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Original Article

Effects of Swimming and Weight Loading on Bone Density and Mechanical Properties of the Mouse Femoral Bone

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Abstract

Effects of swimming and weight loading on femoral bone density and mechanical properties of the bone were investigated. ICR 8-week old female mice were divided into a swimming, weight-loading and control groups consisting 10 mice in each group. All mice were fed a standard diet and water *ad libitum* during the entire experiment. Body weight was significantly lower in the swimming group ($p < 0.05$) and the weight-loading group ($p < 0.05$) than in the control group, when the mean values were compared. The level of bone density was significantly higher ($p < 0.05$) in the weight-loading group than in the control group. As for the mechanical properties of the femur, there were no significant differences in the level of maximum breaking force, ultimate stress and elasticity between both the exercise groups and the control. Deformation, however, was significantly higher in both the exercise groups than in the control group. No significant difference in serum calcitonin was found between both the exercise groups and the control group. However, parathyroid hormone level had a tendency to be higher in the weight-loading group than in the control group. These results suggest that the effect of exercise on bone was stronger in weight loading than in swimming.

Key words: Swimming, Weight loading, Bone density, Mechanical properties of bone, Mice

Introduction

Osteoporosis is a result of a reduction in the bone mass caused by resorption in excess of bone formation over a long period. The incidence of osteoporosis has increased rapidly in Japan with the increase in the aged population [1]. Since osteoporosis is a cause of poor QOL in aged individuals as well as of an increase in the medical expenditure [1-3], its prevention is an important issue.

Exercise is reported to be effective for prevention of osteoporosis, because it increases the bone density or reduces its age-associated decrease [4-6]. However, the effect of exercise is unequal according to the kind of exercise [7,8] and the bone type [8,9]. Effects of exercise on bone are considered to be derived from compression stimulation by weight loading and from stimulation by muscle contraction, and the variability of the effect of exercise is considered to be due to differences in the force (stress) exerted on the bone. Therefore, evaluation of the effects of weight loading and muscle contraction on bone may contribute to prevention of osteoporosis.

Mechanical properties of bone, which may reflect its supportive function, are affected by the bone mass [10], and are important factors in prevention of osteoporosis as is bone density. However, evaluation of the effects of exercise on mechanical properties of bone has not been sufficient.

In this study, effects of exercise in the growth period on the bone were evaluated in 3 groups of mice, namely, a swimming group, a weight-loading group, and a control group. Two-limb weight-loading limb amputation in limb-amputated models [9] and application of a weight jacket [11] have been used for controlled weight loading, but these methods are excessively invasive or stressful. In this study, therefore, weight loading was achieved simply by attaching a weight to the tail. Swimming increases stimulation of muscle contraction without weight loading while weight loading increases the weight load of the animals. Effects of these stimuli on bone density and mechanical properties of the femur were evaluated in these animals.

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Methods

1. Animals and their rearing

Thirty 7-week-old female ICR mice were purchased from Nisseizai Center (Tokyo) and were housed in an artificial climate room adjusted to a temperature of 25 ± 0.50 ℃ and a humidity of 60 ± 5 % with 12-hour light and dark cycles (7:00-19:00 light period). Food (CE-2; Nihon Clea, Tokyo) and water (distilled water) were given *ad libitum*. After acclimation for 1 week, the animals were divided into a swimming group (10 animals), a weight-loading group (10 animals), and a control group (10 animals) at the age of 8 weeks. The bone growth in mice is known to be completed at about the age of 15 weeks[12]. In this study, the animals were observed for 7 weeks until the age of 15 weeks to evaluate the effects of exercise in the growing period. In the swimming group, the animals were made to swim (water temperature 30 ℃) for 40 minutes a day from 10:00 am 5 days a week for 7 weeks. In the weight-loading group, a weight of about 6 g was attached to the base of the tail, and the animals were reared in this state for 7 weeks. No forced exercise or weight loading was imposed on the control group.

