Jump exercise during remobilization restores integrity of the trabecular architecture after tail suspension in young rats

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Ju Y, Sone T, Okamoto T, Fukunaga M. Jump exercise during remobilization restores integrity of the trabecular architecture after tail suspension in young rats. J Appl Physiol 104: 1594–1600, 2008. First published April 17, 2008; doi:10.1152/japplphysiol.01004.2007.—Three-dimensional trabecular architecture was investigated in the femora of tail-suspended young growing rats, and the effects of jump exercise during remobilization were examined. Five-week-old male Wistar rats (n = 35) were randomly assigned to five body weight-matched groups: tail-suspension group (SUS; n = 7); sedentary control group for SUS (S/CON; n = 7); spontaneous recovery group after tail suspension (S+/R/CON; n = 7); jump exercise group after tail suspension (S+/R/JUM; n = 7); and age-matched control group for S+/R/CON and S+/R/JUM without tail suspension and exercise (S/CON+/R/CON; n = 7). Rats in SUS and S/CON were killed immediately after tail suspension for 14 days. The jump exercise protocol consisted of 10 jumps/day, 5 days/wk, and jump height was 40 cm. Bone mineral density (BMD) of the femur and three-dimensional trabecular bone architecture at the distal femoral metaphysis were measured. Tail suspension induced a 13.6% decrease in total femoral BMD (P < 0.001) and marked deterioration of trabecular architecture. After 5 wk of free remobilization, femoral BMD, calf muscle weight, and body weight returned to age-matched control levels, but trabeculae remained thinner and less connected. On the other hand, S+/R/JUM rats showed significant increases in trabecular thickness, number, and connectivity compared with S+/R/CON rats (62.8, 31.6, and 24.7%, respectively; P < 0.05), and these parameters of trabecular architecture returned to the levels of S/CON+/R/CON. These results indicate that suspension-induced trabecular deterioration persists after remobilization, but jump exercise during remobilization can restore the integrity of trabecular architecture and bone mass in the femur in young growing rats.

Jump exercise; tail suspension (unloading); remobilization; trabecular architecture; microcomputed tomography

SKELETAL UNLOADING OR LOSS of normal weight bearing, as seen during space flight or prolonged bed rest, causes bone mass in humans (28, 52). Studies using rats have also demonstrated marked bone loss, impairment of bone mechanical properties and disruption of bone architectures following spaceflight (20), immobilization by tenotomy or sciatic neurectomy (36), or tail suspension (17).

The recovery potential of bone has been studied after spaceflight (45) and bed rest (29) in humans, and after immobilization (46) or tail suspension-induced bone loss (49) in animals. The time required to recover bone mass caused by mechanical unloading may be longer than the duration of unloading. Moreover, trabecular bone is more susceptible to changes in mechanical environment than cortical bone. Loss of trabecular bone in the proximal tibial metaphysis induced by skeletal unloading is apparently more pronounced and remains longer after reloading than that of cortical bone (44, 50). A previous study indicated that, although subsequent reloading for 14 days restored the reduced trabecular bone formation and suppressed the increased trabecular bone resorption, trabecular bone mass showed insufficient recovery during the reloading period after tail suspension (42). Even though subsequent normal weight bearing after a period of unloading results in increased bone formation to baseline levels, reconnecting trabecular bone architecture after initial disruption remains difficult. The development of countermeasures to prevent skeletal unloading-induced disorganization of bone architectures is of vital importance.

The recovery potential of bone after immobilization also depends on the age of the animal. In old rats, long-term disuse-induced bone loss is hardly restored after reloading (29, 34), whereas in young rats, bone can be recovered after various periods of immobilization (30, 31, 55). However, how exercise applied during a recovery period after mechanical unloading influences bone mass and architecture has not been clarified in detail. In particular, the effects of jump exercise on trabecular architecture in unloading-induced osteopenia has never been evaluated from a three-dimensional (3D) perspective. Weight-bearing exercise is known to be effective in increasing bone mass, and high-impact exercise, such as jumping, is considered particularly beneficial for bones (5, 22, 48). To the best of our knowledge, only one other study has evaluated the effects of physical exercise on restoration of bone architecture after tail suspension-induced osteopenia in young rats (4). They showed that the treadmill exercise during remobilization restored a normal trabecular network. However, that study was two dimensional (2D), and the 3D details of heterogeneous trabecular structures have not been clarified.

