

## Running Exercise for Short Duration Increases Bone Mineral Density of Loaded Long Bones in Young Growing Rats

Yoshinobu Hagihara,<sup>1\*</sup> Arata Nakajima,<sup>1\*</sup> Satoshi Fukuda,<sup>2</sup> Sumio Goto,<sup>1\*</sup> Haruzo Iida<sup>2</sup> and Masashi Yamazaki<sup>1</sup>

<sup>1</sup>Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine, Chiba, Japan

<sup>2</sup>International Space Radiation Laboratory, National Institute of Radiological Sciences, Chiba, Japan

Running exercise is an effective therapy for the prevention of osteoporosis; however, appropriate duration of exercise has not been determined. We therefore investigated the effect of exercise duration on bone mineral density (BMD) and systemic bone metabolism using young growing rats. Fifteen 8-week-old female Wistar rats were divided into three groups according to running load: control group (no running), short duration (30 min/day) and long duration (180 min/day), and animals ran on a treadmill 5 days per week over an 8-week period. BMD of the tibia was measured using peripheral quantitative computed tomography, and serum levels of tartrate-resistant acid phosphatase (TRAP), a bone resorption marker and alkaline phosphatase (ALP), a bone formation marker were measured to know whether the treadmill exercise would affect systemic bone metabolism. Short-duration running exercise (30 min/day) caused a significant increase in BMD of the metaphyseal trabecula ( $p < 0.05$ ) with a reduction of serum TRAP levels ( $p < 0.01$ ) and an increase in serum levels of calcium ( $p < 0.05$ ) and phosphorus ( $p < 0.01$ ). Conversely, long-duration exercise (180 min/day) significantly reduced BMD of the diaphyseal and metaphyseal cortex and that of the diaphyseal trabecula with a significant reduction of serum ALP levels and a significant increase in serum phosphorus. These findings suggest that short-duration exercise may increase BMD through suppression of bone resorption, whereas long-duration exercise may reduce BMD through suppression of bone formation. Exercising for short duration but not prolonged exercise is recommended to increase BMD of loaded long bones. ——— running exercise; duration; bone mineral density; bone formation; bone resorption.

Tohoku J. Exp. Med., 2009, **219** (2), 139-143. © 2009 Tohoku University Medical Press

Current strategies for the prevention of osteoporosis focus on maximizing the accumulation of bone mass early in life during growth and maturation, and minimizing bone loss later in life (Kulak and Bilezikian 1998; Rozenberg et al. 1999; Iwamoto et al. 2009). Achieving the maximum bone mass at skeletal maturity is the best protection against osteoporotic fractures (Matkovic et al. 1990; Iwamoto et al. 2009). Because physical activity during childhood and adolescence may be one of the most important determinants of peak bone mass, exercise should be emphasized during this period (Iwamoto et al. 2009).

Several studies have demonstrated that moderate exercise in the pre-menopausal period, including puberty, increases bone mineral density (BMD) (Myburgh et al. 1990, 1993; Prior et al. 1990; Welten et al. 1994; Friedlander et al. 1995; Goto et al. 1995); however, excessive exercise in this period reduces BMD (Yingling et al. 2001; Barry and Kohrt 2008). Using young growing rats,

we previously verified the effects of moderate exercise in the form of running on a treadmill at a speed of 15 m/min for 30 min per day, over an 8-week period, on BMD of the long bones. We determined that the appropriate exercise frequency to increase BMD of the long bones was 4 to 5 days per week (Hagihara et al. 2005). However, the optimal combination of exercise frequency and duration to maximize BMD has not been established.

The purpose of the present study was to examine the effects of running for short (30 min) and long (180 min) durations on BMD of the loaded long bones and bone metabolic markers in young growing rats. These rats were subject to loading of a moderate magnitude (treadmill running at a speed of 15 m/min) over an 8-week period.

