J Bone Miner Metab (2008) 26:456–460
DOI 10.1007/s00774-007-0848-7

ORIGINAL ARTICLE

**Yoshihisa Umemura · Seigo Nagasawa · Akiko Honda
Rabindarjeet Singh**

High-impact exercise frequency per week or day for osteogenic response in rats

Received: August 21, 2007 / Accepted: December 28, 2007

Abstract The frequency per week or day of high-impact, low-repetition jump exercise for osteogenic response was assessed by two experiments. In the first experiment, 48 11-week-old rats were randomly divided into five groups: a sedentary control (W0: $n = 8$), one exercise session per week (W1: $n = 10$), three exercise sessions per week (W3: $n = 10$), five exercise sessions per week (W5: $n = 10$), and seven exercise sessions per week (W7: $n = 10$). In the second experiment, 30 11-week-old rats were randomly divided into three groups: a sedentary control (D0: $n = 10$), one exercise session per day (D1: $n = 10$), and two exercise sessions per day (D2: $n = 10$). One exercise session consisted of 10 continuous jumps. After 8 weeks of the exercise period, the jump exercise increased the fat-free dry weight of the tibia in the W1 (7.5%, n.s.), W3 (12.6%, $P < 0.01$), W5 (12.0%, $P < 0.01$), and W7 (19.8%, $P < 0.001$) groups compared with the W0 group. The jump exercise also increased the fat-free dry weight in the D1 (12.0%, $P < 0.001$) and D2 (13.0%, $P < 0.001$) groups compared with the D0 group. These increases were accompanied by increased bone strength and cortical area at the mid-shaft. The results in the present study suggest that for bone gain, it is not always necessary to do high-impact exercise every day, although exercising every day does have the greatest effect. The results in this study also suggest that there is little additional benefit if bones are loaded by two separate exercise sessions daily.

Key words mechanical loading · exercise frequency · mechano-sensitivity · high-impact low-repetition load · bone strength

Y. Umemura (✉) · S. Nagasawa · A. Honda
Laboratory for Exercise Physiology and Biomechanics, School of Health and Sport Sciences, Chukyo University, 101 Tokodachi, Kaizu-cho, Toyota, Aichi 470-0393, Japan
Tel. +81-565-46-1211; Fax +81-565-46-1272
e-mail: yumemura@sass.chukyo-u.ac.jp

R. Singh
Research Unit, National Institute of Sports, Malaysia

Introduction

Mechanical loading is known to play a key role in the change of bone mass and strength, where an adequate physical exercise program can promote bone development or maintain bone against age-related bone loss [1,2]. High-impact exercise is considered to be one of the effective exercises on osteogenic response, because of its intermittent dynamic loading that is known to be more effective on bones than static loading [3,4]. Moreover, the dynamic loading in high-impact exercise can produce a high magnitude strain and strain rate on bones, which are important factors for osteogenic response [5–8].

Jump exercise in rodents is considered to be a high-impact exercise model, because it gives large ground reaction forces accompanied with muscular contraction force to limb bones on landing [9–11]. We reported that many loadings per day were not needed in the high-impact exercise for bone development, because bone mass and strength increased with only five or ten sequential loadings per day in the rat jump exercise model [9]. Several studies also proved the effectiveness of high-impact, low-repetition exercises on bone mineral density in humans [12–14]. To our knowledge, however, there is not consistent agreement about the optimal high-impact low-repetition exercise frequency.

In this study, we investigated the relationship between loading frequency and osteogenic response using a jump exercise model in rats. For this purpose, we examined the effects of exercise frequency per day and per week.

Materials and methods

Animals

Ten-week-old female Wistar rats, weighing approximately 210 g (Japan SLC, Hamamatsu, Japan) were housed individually in standard cages (15 × 30 cm) on a 12 h light/dark cycle, with food (standard chow) and water ad libitum. The ambient temperature was 23°C, and the relative humidity was 55%. Because the jump exercise had similar effects on bones without regard to age [15], we used young adult rats in this study. After 1 week of acclimatization, the rats were weight matched, randomly assigned to various groups, and trained for 8 weeks. At the end of the experiment the rats were anesthetized with diethylether and killed by exsanguination. After scarification, the right tibia was dissected and all soft tissue was carefully removed for mechanical test, mass, and cross-sectional area analysis. The animal care and experimental protocol was approved by the Animal Subjects Committee of Chukyo University Graduate School of Health and Sport Sciences.