The strength of cancellous bone depends on the 3D trabecular architecture as well as the amount and quality of bone tissue. Thus the recovering capacity of 3D trabecular architecture would play an important role to restore the bone strength after immobilization. The present study investigated 3D trabecular architecture in the femur using tail-suspended rats as a hindlimb-unloading model and examined the effects of jump exercise during remobilization.

MATERIALS AND METHODS

Animal care. The experiment protocol and all animal procedures were in compliance with the guidelines set forth in the Care and Use 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.
of Laboratory Animals in the Field of Physiological Sciences approved by the Council of the Physiological Society of Japan (http://www.soc.nii.ac.jp/psj/psj/doubutu.html). Male 4-wk-old Wistar rats (n = 35) were purchased from Clea Japan (Osaka, Japan) and individually housed in 35 × 35 × 35-cm metal-grill cages under standard laboratory conditions at a room temperature of 22 ± 1°C and a humidity of 60 ± 5% with a 12-h light-dark cycle (light cycle starting at 0800). Rats were fed standard laboratory animal chow (MF; Oriental Yeast, Chiba, Japan) containing 1.15% calcium and 0.88% phosphorus. After 1 wk of acclimatization to the diet and new environment, rats were randomly assigned into five groups (n = 7 each): tail-suspended group (SUS); control group for SUS (S CON); spontaneous recovery group after tail suspension (S + R CON); jump exercise group after tail suspension (S + R JUMP); and age-matched control group for S + R CON and S + R JUMP without tail suspension and exercise (S CON + R CON). Food intake for intakes of groups of rats was monitored, and controls were pair-fed throughout the experiment. Access to water was unrestricted. Body weight and food intake were measured 3 times/wk. The groups of SUS and S CON rats were immediately killed by exsanguination under pentobarbital sodium anesthesia (0.1 ml/100 g of body wt IP) after 14 days of tail suspension. Groups of S + R CON, S + R JUMP, and S CON + R CON rats were killed 5 wk after the end of the experimental period (jump exercise protocol period). After death, the right hind limb muscles (gastrocnemius and soleus) of each rat were collected and immediately weighed. The right femur was excised from each rat and cleaned of soft tissue. Femoral length was measured using a digital caliper. The femur was stored at −80°C until required for further measurements.

Tail suspension. The tail-suspension procedure was performed according to the recommendations of Morey-Holton and Globus (35), with slight modification. In brief, the tails were cleaned with 70% alcohol, removing all dead or dirty skin, then allowed to dry. Rats were not anesthetized. Traction tape was loosely wrapped around the tail in a helical pattern starting at 1 cm from the base of the tail. A strip of filament tape was attached to two-thirds the length of the tail along the lateral sides, and then secured by two strips of filament tape. One strip of filament tape was placed around the end of the body side of the traction tape, and a second strip was added about halfway up the tail. The filament tape was loosely applied to allow normal blood circulation but tight enough so that the traction tape would not peel from the tail. The metal connector was connected by a wire to a swivel mounted at the back of the rat. The traction tape would not peel from the tail. The metal connector was connected by a wire to a swivel mounted at the back of the rat. The traction tape would not peel from the tail.

Exercise protocol. After removal from the tail suspension apparatus, rats rested for 24 h. The jumping exercise protocol was then implemented according to the method previously reported by Umemura et al. (48). Rats in the jump exercise groups were individually placed at the bottom of a special wooden box surrounded with boards. The height of this box can be adjusted. Rats were initially forced to jump by electric stimulus and to grasp the top of the board with the forelimbs and climb up the board. The rat was then returned to the floor of the cage to repeat the procedure. As rats became accustomed to the jump exercise, the electric stimulus was used less frequently. The jump exercise program comprised 10 jumps/day, 5 days/wk for 5 wk. Initial height of the box was 25 cm, and this was gradually increased to 40 cm during the first week. The time required for 10 jumps was ~1 min.