---

Received August 4, 2009; revision accepted for publication August 20, 2009. doi:10.1620/tjem.219.139

\*Present Addresses: Y.H., Department of Orthopaedic Surgery, Joto Social Insurance Hospital, Tokyo; A.N., Department of Orthopaedic Surgery, Chiba Aoba Municipal Hospital, Chiba; S.G., Waku Orthopaedic Clinic, Chiba.

Correspondence: Masashi Yamazaki, M.D., Ph.D., Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.

e-mail: masashiy@faculty.chiba-u.jp

Table 1. Mean body weights of rats at the start and end of the experiment, and weight gained during the experimental period.

Group	Start (g)	End (g)	Weight gained (g)
Control	212.0 ± 11.40	263.0 ± 16.14	66.8 ± 6.14
SD	214.6 ± 8.65	267.4 ± 16.94	68.4 ± 9.40
LD	210.4 ± 11.95	258.2 ± 13.41	62.2 ± 6.98

SD, short duration; LD, long duration.

## Materials and Methods

### Animals and housing conditions

Fifteen 6-week-old female Wistar rats, with a mean weight of 156.8 g, were purchased. Animals were housed five to a cage and kept in a room with a temperature of 22 ± 2°C; relative humidity, 55 ± 5%; and a 12-h dark and light cycle. They were allowed free access to drinking water and commercial solid food (BM-1; Funabashi Farm, Funabashi, Japan) containing 1.2% calcium.

### Running exercise and procedures

A treadmill device (Shinano, Tokyo, Japan) was used to provide a running load to the rats. Rats were pre-exercised for 2 weeks to habituate them to the device and to determine suitable running conditions, as described later. The rats were then divided into the following groups: control (no running); short duration (30 min/day) load (SD); long duration (180 min/day) load (LD). Each group consisted of five animals. The running load was initiated when the rats were 8 weeks old. The running conditions were determined according to our previous publications (Hagihara et al. 2000, 2005). All rats ran at a treadmill speed of 15 m/min, a speed at which they ran voluntarily and did not drop out. Animals ran 5 days per week (running on Monday through Wednesday and Friday through Saturday, resting on Sunday and Thursday), over a period of 8 weeks. This study was approved by the ethics committee of Chiba University.

### Measured items

Body weights and food intakes of the rats were measured every day before and after running to check for untoward findings arising from the load. To determine whether the treadmill load affected the 4-day estrous cycle of the rats, vaginal smears were collected at 11 : 00 a.m. daily for 5 days in the first and third weeks, and for 10 consecutive days from the sixth week of the experiment. The collected vaginal smears were stained with Giemsa, and cells were observed under a light microscope.

The rats were killed under anesthesia using chloroform. The blood was collected from the posterior vena cava after celiotomy and centrifuged at 3,000 rpm. The serum was frozen and stored at -20°C. Concentrations of total serum calcium (Ca), phosphorus (P), and tartrate-resistant acid phosphatase (TRAP) were determined by the o-cresol phthalein complexone (OCPC), molybdenum blue, and p-nitrophenylphosphate (PNPP) substrate method, respectively.

Tibiae were collected and fixed in 70% ethanol. Measurements of BMD and the bone cross-sectional area were performed using peripheral quantitative computed tomography (pQCT) (voxel: 148 mm, 18.9 mm; XCT-960A) (Norland and Stratec, Germany). In this system, values of less than 395 and more than 690 mg/cm<sup>3</sup> are regarded as trabecular BMD and cortical BMD, respectively. The trabecular BMD was measured at a position 3 mm from the center of the carti-

Table 2. Concentration of calcium and phosphorus in the serum.

Group	Ca (mg/dl)	P (mg/dl)
Control	10.58 ± 0.48	9.06 ± 0.87
SD	13.80 ± 2.22*	11.48 ± 1.15**
LD	11.58 ± 1.87	10.92 ± 1.13*

SD, short duration; LD, long duration.

\*Significantly different from controls,  $p < 0.05$ ; \*\* $p < 0.01$ .

