Effects of intervals between jumps or bouts on osteogenic response to loading

YOSHIHISA UMEMURA, NAOTA SOGO, AND AKIKO HONDA

Laboratory for Exercise Physiology and Biomechanics, School of Health and Sport Sciences, Chukyo University, Toyota 470-0393, Japan

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It is widely accepted that physical exercise increases bone mass and strengthens bone or that it maintains bone mass and strength against age-related bone loss (2, 11). This anabolic effect of exercise on bones is mainly due to mechanical stress elicited by physical action. During exercise, not only external forces (e.g., ground reaction force) but also internal forces (forces generated by muscle contraction) are imposed on the skeleton. It is thought that bones are strained by these forces and respond to these strains (5). Dynamic and high-magnitude loading, which elicits a high great strain rate in bones, is known to be effective for anabolic loading (10, 12, 13, 18, 22). Therefore, high-impact exercise is considered to be very beneficial for bones. In previous studies, our laboratory reported that jump exercise greatly increased bone mass and strength in rats (8, 23, 24). This was because high-impact loading elicited by ground reaction forces, as well as muscle contraction forces, was imposed on lower limb bones during jumping. In human studies, the effectiveness of high-impact exercise is also becoming widely accepted. In cross-sectional studies, it was reported that athletes doing high-impact exercise had greater bone mineral density than those who did low-impact exercise or nonathletes (4, 7). In longitudinal studies, it was also demonstrated that high-impact exercises increased bone mineral density (1, 6, 9).

However, it is not well documented how exercise protocols can be optimized to further the anabolic effect of high-impact loading. In animal studies, in loading protocols with one bout per day, loading with many repetitions (>40) had little additional effect on bones compared with loading with several repetitions (17, 24). From this result, it is considered that many loading repetitions make bones less sensitive to loading. Therefore, many repetitions per bout are not needed. Robling et al. (14, 15) reported that loading protocols consisting of a few bouts separated by several hours per day were more effective on bone than a protocol consisting of one bout per day in rats loaded by a bending device; nevertheless, the total number of loadings per day was the same between protocols. It is thought that mechanosensitivity recovers during bout intervals. This effectiveness of the interval between bouts was reported in 1-wk loading studies that were assessed by using the bone formation rate measured by fluorochrome labeling (14, 15), as well as in a 16-wk loading study that was assessed by bone mineral density and content (16). It is supposed that the effect of the bout interval on osteogenic response can be observed in high-impact exercise training. However, the number of studies on loading bouts per day is limited, so it is unclear whether the same results can be attained if the characteristics of loading (e.g., loading magnitude) are varied. Robling et al. (15) reported in another experiment that the interval between individual loadings in an identical bout could also enhance the anabolic effects on bone in rats. They showed that in rats loaded with one bout daily with 36 repetitions of loading for ~2 wk, a 14-s interval between individual loadings could enhance the bone formation rate compared with 0- to 7-s intervals. It is also supposed that the effect of the loading interval on osteogenic response...
can be observed in high-impact exercise training. However, the number of studies on the interval between individual loading is also limited. Therefore, it is unclear whether the same results can be attained if the loading characteristics are varied or if the loading period is longer than 2 wk.

In this study, we investigated these two interval effects on bone mass, strength, and cortical area at the midshaft of the femur and tibia in a rat jumping model as follows: first, when rats jumped 20 times per day, to assess whether the protocol with 2 bouts of 10 jumps separated by a 6-h interval was more effective on osteogenic response than a protocol with 1bout of 20 jumps; and second, when rats jumped 20 times per day in 1 bout, to assess whether a 30-s interval between individual jumps was more effective than a 3-s interval.

MATERIALS AND METHODS

Forty female Fisher 344 rats aged 4 wk were obtained from Japan SLC (Hamamatsu, Japan). The rats were individually housed in wire cages and provided with standard rat chow and water ad libitum throughout the experiment. All procedures were approved by the Animal Subjects Committee at Chukyo University Graduate School of Health and Sport Sciences.

Experimental design. After 1 wk of acclimatization, the rats were divided randomly into three exercise groups (n = 10/group) or one sedentary control group (n = 10). The rats in the three exercise groups were loaded with jumping of 20 repetitions per day, 5 days/wk, for 8 wk with different protocols. The first protocol was 20 jumps in a single bout with a 3-s interval between each jump (S3), the second was 20 jumps in a single bout with a 30-s interval between each jump (S30), and the third was 20 jumps in 2 bouts (10 × 2) separated by a 6-h interval with a 3-s interval between each jump (D3).

The jump-training procedure was reported in detail in previous studies (23, 24). Briefly, each rat in the jump exercise group was placed at the bottom of a special wooden box. The jumping exercise was initiated by electrical stimulation from the floor of the box, which was used less after a few exercise days because the rats became accustomed to the jump training. Each rat jumped from the floor of the box to the top edge of the box, reaching with its forepaws and climbed up. Each rat was rested during the jump interval and was returned to the floor of the box to repeat the procedure. The initial height of the box was 25 cm, and this was progressively increased from 40 cm up to the fourth week. All rats were allowed normal cage activity except for jumping bouts.