Bone mineral density measurements. Bone mineral density (BMD) of the femur was measured by dual-energy X-ray absorptiometry using a QDR-2000 unit (Hologic, Waltham, MA) at small animal ultra-high resolution scan mode, starting from the distal end of the femur. For subregional analysis, the total femoral region was divided along the femoral long axis into seven equal regions, as reported previously (21): R1 (region including femoral head, neck, and greater trochanter), R2 (intertrochanter), R3 (proximal diaphysis), R4 (mid-diaphysis), R5 (distal diaphysis), R6 (distal metaphysis), and R7 (distal epiphysis).

Micro-CT scanning and 3D architectural parameters. The bone microarchitecture of the right femur was evaluated using a micro-CT system (Ele Scan; Nittetsu Elex, Tokyo, Japan). This apparatus is based on fan-beam tomography and is able to function in multislice mode. An X-ray tube with a microfocus (spot size of 6 × 8 μm) was used and maximum resolution of 4 μm (pixel size) was attainable. Parameters selected for this study included source energy 30 kVp and 100 mA to obtain optimum contrast between bone and soft tissue. The sample area selected for scanning was positioned at a distance of 4–4.5 mm proximal to the distal femoral end, including the border between the distal metaphysis and growth plate. The distal femur was selected over the proximal femur because of the larger volume of cancellous bone available for 3D analysis in the distal femur. A total of 250 consecutive tomographic slices with a slice thickness of 14.1 μm (~3.5 mm) were acquired. Digital data were reconstructed to obtain CT images with a pixel size of 17.6 μm in 512 × 512 matrices.

After micro-CT scanning, the original image data were transferred to a workstation, and structural indexes were calculated using 3D image analysis software (TRI/3D-BON; Ratoc System Engineering, Tokyo, Japan). TRI/3D-BON builds 3D models from serial tomographic datasets for visualization and morphometric analysis (21). This software calculates 3D morphometric parameters of cancellous bone based on micro-CT scan datasets. The volume of interest was defined as the 180 slices above the most proximal portion of growth plate. The resulting gray-scale images were segmented using a 3 × 3 median filter to remove noise, and a fixed threshold of 120 (0–255 range) to extract the mineralized bone phase. The isolated small particles in marrow space and the isolated small holes in bone were removed using a cluster-labeling algorithm.

Cortical and trabecular bone were subsequently separated and structural indexes calculated. Bone surface area (BS), bone volume (BV), and total tissue volume (TV) were estimated using standard procedures (7, 32). Trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp) were measured by distance-transform method, which is independent of an underlying model (13). Fractal dimension of trabecular bone was measured as a representative of complexity using a box-counting method (7) developed three dimensionally. Connectivity density (B/1/TV) (8) and trabecular bone pattern factor (TBPF) (10) were calculated directly from segmented voxel representations. TBPF reflects a concave/convex structure of the trabecular bone surface and lower values imply a highly connected state among trabeculae (9). The parameter B/1/TV also represents trabecular connectivity, indicating more directly the state of trabecular connections. Structural model index (SMI) was calculated according to the methods described by Hildebrand and Rüegsegger (14). SMI defined as a value between 0 and 3 is used to estimate rod-like and plate-like characteristic of trabecular structures. Degree of anisotropy (DA), reflecting trabecular orientation, was determined from the ratio between maximal and minimal radii of the mean intercept length ellipsoid (11).