Exercise program

Two types of experiments were conducted. In the first experiment, to examine the effect of training frequency per week on osteogenic response, the rats were divided into four exercise groups and a sedentary group (W0; $n = 8$). The rats in the exercise groups were trained by the jump exercise once (W1; $n = 10$), three times (W3; $n = 10$), five times (W5; $n = 10$) or seven times (W7; $n = 10$) per week. The W1 group was trained every 7th day, the W3 group was trained every 2 or 3 days, the W5 group was trained every 1 or 2 days, and the W7 group was trained every day. All rats, including the control rats, were handled every day to equalize the handling stress. All rats were allowed normal cage activity.

In the second experiment, to examine the effect of training frequency per day, the rats were divided into two exercise groups and a sedentary group (D0; $n = 10$). The rats in the exercise groups were trained by jump exercise once (D1; $n = 10$) or twice (D2; $n = 10$) per day. The D1 group was trained in the morning, 5 days/week, which was the same frequency as our previous studies [9,10,15], and the D2 group was trained in the morning and afternoon, with a 6-h interval between training, 5 days/week. All rats, including the control rats, were handled twice a day, 5 days/week. All rats were allowed normal cage activity.

Each session of jump exercise consisted of ten continuous jumps that took less than 1 min using a previously described protocol [9–11]. Briefly, each rat in the exercise group was placed at the bottom of a special cage, and jumped when an electrical current was applied to the floor of the box. The rats jumped from the floor to catch the top edge of box, which was 45 cm high. The electrical current was used less over time, because rats learned to jump before the current was applied.

Mechanical testing procedures

After the length of the right tibiae was measured with a sliding caliper, the maximum load at the breaking point of the bones was measured with a three-point bending test (RX1600; I. Techno, Tokyo, Japan). Tests were conducted in a lateral-to-medial direction at midlength. The distance between the bottom support was 16 mm, and the crosshead speed was 10 mm/min.

Bone mass and cross-sectional area

After the fracture test, the tibiae were immersed in solvent (2 vol chloroform combined with 1 vol methanol) for 1 week and then dried at 80°C for 24 h, and later weighed (fat-free dry weight). The bones were then embedded in polyester resin (Rigolac 2004; Okenshoji, Japan) by submersion at room temperature for 3 days after restoration with a bonding agent. The midshaft cross section of each bone was then obtained, which was cut near the site of the fracture. The cross sections were photographed and enlarged. A digitizing pad was used to determine the medullary and cortical areas, and the endosteal and periosteal perimeters. Then, moment of inertia and bending stress were calculated.

Statistical procedures

Analyses of variance (ANOVA) was used to reveal the differences among groups. When the ANOVA revealed a significant difference, post hoc comparisons (Tukey's honestly significant difference test) were used to determine the differences between specific means. All data are reported as means and standard deviations. A significance level of $P < 0.05$ was used for all statistical tests.

Results

Body weight and bone length

Jump exercise did not affect the body weight in the weekly exercise groups and in the daily exercise groups (Table 1). Jump exercise also did not affect the length of the tibia (Table 1).

Bone mass and bone strength

Data for the fat-free dry weight of the tibia and the maximum load at the fracture test are presented in Figs. 1 and 2. In the weekly exercise group, the fat-free dry weight and the maximum load were greater in all exercise groups than sedentary groups, although the difference in the fat-free dry weight between W0 and W1 did not reach statistical significance. W7 had greatest fat-free dry weight and maximum load among all groups, and differences between W7 and some other exercise groups were significant. In the daily exercise group, the fat-free dry weight and the maximum load were also greater in two exercise groups than in the sedentary groups. They were almost the same in the D1 and D2 groups, although the maximum load of the D2 was slightly higher than that of D1.

