td>
</tr>
<tr>
<td>Medullary area, mm²</td>
<td>1.15 ± 0.08</td>
<td>1.18 ± 0.08</td>
<td>0.83</td>
</tr>
<tr>
<td>Cortical area, mm²</td>
<td>6.90 ± 0.16</td>
<td>6.52 ± 0.19</td>
<td>0.17</td>
</tr>
<tr>
<td>Cancellous bone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area, %</td>
<td>21.5 ± 2.0</td>
<td>9.6 ± 1.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Perimeter, mm/mm²</td>
<td>6.89 ± 0.49</td>
<td>4.55 ± 0.59</td>
<td>0.02</td>
</tr>
<tr>
<td>Trabecular thickness, μm</td>
<td>278.1 ± 18.0</td>
<td>192.3 ± 16.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>3.44 ± 0.25</td>
<td>2.27 ± 0.29</td>
<td>0.02</td>
</tr>
<tr>
<td>Osteoid, %total perimeter</td>
<td>13.8 ± 1.1</td>
<td>7.8 ± 0.9</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 Ex rats and 4 Con rats.
levels for osteocalcin. When expressed relative to the Con group, mRNA levels for osteocalcin in the Ex group were approximately fourfold higher ($P < 0.01$) (Fig. 1).

**DISCUSSION**

To our knowledge, this is the first study to utilize resistance training exercise and a rat model to assess skeletal changes. The data indicate that 6 wk of resistance exercise by using progressively heavier loads can result in a significant increase in cancellous bone area in the tibial metaphysis of adult male rats. Cancellous bone perimeter, trabecular thickness, and trabecular number were all significantly greater in Ex animals than in the Con group.

The magnitude of the increase in cancellous bone area is greater than that observed in other exercise training studies (19, 24, 37) and is, in all likelihood, due to exercise modality. Previous training studies in rats have incorporated treadmill running (2, 4, 7, 30, 37) or voluntary wheel running (19), and increases in bone have been on the order of 10%, depending on the exercise protocol and the techniques used to measure skeletal changes. We would also speculate that the large increase in cancellous bone area in the present study was due to the increase in workload with each exercise session. Bourrin and colleagues (4) observed increased bone volume and trabecular number after 3, 4, and 5 wk of treadmill running. They suggested that the efficiency of the training protocol in increasing bone mass over a short time frame was due to continuous adjustment (increased speed) over the training regimen to maintain the same relative intensity. Sogaard et al. (30) reported an increase in bone volume and femoral neck strength with 4 mo of treadmill running but no further increase in animals that ran for an additional 6 mo. This may be related to the fact that the training protocol was not changed during months 6–12, and thus a new skeletal homeostasis was reached and there was no further need for increased bone formation. Raab-Cullen and colleagues (25) have suggested that it is not the intensity of the exercise, per se, but rather the “change” in the exercise level that is necessary to stimulate bone formation. Our data are consistent with this speculation. Workloads were changed with each training session by increasing either the weight carried by the animals or the number of repetitions that were performed; and, as such, each session could be considered “novel.”

We saw no difference in the cross-sectional or cortical area between Ex and Con animals, although there was a trend toward a greater value in both parameters in the Ex group. The small group sizes of four and eight reduced our power to discern a difference and point to the potential of a type II error. Other studies have reported increased modeling and an increase in cortical area or density (7, 23, 37). Our laboratory has previously shown that mechanical unloading due to spaceflight, unilateral sciatic neurotomy, and hindlimb elevation results in bone loss from cancellous but not cortical bone sites (35). Furthermore, the cancellous bone loss is limited to the metaphyseal region; bone is not lost from the epiphysis. Finite element analysis has demonstrated that the cortical bone is under much higher strain energy density levels than cancellous bone and that the trabeculae in the epiphysis are under higher strain energies than in the metaphysis (35). The inverse relationship between strain energy density and change in bone mass previously observed with reductions in mechanical usage (35) and supported by observations in the present study after increased mechanical usage may be due to the relatively larger changes in magnitude occurring at skeletal sites that are normally subjected to low strain energies. Alternatively, these sites may have a smaller strain threshold before initiation of adaptive bone modeling. The animals in the present study were part of a larger study (10, 11), and, therefore, it was not desirable to administer fluorochrome labels for dynamic histomorphometry because of possible side effects on other organ systems. However, mRNA levels for bone matrix proteins are highly correlated with measurements made by dynamic histomorphometry (31, 32) and are reduced in situations where bone formation is depressed (1, 33) and upregulated in conditions of increased bone formation (28, 34, 35). Thus RNA analysis provides a sufficient substitute for dynamic histomorphometry to evaluate the effects of resistance training on bone formation. The present finding that mRNA levels for osteocalcin were significantly higher in the Ex animals suggests that the observed changes were a result, at least in part, of increased bone formation. The significantly greater percentage of cancellous bone covered with osteoid would also support an increase in bone formation.

Studies in humans utilizing resistance training exercise modalities to effect skeletal changes have yielded inconsistent results. Reports of no change (13, 21, 22), detrimental effects (26), or positive effects (17, 18, 29) have been documented. Inconsistent findings regarding the efficacy of resistance training modality may be due to differences in training regimen (intensity, frequency, duration), subject differences (age, gender, nutritional or hormonal status), and sensitivity of the measurement techniques.
Two studies, in which each subject served as a control and the confounding factors of cross-sectional designs (genetics and hormonal and/or nutritional status) were eliminated, were recently conducted. In each, subjects participated in a 12-mo unilateral resistance exercise training program (15, 16). Kerr et al. (16) reported increased bone mass in lower and upper limb sites of the active side relative to the inactive side of the body, whereas Heinonen and colleagues (15) reported no change in the radius, ulna, and humerus. Conflicting results between the two studies may be due to subject characteristics [mean age = 23 (15) and 58 yr (16)], menopausal status [pre (15), post (16)], type of training [single-dumbbell exercise (15), multiple free and machine-based weights (16)], and/or other considerations. The data of Kerr et al. (16) provide evidence that resistance exercise has a skeletal beneficial effect and that this effect is site specific, but the discrepant results again highlight the need for caution when results are compared and interpretations from different studies are formulated.

The complexity and difficulty in conducting randomized, prospective, long-term exercise studies are well appreciated. Compliance and adherence issues are a large factor, as is the inability to sensitively measure changes in bone and the mechanisms responsible for these changes. Thus the ability to utilize an animal model and a resistance training protocol such as have been described herein has the potential to facilitate understanding the role that resistance/weight training exercise would have to prevent bone loss and potentially to increase bone gained.

In summary, the present study is the first to utilize resistance exercise training in a rat model to document skeletal changes. Results indicate an increase in cancellous bone area and perimeter and an increase in trabecular number and thickness. The positive changes appear to be, at least in part, a result of increased bone formation. Utility of this model in rats of different age, sex, and hormonal and nutritional status may lead to a greater understanding of how resistance exercise may be effective in increasing peak bone mass and in mitigating age- and hormone deficiency-associated bone loss.

This work was supported by National Institutes of Health Grants DK-07352 (K. C. Westerlind), AR-43127 (P. A. Farrell), and AR-35651 (R. T. Turner).

Address for reprint requests: K. C. Westerlind, AMC Cancer Research Center, 1600 Pierce St., Denver, CO 80214.

Received 3 April 1997; accepted in final form 22 September 1997.

References