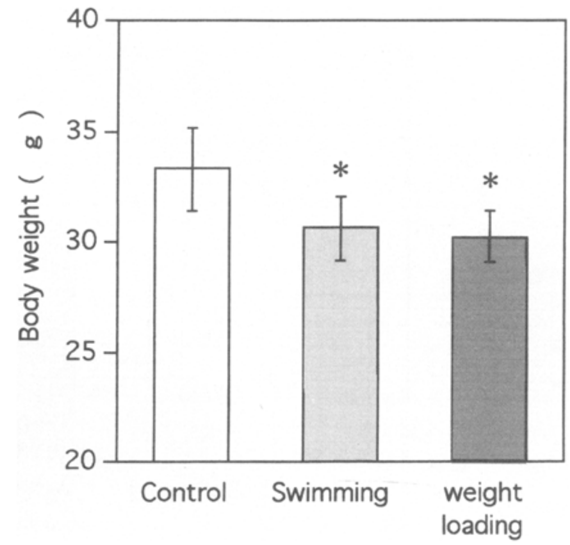

Fig 1. Body weight of each group after experiment
Values are mean ± SD
* $p < 0.05$ Significant difference vs control

2. Items and methods of measurements

1) *Bone density*

At the end of the 7-week rearing period, animals of all groups were sacrificed by cervical dislocation after blood sampling under ether anesthesia. The right femur was excised, soft tissues were detached from the bone, and lateral soft X-ray images of the femur were obtained by exposure for 1 minute at 40 kV, 5 mA, and 45 cm film-focus distance. The bone density and cortical thickness index (CTI) in the center of the femur were measured[13] in the soft X-ray films using a microdensitometer (PDS-15; Konica, Tokyo). This method is referred to as the MD method in this report.

2) *Mechanical properties of the bone*

The maximum breaking force, ultimate stress, deformation, and elasticity were measured by the three-point bending test of the femur[14] using an autograph (Shimadzu, Tokyo). The test was performed by adjusting the position of the femur so that the plunger would work at the center of the anterior surface of the bone. The interfulcral distance was 5 mm, and the bending rate was 1 mm/min.

3) *Blood hormone levels*

Serum was separated immediately from fresh whole blood and stored at -80 ℃ until measurement of C-terminal parathyroid hormone (C-PTH) and calcitonin (CT) levels. A radioimmunoassay kit (Eiken Kagaku, Tokyo) was used for the measurements.

Statistical analyses were performed using the Halbau statistical package. Following one-way analysis of variance, inter-group comparisons were made by Scheffe's method at the 5 % level of significance.

Results

1. Body weight

Figure 1 shows the body weight of the animals at the end of the study. It was 30.6 ± 1.5 g (mean ± standard deviation) in the swimming group and 30.2 ± 1.2 g in the weight-loading group, both significantly lower ($p < 0.05$) than the 33.3 ± 1.9 g in the control group.

2. Bone density

Figure 2 shows the bone density and CTI measured by the MD method. The bone density was 0.69 ± 0.08 in the swimming group, 0.71 ± 0.05 in the weight-loading group, and 0.63 ± 0.07 in the control group, with a significant difference between the weight-loading group and the control group (Fig. 2A). However, CTI was 0.34 ± 0.03 in the control group, 0.32 ± 0.03 in the swimming group, and 0.32 ± 0.03 in the weight-loading group, with no significant difference among the groups (Fig. 2B).

3. Mechanical properties of the femur

Figure 3 shows the mechanical properties of the femur. The maximum breaking force, or the force that caused breaking of the bone, was 2.6 ± 0.5 kg in the control group, 2.8 ± 0.3 kg in the swimming group, and 2.8 ± 0.3 kg in the weight-loading group (Fig. 3A). The ultimate stress was 1.5 ± 0.3, 1.5 ± 0.2 and 1.5 ± 0.2 kg/mm^2, respectively (Fig. 3B), and the elasticity was 55.2 ± 5.3, 50.9 ± 9.1, and 55.1 ± 10.0 kg/mm^2, respectively (Fig. 3C). No significant difference was observed in the maximum breaking force, ultimate stress, or elasticity among the three groups. However, deformation was 0.56 ± 0.12 mm in the swimming group and 0.46 ± 0.08 mm in the weight-loading group, significantly greater ($p < 0.05$) in both groups than the 0.30 ± 0.15 mm in the control group (Fig. 3D).

4. Blood hormone levels

The C-PTH level was 0.834 ± 0.287 ngEq/ml in the control group, 0.743 ± 0.189 ngEq/ml in the swimming group, and 1.026 ± 0.319 ngEq/ml in the weight-loading group, with no significant difference among the three groups although it was higher in the weight-loading group ($F = 2.553$, $0.1 > p > 0.05$)(Fig. 4A). The CT level was 101.4 ± 31.2, 84.1 ± 36.5, and 76.3 ± 22.4 pg/ml, respectively, with no significant difference among the groups (Fig. 4B).