Table 3. Concentration of TRAP and ALP in the serum.

Group	TRAP (IU/ml)	ALP (IU/ml)
Control	39.04 ± 5.91	359.0 ± 60.8
SD	26.16 ± 3.12**	331.4 ± 64.5
LD	31.98 ± 6.71	250.6 ± 56.7*

SD, short duration; LD, long duration.

\*Significantly different from controls,  $p < 0.05$ ; \*\* $p < 0.01$ .

lage plate image in the proximal epiphysis of the tibia. Cortical BMD was measured 12 mm away from the respective cartilage plate.

### Statistical analyses

Differences between groups were determined by an ANOVA. Where differences existed, the Fisher protected least significant difference test was used to determine significance. A value of  $p < 0.05$  was considered statistically significant. All values were expressed as means ± s.d.

## Results

There were no significant differences in food intake between groups. Also, there were no alterations or differences in the estrous cycle among the groups. The increase in body weight of group SD was higher than that of the other two groups, but no significant differences were seen among the groups (Table 1).

Regarding serum biochemistry, there was a significant increase in serum calcium values in group SD, and in serum phosphorus values in groups SD and LD compared to the control group (Table 2). The ALP level, a bone formation marker, was significantly decreased in group LD, but not altered in group SD (Table 3). It was of great interest that the TRAP level, a bone resorption marker, was significantly reduced in group SD (Table 3).

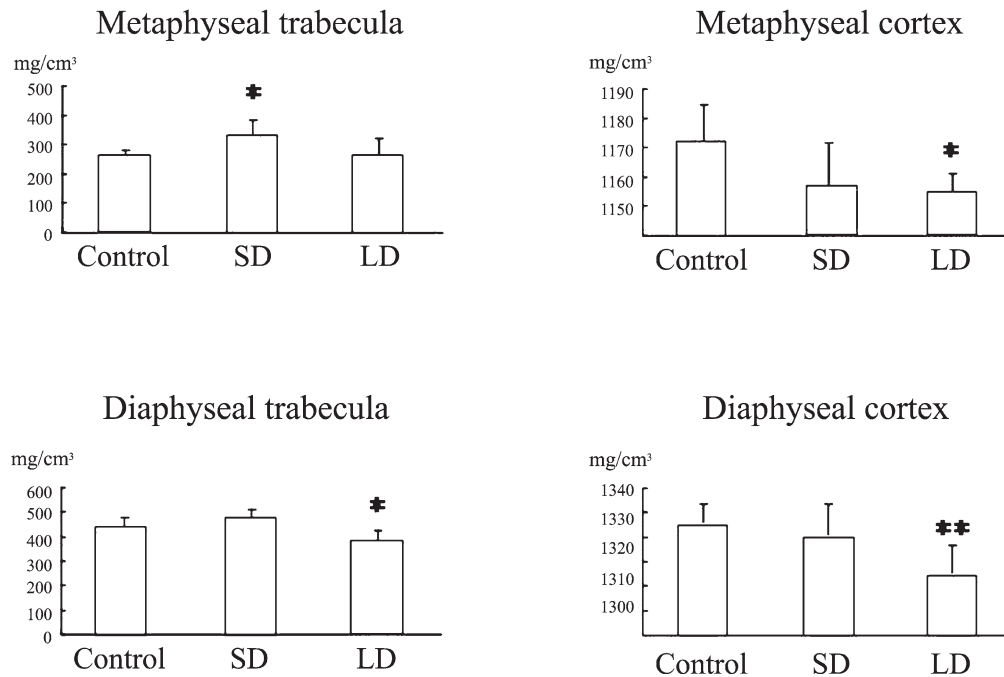


Fig. 1. BMD of the metaphyseal trabeculae and cortex of the tibia, and that of the diaphyseal trabeculae and cortex. Thirty-minute exercise periods (short duration) significantly increased BMD of the metaphyseal trabecula compared to the control (no running) group. In contrast, 180-minute periods (long duration) significantly reduced BMD of the diaphyseal and metaphyseal cortex, and that of the diaphyseal trabecula compared to controls. \*Significantly different from controls,  $p < 0.05$ ; \*\* $p < 0.01$ . SD, short duration; LD, long duration.