Data analysis. All statistical analyses were performed using SPSS software version 14.0 for Windows (SPSS, Chicago, IL). The significance of differences between values in SUS and S CON groups was determined using Student’s t-test. Differences among treatment groups (S + R CON, S + R JUMP, and S CON + R CON groups) were evaluated by...
RESULTS

Body and muscle weights and femoral length. Body weight and hind limb muscle weights, but not femoral length, were significantly lower in tail-suspended rats than in sedentary control rats (12%, 21%, and 0.5% lower in SUS compared with SCON, respectively) (Table 1). After 5 wk of free remobilization, body weight and hind limb muscle weight had recovered rapidly in the S+RCON group to the same level as age-matched control rat values. However, final body weight in the S+RJUM group was significantly lower compared with SCON and S+RCON groups (6.8% and 5.6% difference, respectively). Jump exercise did not affect either hind limb muscle weight or femoral length.

BMD in subregions of the femur. The BMD with respect to anatomical location along the length of the femur are shown in Table 2. After 14 days of tail suspension, total and subregional BMD values of the femur were significantly lower compared with sedentary control rats (10–53% difference; P < 0.001). The BMD recovered with free mobilization at R2, R3, R5, and total femoral regions but not at R1, R4, R6, and R7 (4.1–6.5% difference; P < 0.05–0.01). Conversely, in the S+RJUM group, BMD in all regions had recovered to above the level of the S+RCON group after 5 wk of jump exercise (5.2–14% difference; P < 0.05–0.001). Moreover, total femoral, R5, and R6 BMD in the S+RJUM group were significantly higher even compared with the S+RCON group (4.3–6.9% difference; P < 0.05–0.01).

Microstructural properties. Tail suspension induced a marked deterioration of trabecular architecture in the distal metaphysis of the femur (Table 3). After 5 wk of spontaneous recovery, trabecular BV, BS, BV/TV, Tb.N, β1/TV, and fractal dimension were significantly lower, whereas BS/BV, Tb.Sp, TBPf, and SMI were significantly higher in the S+RCON group compared with SCON+RCON group. No significant differences in Tb.Th and DA were observed between S+RCON and SCON+RCON groups. These changes were returned by jump exercise during the recovery period except for TV. DA was significantly higher in S+RJUM compared with S+RCON.

Figures 1 and 2 show typical features of 3D trabecular microstructure in the distal femoral metaphysis for a rat from each group. These images demonstrate that tail suspension elicited marked deterioration in trabecular architecture and that these changes recovered with jump exercise.

Table 1. Physical parameters of the experimental rats

<table>
<thead>
<tr>
<th></th>
<th>SCON (n = 7)</th>
<th>SUS (n = 7)</th>
<th>SCON+RCON (n = 7)</th>
<th>S+RCON (n = 7)</th>
<th>S+RJUM (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight before tail suspension, g</td>
<td>115.15±5.76</td>
<td>115.43±1.86</td>
<td>114.01±0.88</td>
<td>113.59±5.62</td>
<td>111.76±4.72</td>
</tr>
<tr>
<td>Body weight after tail suspension, g</td>
<td>200.66±7.50</td>
<td>176.56±5.03*</td>
<td>203.03±11.88</td>
<td>177.43±9.89‡</td>
<td>169.04±9.08‡</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>317.77±12.43</td>
<td>313.06±9.15</td>
<td>317.77±12.43</td>
<td>313.06±9.15</td>
<td>295.57±17.49§</td>
</tr>
<tr>
<td>Hind limb muscles weight, g</td>
<td>0.90±0.02</td>
<td>0.71±0.06*</td>
<td>1.70±0.11</td>
<td>1.67±0.05</td>
<td>1.61±0.17</td>
</tr>
<tr>
<td>Femoral length, mm</td>
<td>30.83±0.54</td>
<td>30.68±0.49</td>
<td>34.86±0.66</td>
<td>35.15±0.51</td>
<td>34.54±0.60</td>
</tr>
</tbody>
</table>

Values are means ± SD. SCON, 14-day sedentary control group; SUS, 14-day tail suspension group; SCON+RCON, spontaneous recovery group after tail suspension; S+RCON, age-matched controls group without tail suspension; S+RJUM, jump exercise group after tail suspension. *Significant difference vs. SUS group by Student’s t-test (P < 0.001). Significant difference vs. SCON+RCON group (post hoc test); †P < 0.01; ‡P < 0.001. Significant difference vs. S+RCON group (post hoc test) (§P < 0.05).