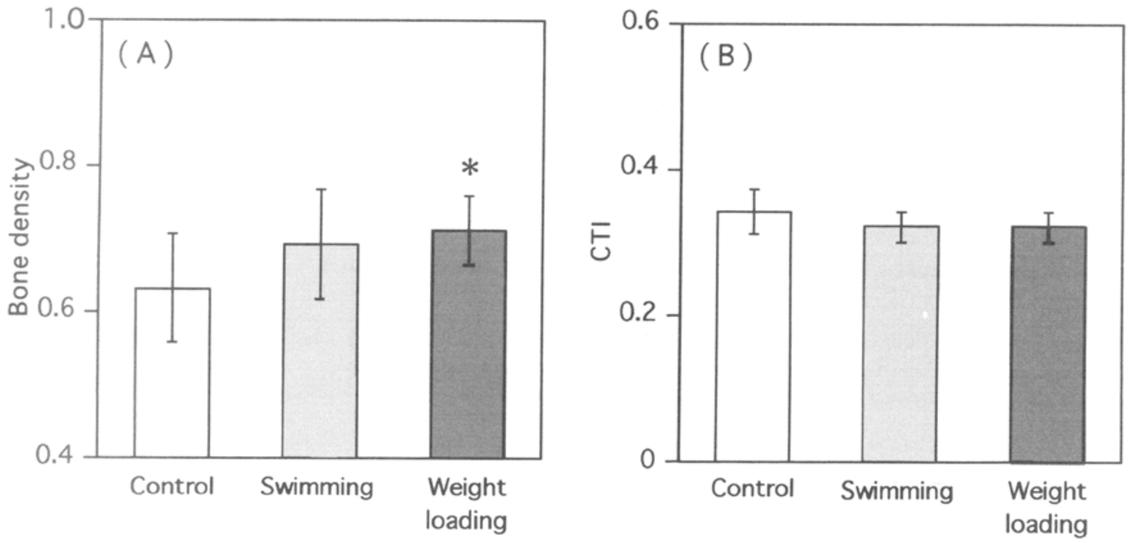

Fig 2. Bone density and CTI measured by microdensitometric examination (MD method) in each group
Values are mean ± SD
* $p < 0.05$ Significant difference vs control
CTI: Cortical Thickness Index