Most importantly, BMD of the metaphyseal trabecula of the tibia was significantly increased in group SD, but no significant differences were noted between group LD and the control group (Fig. 1). BMD of the metaphyseal cortex was lower in groups SD and LD than in the control group; however, the reduction was only significant in group LD (Fig. 1).

In contrast, BMD of the diaphyseal trabecula was not altered between group SD and the control group, while it was reduced significantly in group LD (Fig. 1). Similarly, there were no significant differences in BMD of the diaphyseal cortex between group SD and the control group, but there was a significant decrease in that of group LD (Fig. 1).

### Discussion

In the present study, we examined the effect of exercise duration on BMD of the tibia. Running for a short duration significantly decreased TRAP levels in the serum, leading to a significant increase in BMD of the metaphyseal trabecula. Conversely, extended running significantly reduced serum ALP levels, resulting in a significant decrease in BMD of the metaphyseal cortex and that of the diaphyseal trabecula and cortex.

Several studies investigating the relationship between exercise and BMD have shown that moderate exercise increases BMD, while excessive exercise such as marathon running induces a decrease in BMD (Myburgh et al. 1990, 1993; Prior et al. 1990; Welten et al. 1994; Friedlander et al. 1995; Goto et al. 1995). Although we did not precisely

define optimum running conditions for maximizing BMD, we did demonstrate clearly that running for a short duration (30 min/day) was more effective at increasing BMD of the metaphyseal trabecula of the tibia than running for a longer duration (180 min/day). Previously, we found no changes in adrenal gland weights after rats were subjected to a running exercise load of 15 m/min (Fukuda et al. 2002), the speed used in the present study. In our prior study, the 8-week-old male rats were run for 90 min/day for 35 days and we inferred that this regimen did not subject the rats to significant stress. Thus the speed of 15 m/min was considered to be a moderate load for the 8-week-old rats used in the present study. Though the durations tested were different from the prior study, we considered the overall stress to which the animals were exposed to be similar to the previous study.

It is of great significance that running for a short duration increased BMD of the metaphyseal trabecula but not BMD of the cortical bone. We previously reported that BMD of the metaphyseal trabecula in rat tibiae subjected to a moderate running load was significantly higher than that of controls. There were no significant differences in BMD of the diaphyseal cortex (Hagihara et al. 2005), consistent with the results presented here. Iwamoto et al. (2000) previously demonstrated that treadmill exercise significantly increased proximal tibial cancellous bone volume but not tibial shaft cortical bone area in young growing rats, supporting our results that showed the differential effect of running exercise on the trabecular and cortical bone.

In this study, running for a short duration increased BMD of the metaphyseal trabecula of the tibia and decreased serum TRAP levels but did not alter the ALP levels. This suggests that exercising for a short duration may have a negative effect on bone catabolism, with no significant effects on bone anabolism. Recently, Iwamoto et al. (2005) showed that treadmill exercise increases cortical and cancellous bone mass of the tibia as a result of increased bone formation and decreased bone resorption in young and adult rats. In addition, they revealed that treadmill exercise prevents cancellous bone loss at the tibia as a result of suppressed bone resorption, and increases bone mass of the tibia and mechanical strength of the femur as a result of suppressed bone resorption and increased bone formation in osteopenic rats after ovariectomy (Iwamoto et al. 2005). Although neither short nor long duration exercise increased serum ALP levels in the present study, these findings, at least in part, support our results. Given that the mechanism by which the BMD was increased was due to inhibition of bone resorption and not promotion of bone formation, running may have more of an effect on trabecular bones than cortical bones because trabecular bones contain more osteoclasts.