Table 1. Body weight and bone length of the tibia

	Weekly exercise study					Daily exercise study		
	W0 ($n = 8$)	W1 ($n = 10$)	W3 ($n = 10$)	W5 ($n = 10$)	W7 ($n = 10$)	D0 ($n = 10$)	D1 ($n = 10$)	D2 ($n = 10$)
Body weight (g)	259 ± 12	267 ± 15	267 ± 17	267 ± 24	273 ± 12	272 ± 14	273 ± 20	276 ± 17
Length of Tibia (mm)	38.0 ± 0.7	38.2 ± 0.5	38.5 ± 0.7	38.3 ± 0.9	38.6 ± 0.7	37.7 ± 0.4	38.3 ± 0.5	38.2 ± 0.7

All values are mean ± SD
W0, sedentary; W1, one session per week; W3, three sessions per week; W5, five sessions per week; W7, seven sessions per week; D0, sedentary; D1, one session per day; D2, two sessions per day
There were no significant differences between groups

Table 2. Cross-sectional analyses and mechanical properties of the tibia

	Weekly exercise study					Daily exercise study		
	W0 ($n = 8$)	W1 ($n = 10$)	W3 ($n = 10$)	W5 ($n = 10$)	W7 ($n = 10$)	D0 ($n = 10$)	D1 ($n = 10$)	D2 ($n = 10$)
Cortical area (mm^2)	3.18 ± 0.51	3.92 ± 0.46*	4.33 ± 0.42*	4.46 ± 0.46*	4.67 ± 0.43*,†	3.52 ± 0.33	3.98 ± 0.34*	4.28 ± 0.29*
Periosteal perimeter (mm)	8.11 ± 0.74	8.88 ± 0.56*	9.00 ± 0.38*	8.99 ± 0.46*	9.31 ± 0.53*	8.94 ± 0.50	9.41 ± 0.31*	9.47 ± 0.31*
Endosteal perimeter (mm)	4.33 ± 0.32	4.65 ± 0.38	4.60 ± 0.53	4.42 ± 0.30	4.60 ± 0.47	5.07 ± 0.35	5.39 ± 0.40	5.21 ± 0.41
Moment of inertia (mm^4)	1.21 ± 0.32	1.83 ± 0.37*	2.10 ± 0.36*	2.13 ± 0.44*	2.25 ± 0.41*	1.67 ± 0.34	2.12 ± 0.26*	2.24 ± 0.21*
Bending stress ($N{\cdot}mm^2$)	256 ± 55	229 ± 34	210 ± 30	224 ± 37	225 ± 29	211 ± 52	212 ± 33	200 ± 11

All values are mean ± SD
W0, sedentary; W1, one session per week; W3, three sessions per week; W5, five sessions per week; W7, seven sessions per week; D0, sedentary; D1, one session per day; D2, two sessions per day
* Significantly different from each sedentary group; † significantly different from the W1 group

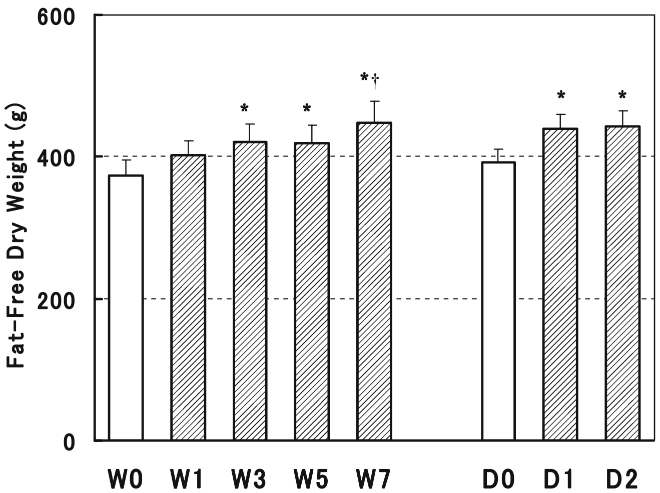

Fig. 1. Effect of exercise frequency per week or per day on fat-free dry weight of tibia. Data are shown as mean ± SD. *W0*, sedentary; *W1*, one session per week; *W3*, three sessions per week; *W5*, five sessions per week; *W7*, seven sessions per week; *D0*, sedentary; *D1*, one session per day; *D2*, two sessions per day. *, significantly different from each sedentary group; †, significantly different from the W1 group