DISCUSSION

The goal of this study was to assess the ability of jump exercise to restore bone mass and trabecular bone structure to age-matched control levels after deterioration induced by tail suspension in young growing rats. The restoration reflects the ability to combat the disuse-induced deterioration as well as growth retardation of bone. Five weeks of spontaneous recovery after 2 wk of skeletal unloading by tail suspension restored total femoral bone density, whereas integrity of femoral trabecular architecture in young growing rats was not sufficiently recovered. However, subsequent jump exercise after tail suspension completely restored the reduced bone mass and microarchitectural deterioration of bone with a consequent increase in Tb.Th, Tb.N, and connectivity, and a decrease in rod-like structures. These results thus suggest that jump exercise applied during remobilization period allowed full recovery of the integrity of trabecular microarchitecture as well as overall bone mass at the femur in young growing rats.

Chronic reductions in mechanical loading, such as immobilization, bed rest, spinal cord injury, and exposure to microgravity, are well known to precipitate generalized skeletal loss, particularly in bones that bear weight under normal conditions. Conversely, mechanical loading from physical activity is known to play a key role in the development of bone mass in both humans and animals. In particular, among the various types of exercise regimens, high-impact training seems to be the most beneficial for the skeleton. However, previous exercise intervention studies on immobilization-induced osteopenia in rats have mostly used treadmill running exercise and have produced conflicting results (4, 24, 46). Several studies have reported that the strains created by running exercise might be below or a little over the minimum effective strain (3, 26). In addition, running exercise yielded different results depending on factors such as training intensity, duration, period, and frequency (41). On the other hand, jump exercise compared with running yielded greater increases in bone mass and strength because of the greater mechanical stress and higher strain rate (47). However, little is known about the effect of jump exercise in restoring trabecular bone architecture after tail suspension-induced osteopenia. To the best of our knowledge, this study is the first to use jump exercise to assess the recovery of trabecular bone architecture in a tail-suspended rat model.

To clarify the mechanisms underlying the loss of bone mass induced in microgravity environments, a number of in vivo and in vitro studies have been conducted during spaceflight. However, the availability of spaceflight experiments is extremely
imply that the decrease in cancellous bone induced by skeletal
Trabecular bone parameters in distal femoral metaphysis
(data reported in the present study using 3D micro-CT analysis
a reduction in BV/TV by 50%, Tb.N by 50%, and Tb.Th by
In histomorphometric analyses of the proximal tibia isolated
of simulated bone changes induced by weightlessness (33, 54).
A hind limb elevation model of rat by tail suspension
investigate the mechanisms of osteopenia induced by weight-
limited. Ground-based studies have thus been conducted to
investigate the mechanisms of osteopenia induced by weight-
lessness. A hind limb elevation model of rat by tail suspension
is supported by a previous histomorphometric study (4).
ery in total femoral BMD in spontaneous recovery rats after 5
sion is primarily due to increased Tb.Th rather than to notice-
that the increase in trabecular bone mass by mechanical exer-
sis revealed that the microarchitecture of the distal femoral
metaphysis had not completely recovered. Our results concur
with the findings of a previous study (23), in which true
trabecular disappearance could not be restored during remobil-
ization. Sakata et al. (42) proposed two possibilities for the
insufficient recovery of trabecular bone mass during reloading:
that the reloading period was too short for sufficient recovery
or that increased trabecular perforation due to rapid trabecular
thinning caused deterioration of the trabecular bone packet and
a decrease in possible sites of bone formation.
Several previous histomorphometric analyses have found
that the increase in trabecular bone mass by mechanical exercise
is primarily due to increased Tb.Th rather than to noticeable
changes in numbers of trabeculae (16, 38). In the present
study, jump exercise during the remodeling period induced a
significant increase in Tb.N of 31% and Tb.Th of 63% when
compared with the spontaneous recovery group, resulting in a
total increase in cancellous bone mass. These results imply that
the cancellous bone gain induced by jump exercise during
remobilization is predominantly attributable to increases in Tb.Th with a slight increase in Tb.Sp by 179% in the secondary
spongiosa (2). These values are very similar in magnitude to
data reported in the present study using 3D micro-CT analysis
(−51%, −51%, −21%, and 58%, respectively). Our results
imply that the decrease in cancellous bone induced by skeletal
unloading is primarily due to trabecular disappearance (−51%) rather than by a thinning of trabeculae (−21%). This conclusion
is supported by a previous histomorphometric study (4).
Most studies investigating recovery potential after skeletal
unloading have not demonstrated complete restoration of bone
morphology (42, 49). In the present study, although the results of
dual-energy X-ray absorptiometry analysis indicated recovery
in total femoral BMD in spontaneous recovery rats after 5
wk of remobilization without jump exercise, micro-CT analy-