Also noteworthy was the observation that prolonged exercise significantly diminished BMD both for trabecular and cortical bone in the tibial diaphysis. Considering that prolonged exercise significantly reduced serum ALP levels, it seems conceivable that the BMD would be reduced more significantly in trabeculae than cortices because trabeculae contain more osteoblasts; however, BMD of the cortex (both metaphyseal and diaphyseal) was significantly reduced. We speculate two possible explanations for this inconsistency. Like trabecular osteoblasts, periosteal osteoprogenitor cells are considered to have high levels of ALP activity in young growing rats (Mizuno et al. 2006; Park et al. 2007). Thus, it is possible that periosteal osteoprogenitor cells rather than trabecular osteoblasts were affected more strongly by the long duration exercise-induced decrease in bone formation, resulting in significant loss of BMD of the cortex. Another is the issue that we may not be able to simply correlate decrease in serum ALP levels with decrease in bone formation because we did not measure bone-specific ALP. To establish mechanisms by which long duration exercise reduced BMD of the cortex, analyses for bone-specific markers and dynamic parameters by bone histomorphometry will be necessary.

Exercise has an effect on calciotropic hormones. In rats, it promotes a positive calcium balance and increases skeletal mass, largely as a result of an increase in 1,25-dihydroxyvitamin D<sub>3</sub> and enhancement of intestinal calcium absorption (Yeh et al. 1989; Yeh and Aloia 1990). Consistent with this observation, in the present study animals exercised for a short period exhibited a significant increase in serum calcium levels; however, those run for a prolonged duration showed no significant hormonal changes. This suggests that running for a short period increases

serum 1,25-dihydroxyvitamin D<sub>3</sub> levels and intestinal absorption of calcium, which in turn, decreases absorption of calcium from bone, resulting in inhibition of bone resorption. Iwamoto et al. (2004) investigated effects of treadmill exercise on calciotropic hormones in young growing rats, and demonstrated that exercise increases the serum 1,25-dihydroxyvitamin D<sub>3</sub> level and decreases the serum parathyroid hormone level, resulting in an increase in bone mass with stimulation of longitudinal bone growth, especially at weight bearing sites. Although the serum calcium level was not altered in exercised rats and controls in their study, these results appear to support our results and explanation to why short duration exercise-induced decrease in serum calcium levels lead to the decrease in bone resorption.

A limitation of the present study is the lack of bone (including both trabecular and cortical bones) histomorphometric analyses. Therefore, the alternations in local bone formation and bone resorption, cellular activities and bone architecture in the tibia remain uncertain. Further studies are essential to establish the effects of exercise duration on these dynamic parameters in loaded long bones such as the tibia.

In conclusion, short duration exercise increased BMD of the tibia while prolonged exercise did not. The mechanism by which exercise for a short duration increases BMD may be inhibition of systemic bone resorption. Prolonged exercise may reduce BMD through suppression of systemic bone formation. To increase the peak bone mass during the pre-menopausal period exercise duration must be neither too short nor too long. The optimal duration and load in humans, however, remains to be established.

## References

- Barry, D.W. & Kohrt, W.M. (2008) BMD decreases over the course of a year in competitive male cyclists. *J. Bone Miner. Res.*, **23**, 484-491.
- Friedlander, A.L., Genant, H.K., Sadowsky, S., Byl, N.N. & Gluer, C.C. (1995) A 2-year program of aerobics and weight training enhances bone mineral density of young women. *J. Bone Miner. Res.*, **10**, 574-585.
- Fukuda, S., Iida, H., Hyakutake, S. & Yan, X. (2002) Preventive effects of running exercise on bones in heavy ion particle irradiated rats. *J. Radiat. Res.*, **43** (suppl), S233-238.
- Goto, S., Hyakutake, S. & Shigeta, H. (1995) Significance and effects of athletics for prevention of osteoporosis (in Japanese). *Osteoporosis Japan*, **3**, 110-118.
- Hagihara, Y., Fukuda, S., Goto, S., Hasegawa, S., Iida, H., Yamagata, M., Saisu, T. & Moriya, H. (2000) Effects of continuous running exercise on bone mineral density in young rats (in Japanese). *Osteoporosis Japan*, **8**, 220-223.
- 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.
- Iwamoto, J., Sato, Y., Takeda, T. & Matsumoto, H. (2009) Role of sport and exercise in the maintenance of female bone health. *J. Bone Miner. Metab.*, Apr. **7**. (Epub ahead of print).
- Iwamoto, J., Shimamura, C., Takeda, T., Abe, H., Ichimura, S., Sato, Y. & Toyama, Y. (2004) Effects of treadmill exercise on bone mass, bone metabolism, and calciotropic hormones in young growing rats. *J. Bone Miner. Metab.*, **22**, 26-31.