Bone mass, strength, and morphometry. After the 8-wk training period, the rats were anesthetized with diethyl ether and killed by exsanguination. The right femur and tibia were dissected and cleaned of soft tissue. Because the rats used both lower limbs in the same manner during the jumps, we sampled only one side. After the lengths were measured, the maximum breaking force at the midshaft of the bone was measured with a three-point bending test apparatus (model RX1600, I. Techno, Tokyo, Japan) as described in previous studies (8, 24). The distance between the two bottom supports at this fracture test was 16 mm, and the crosshead speed was 10 mm/min. After the fracture test, the femur and tibia were immersed in chloroform-methanol solution (2:1 vol/vol) for 1 wk, dried at 80°C for 24 h, and then weighed (fat-free dry weight). Then, the bones were restored with a bonding agent, embedded in polyester resin, and cut at the point of fracture. Finally, the cortical and medullary areas at midshaft were obtained from the cross-sectional surface of the cut piece.

RESULTS

The final body weight and the lengths of the femur and tibia were not significantly different among the groups (Table 1). The bone masses (fat-free dry weight) and the bone masses per body weight were greatest in the S30 group, although they were equally elevated in both the S3 and D3 groups compared with the control (Table 1).

The maximum breaking forces of the femur and tibia were also greatest in the S30 group, although they were equally elevated in both the S3 and D3 groups compared with the control (Table 2). The cortical areas of the femur and tibia were significantly greater in the three exercise groups than the control group, but there were few differences among the three exercise groups (Table 2). The medullary area of the femur was

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>S3</th>
<th>S30</th>
<th>D3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body wt, g</td>
<td>170 ± 9</td>
<td>165 ± 9</td>
<td>167 ± 6</td>
<td>161 ± 8</td>
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<tr>
<td>Bone length, mm</td>
<td></td>
<td></td>
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<tr>
<td>Femur</td>
<td>30.0 ± 0.4</td>
<td>30.1 ± 0.7</td>
<td>30.1 ± 0.4</td>
<td>29.7 ± 0.6</td>
</tr>
<tr>
<td>Tibia</td>
<td>34.4 ± 0.4</td>
<td>34.6 ± 0.5</td>
<td>34.7 ± 0.3</td>
<td>34.4 ± 0.6</td>
</tr>
<tr>
<td>Fat-free dry wt, mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femur</td>
<td>313 ± 16</td>
<td>343 ± 18*</td>
<td>359 ± 14†</td>
<td>339 ± 26*</td>
</tr>
<tr>
<td>Tibia</td>
<td>241 ± 10</td>
<td>273 ± 14*</td>
<td>284 ± 12†</td>
<td>270 ± 18*</td>
</tr>
<tr>
<td>Fat-free dry wt/body wt, %</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Femur</td>
<td>0.184 ± 0.010</td>
<td>0.208 ± 0.005*</td>
<td>0.215 ± 0.005†</td>
<td>0.210 ± 0.009*</td>
</tr>
<tr>
<td>Tibia</td>
<td>0.141 ± 0.005</td>
<td>0.165 ± 0.005*</td>
<td>0.171 ± 0.006†</td>
<td>0.167 ± 0.005*</td>
</tr>
</tbody>
</table>

Values are means ± SD for 10 animals in each group. Rats in the exercise group were trained with 20 jumps/day for 8 wk, in a single bout with a 3-s interval between jumps (S3) or with a 30-s interval between jumps (S30), or in 2 bouts (10 × 2) separated by a 6-h interval with a 3-s interval between jumps (D3). *Different from control, P < 0.01. †Different from D3, P < 0.05. ‡Different from S3, P < 0.05.
significantly greater in the two exercise groups (S3 and S30) than the control group. The medullary area of the tibia was not significantly different among the groups (Table 2).

DISCUSSION

The aims of this study were to determine whether the interval between jumps or bouts could enhance the anabolic effects of loading on bones in rats exercised by 20 jumps per day for 8 wk. We found that a 30-s interval between jumps in one bout could enhance the effect on the bone mass of lower limbs compared with a 3-s interval. However, a 6-h interval between two daily bouts did not enhance the effects on bone mass, strength, or cortical area at the midshaft. These data suggested that a longer interval (30 s) between individual loading was more effective for anabolic effects on bone than a shorter interval (3 s) and that two separated bouts (2 × 10 repetitions) were not more effective than a single bout (1 × 20 repetitions) daily.