Table 2. BMD in the subregions of femur measured by DXA

<table>
<thead>
<tr>
<th></th>
<th>SCON (n = 7)</th>
<th>SUS (n = 7)</th>
<th>SCON + RCON (n = 7)</th>
<th>S + RCON (n = 7)</th>
<th>S + RUM (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 BMD, g/cm²</td>
<td>0.260±0.007</td>
<td>0.225±0.013</td>
<td>0.264±0.011</td>
<td>0.251±0.008</td>
<td>0.264±0.010</td>
</tr>
<tr>
<td>R2 BMD, g/cm²</td>
<td>0.242±0.007</td>
<td>0.188±0.020</td>
<td>0.221±0.020</td>
<td>0.205±0.009</td>
<td>0.230±0.016</td>
</tr>
<tr>
<td>R3 BMD, g/cm²</td>
<td>0.187±0.009</td>
<td>0.154±0.009</td>
<td>0.170±0.007</td>
<td>0.167±0.002</td>
<td>0.176±0.005</td>
</tr>
<tr>
<td>R4 BMD, g/cm²</td>
<td>0.234±0.008</td>
<td>0.110±0.011</td>
<td>0.221±0.009</td>
<td>0.212±0.004</td>
<td>0.229±0.006</td>
</tr>
<tr>
<td>R5 BMD, g/cm²</td>
<td>0.257±0.006</td>
<td>0.225±0.014</td>
<td>0.235±0.010</td>
<td>0.232±0.007</td>
<td>0.245±0.002</td>
</tr>
<tr>
<td>R6 BMD, g/cm²</td>
<td>0.260±0.011</td>
<td>0.229±0.009</td>
<td>0.246±0.007</td>
<td>0.230±0.008</td>
<td>0.263±0.011</td>
</tr>
<tr>
<td>R7 BMD, g/cm²</td>
<td>0.198±0.008</td>
<td>0.178±0.007</td>
<td>0.190±0.007</td>
<td>0.178±0.007</td>
<td>0.188±0.008</td>
</tr>
<tr>
<td>Total BMD, g/cm²</td>
<td>0.228±0.007</td>
<td>0.197±0.010</td>
<td>0.214±0.008</td>
<td>0.209±0.003</td>
<td>0.224±0.004</td>
</tr>
</tbody>
</table>

Values are means ± SD. Total femoral region was divided along the femoral long axis into 9 equal regions, and the 3 midregions were averaged, which resulted in 7 regions (R1 to R7 from proximal to distal). BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry. Significant difference vs. SCON group (post hoc test): aP < 0.05; bP < 0.01; cP < 0.001. Significant difference vs. S + RCON group (post hoc test): dP < 0.05; eP < 0.01; fP < 0.001. Significant difference vs. SUS group by Student’s t-test (P < 0.001).