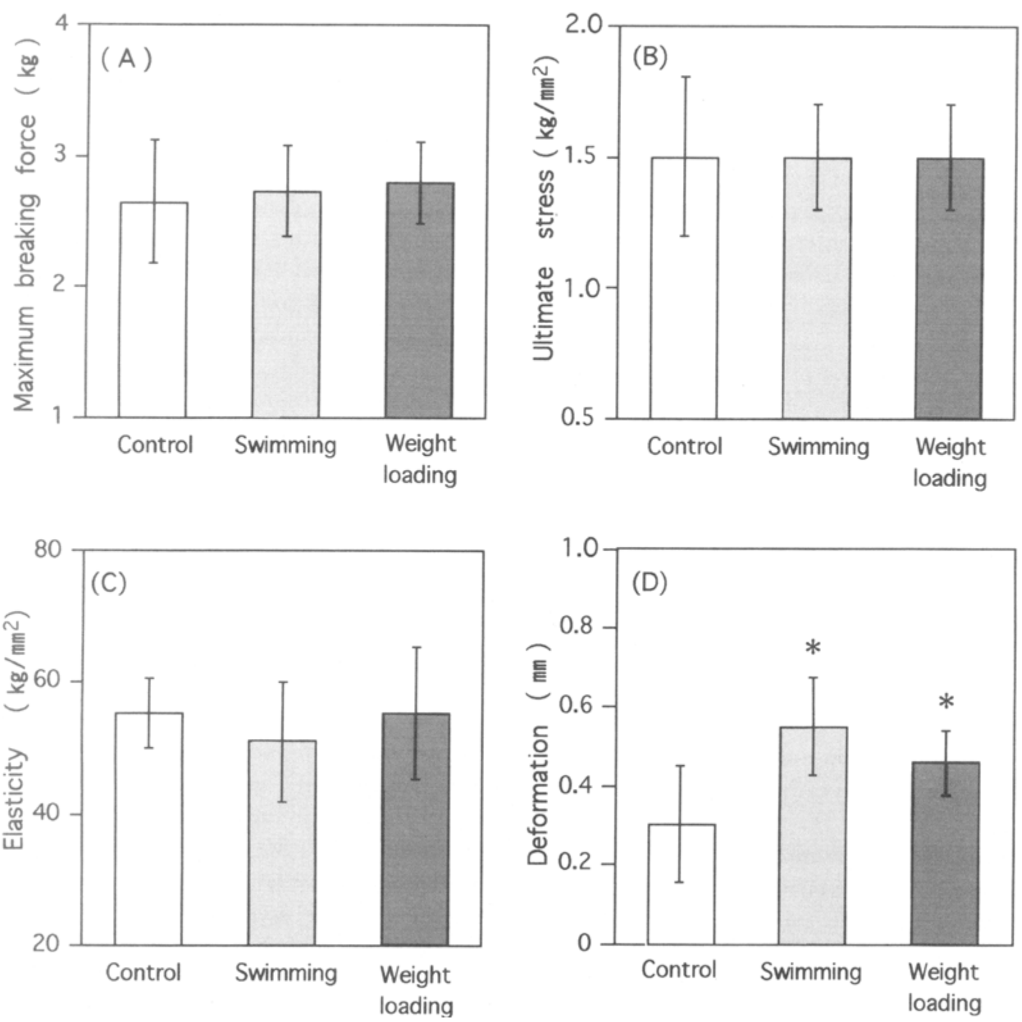

Fig 3. Mechanical properties of femoral bone in each group
Values are mean ± SD
* $p < 0.05$ Significant difference vs control

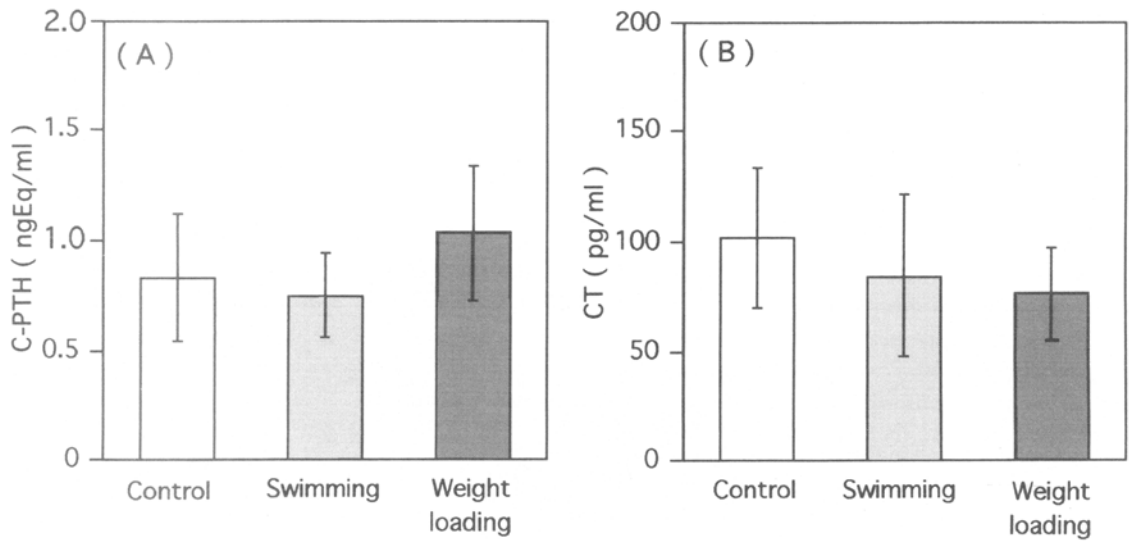

Fig 4. Calcium regulating hormones, C-PTH and CT, in each group
Values are mean ± SD

Discussion

Exercise is widely known to reduce body weight gain[15]. In this study, also, the body weight was significantly lower in the swimming group ($p < 0.05$) and weight-loading group ($p < 0.05$) than in the control group, indicating that both swimming and weight loading suppress body weight gain.