- Iwamoto, J., Takeda, T. & Sato, Y. (2005) Effect of treadmill exercise on bone mass in female rats. *Exp. Anim.*, **54**, 1-6.
- Iwamoto, J., Yeh, J.K. & Aloia, J.F. (2000) Effect of deconditioning on cortical and cancellous bone growth in the exercise trained young rats. *J. Bone Miner. Res.*, **15**, 1842-1849.
- Kulak, C.A. & Bilezikian, J.P. (1998) Osteoporosis: preventive strategies. *Int. J. Fertil. Womens Med.*, **43**, 56-64.
- Matkovic, V., Fontana, D., Tominac, C., Goel, P. & Chesnut, C.H. III (1990) Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. *Am. J. Clin. Nutr.*, **52**, 878-888.
- Mizuno, H., Hata, K., Kojima, K., Bonassar, L.J., Vacanti, C.A. & Ueda, M. (2006) A novel approach to regenerating periodontal tissue by grafting autologous cultured periosteum. *Tissue Eng.*, **12**, 1227-1335.
- Myburgh, K.H., Bachrach, L.K., Lewis, B., Kent, K. & Marcus, R. (1993) Low bone mineral density at axial and appendicular sites in amenorrheic athletes. *Med. Sci. Sports Exerc.*, **25**, 1197-1202.
- Myburgh, K.H., Hutchins, J., Fataar, A.B., Hough, S.F. & Noakes, T.D. (1990) Low bone density is an etiologic factor for stress fracture in athletes. *Ann. Intern. Med.*, **113**, 754-759.
- Park, B.W., Hah, Y.S., Kim, D.R., Kim, J.R. & Byun, J.H. (2007) Osteogenic phenotypes and mineralization of cultured human periosteal-derived cells. *Arch. Oral Biol.*, **52**, 983-989.
- Prior, J.C., Vigna, Y.M., Schechter, M.T. & Burgess, A.E. (1990) Spinal bone loss and ovulatory disturbances. *N. Engl. J. Med.*, **323**, 1221-1227.
- Rozenberg, S., Vandromme, J., Ayata, N.B., Filippidis, M. & Kroll, M. (1999) Osteoporosis management. *Int. J. Fertil. Womens Med.*, **44**, 241-249.
- Welten, D.C., Kemper, H.C.G., Post, G.B., Mechelen, W.V., Twisk, J., Lips, P. & Teule, G.J. (1994) Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. *J. Bone Miner. Res.*, **9**, 1089-1096.
- Yeh, J.K. & Aloia, J.F. (1990) Effects of physical activity on calciotropic hormones and calcium balance in rats. *Am. J. Physiol.*, **258**, E263-268.
- Yeh, J.K., Aloia, J.F. & Yasumura, S. (1989) Effect of physical activity on calcium and phosphorus metabolism in the rat. *Am. J. Physiol.*, **256**, E1-6.
- Yingling, V.R., Davies, S. & Silva, M.J. (2001) The effects of repetitive physiologic loading on bone turnover and mechanical properties in adult female and male rats. *Calcif. Tissue Int.*, **68**, 235-239.
-