It was revealed that loading with many repetitions at one time had little additional effect on bones compared with loading with 10–40 repetitions (17, 24). From this fact, it is thought that the mechanosensor loses sensitivity after several repetitions of constant loading and recovers after a low-loading (sedentary) interval. Robling et al. (15) assessed the bone formation rate measured by fluorochrome labeling in rat tibiae loaded by a four-point bending apparatus for ~1 wk. They reported that the intervals between bouts enhanced the anabolic effects of loading, and ~8 h of recovery were sufficient to restore full mechanosensitivity. In another study, they assessed bone mineral density and content in rat ulnae loaded by a longitudinal loading apparatus for 16 wk (16). They reported that a daily loading protocol with 4 bouts of 90 loading cycles (90 × 4), with each bout separated by 3 h, had a significantly greater effect on bone than a single, uninterrupted bout of 360 loading cycles. Despite these results, in our study we did not observe any differences in osteogenic response between a protocol with a daily single bout of 20 jumps and a protocol with 2 bouts of 10 jumps with a 6-h interval. One reason for the different results may be the magnitude of loading. Although we could not measure or predict the strain elicited by the jump exercise, we assumed that the strain or strain rate was greater than the preceding studies of Robling et al. (14–16), because we observed greater increases in the bone mass of the jumped rats (9.6 and 13.3% in the femur and tibia, respectively, in the daily 1-bout protocol for 8 wk) compared with the sedentary control, whereas, in the study of Robling et al. (16), the bone mineral content increase was ~7% in the ulna in a daily one-bout protocol for 16 wk. A high strain magnitude or strain rate may enhance the mechanosensors’ less sensitive period and require a long recovery period. Thus it was concluded that the 6-h interval could not result in recovery of mechanosensitivity in this study.

Another reason we did not find an interval effect between bouts could have been accommodation to loading. It is thought that bones do not respond further to certain loading after they have adapted to the magnitude of the loading and increased bone mass, because increased bone mass can diminish the bone strain imposed by the loading (5, 20). With rats that jumped one or two bouts per day, their bones may have accommodated to the magnitude of the loading over 8 wk. Cullen et al. (3) reported that, after 12 or 18 wk of constant loading, the bone formation rate decreased to the same level as that of a no-loading control group. An 8-wk period may have been enough to accommodate the bones to jump loading in this study, although why a 16-wk period was not enough for accommodation to loading in the study of Robling et al. (16) is not clear; the reason could possibly be a different loading magnitude or different loading frequency per week (5 days/wk in this study vs. 3 days/wk in the study of Robling et al.).

In contrast to the results for the bout interval, a 30-s interval between jumps enhanced the effect of loading. This result was supported by the study of Robling et al. (15), which assessed the bone formation rate measured by fluorochrome labeling in rats loaded for ~2 wk. From the results in this study, it is concluded that a 30-s interval between jumps improved the sensitivity to mechanical stress in rats. Why the interval between individual loadings can recover sensitivity after loading is not clear, but Robling et al. pointed out the possibility that extracellular fluid dynamics were related to the recovery. Recently, extracellular fluid dynamics have also been considered to be an important medium for transferring mechanical stress to bone.
mechanosensors (21). Srinivasan and Gross (19) reported in an analytic model that extracellular fluid velocity decreased to <60% in the second and subsequent cycles of loading compared with the first cycle. The 30-s interval between jumps might enhance the extracellular fluid dynamics and then the anabolic effect of loading. It was suggested that because the 30-s interval between jumps could enhance the sensitivity to each jump, the bones might sense that each jump was of a greater magnitude compared with the jump with the 3-s interval. Therefore, if the bones were accommodated to the jumps for 8 wk as in this study, the 30-s interval protocol could have large effects.

From the results of this study, it is suggested that an appropriate interval between individual loading is also important for the anabolic effect of loading on bones in humans. The loading during running and walking does not function as efficiently as the magnitude of loading, because the intervals between loading are too short. Jump exercise, resistance exercise training, and other forms of exercise that have appropriative intervals can function efficiently, but more studies are needed to assess whether several daily bouts of exercise with intervals of a few hours have additional effects compared with one daily bout of exercise.

Several limitations of this study should be considered. First, we used young rats aged 3 mo at the end of the exercise, so the results might differ if older rats are used. However, we observed that the effect of jump exercise on bone mass was at almost the same level among rats aged from 3 to 27 mo in a previous study (29). Therefore, we believe that the same results would be obtained in mature rats. Second, we could not measure the magnitude of loading. Although it was not confirmed that rats were loaded constantly, they jumped and hung from the top of the box in the same manner in <10% of failed jumps. If the S30 group jumped more vigorously than the S3 or D3 groups because of the long muscle recovery time, it could explain some of the results in this study. However, our laboratory data from other rats implied that there were not large differences in the ground reaction forces among rats aged from 3 to 27 mo in a previous study.

In conclusion, when rats were loaded by jumping with 20 repetitions per day for 8 wk, a 30-s interval between jumps enhanced the effect on the bone mass of the lower limbs compared with a 3-s interval. However, a 6-h interval between two daily bouts of 10 jumps did not enhance the effects on bone mass, strength, or cortical area at the midshaft. These data suggest that exercise protocols with an adequate loading interval to enhance the osteogenic response are more effective on bones than exercise protocols with a short loading interval, but more studies are needed to clarify whether several exercise bouts have additional effects compared with a single daily bout.

REFERENCES