To increase the peak bone mass in is suggested as a preventive measure against osteoporosis[16], and exercise in the growing period has been shown to increase this[4, 17, 18]. However, in mice and rats that were exercised or those that were fore limb-amputated for exclusive hind-limb weight loading, the bone density increased to a greater degree in the weight-bearing limbs than in non-weight-bearing-limbs[9, 18]. In humans, also, weight lifting is more effective than swimming for increasing the peak bone mass[7, 8], and the effects of exercise are reported to vary with the kind of exercise and the bone examined. In this study, the femur bone mass was significantly increased ($p < 0.05$) only in the weight-loading group, and no clear difference was observed in the swimming group, which is considered to primarily reflect the effects of muscle contraction, as compared with the control group.

The mechanism of the increase in bone density has not been fully elucidated. However, compression stimulation caused by weight loading during exercise is considered to induce a piezo electric effect in the bone, attract Ca ions, and increase the intraosseous blood flow. These changes together are considered to activate osteoblasts and to increase bone density[19, 20]. While compression stimulation and an increase in the blood flow are expected in the weight-loading group, no compression stimulation is present in the swimming group, and this difference may explain the difference in the results. In addition, whereas animals in the swimming group swam only for 40 minutes a day 5 days a week, the weight-loading group always carried the weight attached to the tail, equivalent to about 20 % of body weight, except during resting periods. This difference in the intensity and duration of loading may have affected the results of the study. This is supported by a report that body weight is correlated with bone density[21]. Moreover, food intake was not measured in this study, but the higher bone density in the weight-loading group may have been related to a higher food intake. In consideration of the intensity and duration of loading in the swimming group and weight-loading group, a greater force may be exerted on the bone by weight loading than by swimming, and the differences in the results between the two groups may be ascribed to the differences in the force applied to the bone, although this observation is still speculative. Since bone resorption and bone formation occur in the internal and external periosteum in long tubular bones, CTI serves as an index of the bone mass. There was no difference in CTI despite the increase in the bone density in the weight-loading group as compared with the control group. The short study period of 7 weeks may have been insufficient to cause appreciable changes in CTI.

Mechanical properties of bone, which reflect its supportive function, are affected by the bone mass and trabeculae[10]. Therefore, the high bone density in the weight-loading group may have been related to the mechanical properties of the femur. Concerning effects of exercise, Saville and Smith[22] reported increases in the bone density and breaking force in rats after 80-day two-limb weight loading, and Asami et al.[23] noted an increase in the maximum breaking force in rats allowed to have spontaneous exericise over 170 days. However, Shirasaki et al.[24] found that tan δ, which is a parameter of viscoelasticity, was increased in rats that swam for 1 hour daily 6 days a week for 33 weeks but that the density and mechanical properties of the bone were reduced in those that ran 2.3 km per day on a treadmill 6 days a weeks for 33 weeks as compared with the control group. The results have, thus, varied among investigators. One of the reasons for such a discrepancy is as follows. Appropriate exercise activates osteoblasts, increases bone density, and consequently improves the mechanical properties of the bone. However, excessive exercise may activate osteoclasts more than osteoblasts, causing a decrease in the bone density. Also, excessive running is a cause of fatigue fracture[25]. These findings suggest that excessive exercise causes mechanical properties of the bone to deteriorate. In this study, no differences were observed in the maximum breaking force, which represents the breaking point of the bone, ultimate stress, a parameter of bone strength, or elasticity, which represents the degree of bending, among the three groups. Since no difference was observed in CTI either, the exercise in this study may not have affected the maximum breaking force and other mechanical properties. However, as the deformity, which represents the degree of bone destruction, was high in both the swimming and weight-loading groups, the bone in these groups may have been more resistant to fracture.

Bone is affected by hormones involved in regulation of the Ca metabolism. PTH and CT, which are Ca-regulating hormones, stabilize the blood Ca concentration and thus regulate bone metabolism[26]. However, the blood PTH concentration is reported to be increased by exercise[27]. When PTH increases due to exercise, it induces bone resorption, and bone formation that follows is considered to increase the bone density[20]. In this study, no difference was observed in CT among the groups, but C-PTH tended to be higher in the weight-loading group. Therefore, bone resorption and bone formation appear to have been more active in the weight-loading group than in the control group and to have contributed to the high bone density in this group.

These observations suggest that the effect of weight loading on the femur is greater than that of muscle contraction although the results may vary with the weight load, duration of swimming and training period. Therefore, the results of this study in mice suggest that both swimming and weight loading are effective but that exercise involving weight bearing such as running is more effective than exercise primarily dependent on muscle contraction such as swimming.

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