Table 3. Trabecular bone parameters in distal femoral metaphysis

<table>
<thead>
<tr>
<th></th>
<th>SCON (n = 7)</th>
<th>SUS (n = 7)</th>
<th>SCON + RCON (n = 7)</th>
<th>S + RCON (n = 7)</th>
<th>S + RUM (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV, mm³</td>
<td>20.44±2.08</td>
<td>19.73±0.93</td>
<td>25.95±3.92</td>
<td>23.09±2.12</td>
<td>20.83±1.79</td>
</tr>
<tr>
<td>BV, mm³</td>
<td>7.08±1.44</td>
<td>3.38±1.59</td>
<td>6.76±2.30</td>
<td>4.05±1.04</td>
<td>6.02±1.02</td>
</tr>
<tr>
<td>BS, mm²</td>
<td>217.50±27.89</td>
<td>123.68±40.80</td>
<td>200.77±45.51</td>
<td>143.10±28.52</td>
<td>170.67±24.91</td>
</tr>
<tr>
<td>BS/BV, per mm</td>
<td>31.13±2.89</td>
<td>38.23±4.79f</td>
<td>30.54±3.01</td>
<td>35.82±2.12</td>
<td>28.43±1.34</td>
</tr>
<tr>
<td>BV/TV, %</td>
<td>34.70±6.66</td>
<td>17.02±7.73b</td>
<td>25.62±5.34</td>
<td>17.50±4.23</td>
<td>28.80±2.75</td>
</tr>
<tr>
<td>Tb.Th, μm</td>
<td>76.09±26.39</td>
<td>60.36±30.99</td>
<td>41.42±15.62</td>
<td>30.59±3.26</td>
<td>49.81±18.08</td>
</tr>
<tr>
<td>Tb.N, per mm</td>
<td>0.35±0.06</td>
<td>0.17±0.08b</td>
<td>0.24±0.05</td>
<td>0.19±0.05a</td>
<td>0.25±0.04a</td>
</tr>
<tr>
<td>Tb.Sp, μm</td>
<td>132.53±14.85</td>
<td>209.49±43.49a</td>
<td>160.02±11.38</td>
<td>185.13±16.73</td>
<td>161.43±6.50</td>
</tr>
<tr>
<td>β1/TV</td>
<td>121.69±20.75</td>
<td>55.20±7.20b</td>
<td>77.24±12.21</td>
<td>59.64±12.76</td>
<td>74.37±8.22</td>
</tr>
<tr>
<td>TBPf, per mm</td>
<td>3.66±2.11</td>
<td>11.52±5.29b</td>
<td>5.51±1.74</td>
<td>8.74±1.65</td>
<td>8.45±0.98</td>
</tr>
<tr>
<td>FD</td>
<td>2.45±0.05</td>
<td>2.27±0.09b</td>
<td>2.39±0.03</td>
<td>2.31±0.04c</td>
<td>2.38±0.02e</td>
</tr>
<tr>
<td>SMI</td>
<td>1.00±0.24</td>
<td>1.70±0.20b</td>
<td>1.24±0.16</td>
<td>1.52±0.14b</td>
<td>1.28±0.11b</td>
</tr>
<tr>
<td>DA</td>
<td>1.21±0.05</td>
<td>1.25±0.07</td>
<td>1.29±0.06</td>
<td>1.22±0.04</td>
<td>1.36±0.09</td>
</tr>
</tbody>
</table>

Values are means ± SD. TV, tissue volume; BV, bone volume; BS, bone surface; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; β1/TV, connectivity density; TBPf, trabecular bone pattern factor; FD, fractal dimension; SMI, structure model index; DA, degree of anisotropy. Significant difference vs. SCON + RCON group (post hoc test): aP < 0.05; bP < 0.01; cP < 0.001. Significant difference vs. S + RCON group (post hoc test): dP < 0.05; eP < 0.01; fP < 0.001. Significant difference vs. SUS group by Student’s t-test: gP < 0.01; hP < 0.001.
strated, and Tb.Th plays a particularly important role in bone strength (51). An increase in Tb.Th induced by jump exercise may thus have an intense positive effect on bone strength.