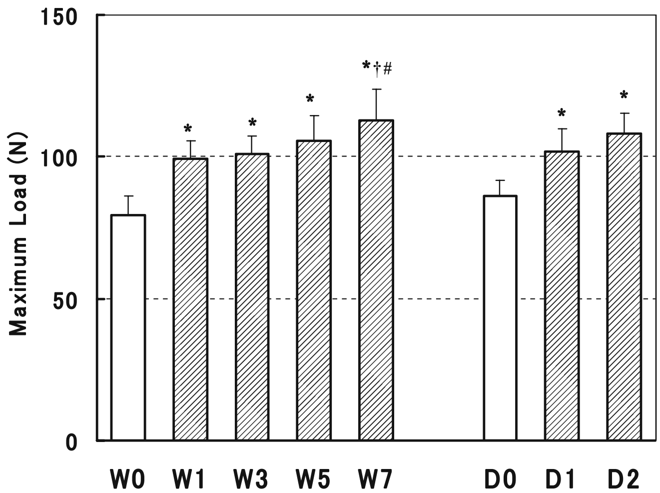

Fig. 2. Effect of exercise frequency per week or per day on maximum load of tibial midshaft. Data are shown as mean ± SD. *W0*, sedentary; *W1*, one session per week; *W3*, three sessions per week; *W5*, five sessions per week; *W7*, seven sessions per week; *D0*, sedentary; *D1*, one session per day; *D2*, two sessions per day. *, significantly different from each sedentary group; †, #, significantly different from the W1 and W3 group, respectively

Cross-sectional analyses and mechanical properties

Data for the cross-sectional analyses are presented in Table 2. The cortical area, periosteal perimeter and moment of inertia were significantly greater in all exercise groups than their respective sedentary groups. In the daily exercise groups, there were no significant differences between the D1 and D2 in these variables. In the weekly exercise groups, W7 had significantly greater cortical area than W1. There were no significant differences among all groups in the endosteal perimeter or bending stress.

Discussion

The results of this study showed that when rats were jump-exercised for ten jumps per session daily in an exercise program, it was more effective on bone mass and strength than jump training once or a few times per week, even though the once a week exercise program also showed a significant osteogenic response. On the other hand, the twice a day exercise program had little additional benefit.

It is known that only a few high-impact mechanical loadings are sufficient for osteogenic response in the daily exercise program [9,16]. In the rat jump model, the differences in the effects on bone mass and strength were not large between the 100 jumps per day protocol and the 10 jumps per day protocol [9]. Similarly, Rubin et al. [16] reported that 1800 loadings per day did not have greater effect on bone mineral content than 36 loadings per day in their loading mode. The relationship between bone mass gain and loading number per day was almost approximated by a logarithmic curve [17]. It is thought that bones have a mechano-sensor that detects bone strains and response to strain magnitude [18,19]. It is thought that many loadings at once diminishes returns because the mechano-sensor decreases sensitivity according to the number of the sequential loadings, and that the sensitivity will recover gradually in a no-loading period [20,21]. For effective loading protocol on bone gain, we need to consider the recovery of the mechano-sensitivity.

We reported in our previous study [10] that when daily jump number was the same (20 jumps), the daily two-session program with a 6-h interval (10 jumps × 2) did not enhance the effect on bone mass and strength compared to the daily one-session program (20 jumps × 1). In this study, we also observed that the daily two-session program (10 jumps × 2) did not enhance significantly the effect on bone mass and strength when compared to the daily one-session program (10 jumps × 1), although in the daily two-session program the rats jumped twice as much as the rats in the one-session program. In contrast to these results, Robling et al. [22,23] reported that when the total daily loading cycles were the same (360 cycles), four daily sessions with a 3-h interval program (60 cycles × 4) were more effective on bone mineral content (BMC) and bone strength than the daily one-session program (360 cycles × 1), where they attempted a 16-week loading experiment using their compressive loading model in the rat ulna. They described that sensitivity of bones to the mechano-stress decreased after continual loadings and recovered after an adequate no-loading interval period, that is, 3 h in their studies [22,23]. In our study, however, the mechano-sensitivity, which decreased by the continual 10 jumps, might not recover in 6 h after the first session of exercise.

The reason for this different result between Robling et al. [22,23] and our study may be the differences of loading magnitude. In our jumping exercise model, the 8 weeks loading increased bone mass about 20% as compared with the no-loaded control, whereas in Robling's loading model, the 16 weeks loading increased BMC about 11%. Thus, the jump exercise may generate great bone strain or strain rate, although we have not measured the bone strain imposed by the jump exercise. In high-impact low-repetition loading, such as jumping in rats, a few hours may not be sufficient for the recovery of the mechano-sensitivity. In our jump training, rats jumped by themselves and were deposited softly by our hands on the bottom of the box. Thus, the effective load was generated only at the take-off of jumping and was not generated at the touching-down to the floor in this study, although Welch et al. [24] reported that the free-fall impact had positive effects on bones.

In the frequency per week exercise groups, we observed that the weekly one-session program had a significant potency to osteogenic response, and as the frequency of training days per week increased, the response was greater. Hagihara et al. [25] reported that exercise more than 4 days per week increased tibial bone mineral density. Their result was consistent with our results, although they did not try less frequent exercise programs. It is interesting to note that once a week (W1) jump-exercise increased bone mass by 7.5%, bone strength by 25.1%, and cortical area by 23.3% when compared to the sedentary control (W0) values, where the rats jumped only 10 times per session to a total of 80 jumps (10 times × 8 weeks) throughout the duration of the experiment. From these results, it can be pointed out that low-frequency exercise may have meaningful effect on osteogenic response. It was supported by the studies that a few loading sessions had a meaningful potency to influence active bone formation [26–28]. On the other hand, 24 h may not be sufficient for the full recovery of the mechano-sensitivity, because differences in the effects on bone mass and strength were not large between W5 and W7.

The effects of the jump exercise were observed in bone mass, strength, and cortical area of the midshaft of the tibia but were not observed in bending stress. This result was consistent with our previous study [9] and implied that the jump exercise evoked quantitative changes rather than qualitative changes. The increased rates of bone strength and cortical area of the midshaft were greater than that of the bone mass in all training groups. These results are consistent with previous studies [22,23] in which the loading enhanced bone strength greatly with little gain in bone mass. In other words, the loading strengthens bone efficiently with little bone mass gained that attaches to the adequate area. For this reason, exercise may have an important potential to prevent bone fracture

In conclusion, the results in the present study suggest that daily high-impact loading is more effective for bone mass and strength than loading once or a few times per week, although loading once a week can have positive effects. The results in this study also suggest that there is little additional benefit if bones are loaded by two separate sessions daily.

References

1. Berard A, Bravo G, Gauthier P (1997) Meta-analysis of the effectiveness of physical activity for the prevention of bone loss in postmenopausal women. Osteoporos Int 7:331–337
2. Wallace BA, Cumming RG (2000) Systematic review of randomized trials of the effect of exercise on bone mass in pre- and postmenopausal women. Calcif Tissue Int 67:10–18
3. Lanyon LE, Rubin CT (1984) Static vs. dynamic loads as an influence on bone remodelling. J Biomech 17:897–905