The loss of trabecular connectivity is known to be associated with a reduction in the physical strength of trabecular bone (43). In the present study, both TBPf and β1/TV indicated significant decreases in trabecular connectivity after tail suspension. In the distal femoral metaphysis of tail-suspended rats, after 5 wk of recovery without exercise, both TBPf and β1/TV had not fully recovered. Conversely, when jump exercise was applied during the recovery period, both TBPf and β1/TV returned to age-matched control rat values. Increases in trabecular connectivity are usually coupled with increases in bone strength (27). Moreover, some studies have shown that restoration of trabecular connectivity is important for strength recovery (18, 27). Changes in TBPf and β1/TV in the present study could thus have contributed to the beneficial effects of jump exercise on cancellous bone strength.

After 14-day tail suspension, SMI exhibited a significantly higher value compared with control rats, indicating a change in trabecular structure from plate-like to rod-like with tail suspension. These findings support the notion that disuse osteoporosis is associated with increased SMI, as found in neurectomized rats (19) and tail-suspended rats (6). Spontaneous recovery did not fully restore changes in the structural type of trabecular bone as shown by a significantly higher SMI compared with age-matched controls. Conversely, when jump exercise was applied during the recovery period, SMI recovered nearly to the level of age-matched control rats, suggesting a concomitant increase in mechanical strength.

Trabecular alignment is another important parameter contributing to the mechanical strength of bone (39). In the present study, DA, reflecting trabecular orientation, was unaffected by tail suspension. However, trabecular bone was oriented more heterotropically in jump-exercised rats than in age-matched control rats. In our previous study using running exercise, trabecular alignments remained unchanged after 10 wk of exercise in rats (21). These findings can be considered to represent quantitative verification of Wolff’s trajectorial theory of trabecular alignment (53).

Changes in body weight and muscle mass (force) may play important roles in the regulation of bone mass. Exercise usually induces body weight loss in male rats, whereas muscle weight is often unchanged (40). In the present study, both body weight and hind limb muscle mass decreased by tail suspension and recovered after 5 wk of remobilization to the same level as in age-matched control rats. Conversely, jump exercise during the remobilization period induced weight loss compared with controls, suggesting a negative effect on bone mass. Nevertheless, final BV was greater in S\textsubscript{JUM} rats than in S\textsubscript{CON} rats. These data could indicate that the recovery of BV observed in jump-exercised rats is derived primarily from the exercise stress itself.

Although it is impressive that the jump training was able to restore trabecular architecture, at the same time it may have endangered the fragile trabecular structure weakened by disuse. The ground reaction force on the lower leg with a 40-cm
jump in rats has been reported about five times of body weight (25). Thus jump training would cause a great mechanical stress on bone. Although in humans the jump training with the ground reaction force of 2.1–5.6 times of body weight effectively increased bone mass (12), its application to fragile bone should be further examined in the context of safety.

Several limitations of this study should be considered. First, we used young growing rats (5 wk old), because the effects of tail suspension in adult rats occur more slowly compared with young rats (1), and in humans and animals, growing subjects usually have greater potential for bone recovery after immobilization (37, 46). Therefore, although Umemura et al. (47) showed that the skeletal effects of jump training were at almost the same level among young (3 mo old) and adult rats (27 mo old), our results might differ quantitatively if adult rats were used in the tail-suspension model. Second, we did not measure braking force in cancellous bone per se at the distal femoral metaphysis. We interpreted the structural change in cancellous bone as being related to the increase in bone strength, since previous studies have demonstrated a positive relationship between bone strength and parameters such as Tb.Th, Tb.N, and connectivity. Finally, the rat is an established model for many aspects of human bone metabolism but displays some limitations in that cortical structure and bone-modeling patterns differ from those in human. Direct extrapolation of data from quadrupedal rats to bipedal humans is thus inappropriate. Nonetheless, the results obtained from the present study using 3D micro-CT analysis suggest the advantage of jump exercise in preventing the trabecular bone loss induced by immobilization and microgravity.

In summary, the results of 3D analysis in the present study demonstrated that suspension-induced trabecular deterioration persists after remobilization and jump exercise during the remobilization period could restore the integrity of trabecular architecture as well as bone mass at the femur in young growing rats.

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REFERENCES