4. Perren SM, Huggler A, Russenberger M, Allgower M, Mathys R, Schenk R, Willenegger H, Muller ME (1969) The reaction of cortical bone to compression. Acta Orthop Scand 125(suppl):19–29
5. Rubin CT, Lanyon LE (1985) Regulation of bone mass by mechanical strain magnitude. Calcif Tissue Int 37:411–417
6. Turner CH, Owan I, Takano Y (1995) Mechanotransduction in bone: role of strain rate. Am J Physiol 269(3 pt 1):E438–E442
7. Mosley JR, March BM, Lynch J, Lanyon LE (1997) Strain magnitude related changes in whole bone architecture in growing rats. Bone (NY) 20:191–198
8. Mosley JR, Lanyon LE (1998) Strain rate as a controlling influence on adaptive modeling in response to dynamic loading of the ulna in growing male rats. Bone (NY) 23:313–318
9. Umemura Y, Ishiko T, Yamauchi T, Kurono M, Mashiko S (1997) Five jumps per day increase bone mass and breaking force in rats. J Bone Miner Res 12:1480–1485
10. Umemura, Y, Sogo N, Honda A (2002) Effects of intervals between jumps or bouts on osteogenic response to loading. J Appl Physiol 93:1345–1348
11. Umemura Y, Baylink DJ, Wergedal JE, Mohan S, Srivastava AK (2002) A time course of bone response to jump exercise in C57BL/6J mice. J Bone Miner Metab 20:209–215
12. McKay HA, MacLean L, Petit M, MacKelvie-O'Brien K, Janssen P, Beck T, Khan KM (2005) "Bounce at the Bell": a novel program of short bouts of exercise improves proximal femur bone mass in early pubertal children. Br J Sports Med 39:521–526
13. Kato T, Terashima T, Yamashita T, Hatanaka Y, Honda A, Umemura Y (2006) Effect of low-repetition jump training on bone mineral density in young women. J Appl Physiol 100:839–843
14. Macdonald HM, Kontulainen SA, Khan KM, McKay HA (2007) Is a school-based physical activity intervention effective for increasing tibial bone strength in boys and girls? J Bone Miner Res 22:434–446
15. Umemura Y, Ishiko T, Tsujimoto H, Miura H, Mokushi N, Suzuki H (1995) Effects of jump training on bone hypertrophy in young and old rats. Int J Sports Med 16:364–367
16. Rubin CT, Lanyon LE (1984) Regulation of bone formation by applied dynamic loads. Bone Joint Surg [Am] 66:397–402
17. Turner CH (1998) Three rules for bone adaptation to mechanical stimuli. Bone (NY) 23:399–407
18. Frost HM (1987) Bone "mass" and the "mechanostat": a proposal. Anat Rec 219:1–9
19. Frost HM (1997) On our age-related bone loss: insights from a new paradigm. J Bone Miner Res 12:1539–1546
20. Robling AG, Burr DB, Turner CH (2000) Partitioning a daily mechanical stimulus into discrete loading bouts improves the osteogenic response to loading. J Bone Miner Res 15:1596–1602
21. Robling AG, Burr DB, Turner CH (2001) Recovery periods restore mechanosensitivity to dynamically loaded bone. J Exp Biol 204:3389–3399
22. Robling AG, Hinant FM, Burr DB, Turner CH (2002) Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. J Bone Miner Res 17:1545–1554
23. Robling AG, Hinant FM, Burr DB, Turner CH (2002) Shorter, more frequent mechanical loading sessions enhance bone mass. Med Sci Sports Exerc 34:196–202
24. Welch JM, Weaver CM, Turner CH (2004) Adaptations to free-fall impact are different in the shafts and bone ends of rat forelimbs. J Appl Physiol 97:1859–1865
25. Hagihara Y, Fukuda S, Goto S, Iida H, Yamazaki M, Moriya H (2005) How many days per week should rats undergo running exercise to increase BMD? J Bone Miner Metab 23:289–294
26. Pead MJ, Skerry TM, Lanyon LE (1988) Direct transformation from quiescence to bone formation in the adult periosteum following a single brief period of bone loading. J Bone Miner Res 3:647–656
27. Forwood MR, Turner CH (1994) The response of rat tibiae to incremental bouts of mechanical loading: a quantum concept for bone formation. Bone (NY) 15:603–609
28. Chow JW, Jagger CJ, Chambers TJ (1993) Characterization of osteogenic response to mechanical stimulation in cancellous bone of rat caudal vertebrae. Am J Physiol 265(2 pt 1):E340–E347