Effects of Exercise at Different Ages on Bone Density and Mechanical Properties of Femoral Bone of Aged Mice

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Hoshi, A., Watanabe, H., Chiba, M. and Inaba, Y. *Effects of Exercise at Different Ages on Bone Density and Mechanical Properties of Femoral Bone of Aged Mice.* Tohoku J. Exp. Med., 1998, 185 (1), 15-24 —— We determined the bone density and mechanical properties of bone specimens from 5 groups of aged mice which had been subjected to voluntary exercise at different ages. ICR 10-week-old female mice were divided into control (C), and exercise-trained during age periods of 10-70 weeks (EE), 10-30 weeks (GPE), 30-50 weeks (MPE) and 50-70 weeks (APE). It was found that in the exercise-trained groups body weight gain was suppressed during the exercise-training period, and that de-training accelerated weight gain. Bone density was significantly higher in all the exercise-trained groups than in the C group and cortical thickness index (CTI) was higher in the exercise-trained groups, except for the APE group. Maximum breaking force, ultimate stress and elasticity in the exercise-trained groups were higher than in the C group except for the APE group, whereas deformation in the APE group had a tendency to be higher than in the other groups. Blood C-terminal para-thyroid hormone, calcitonin and calcium concentrations were similar among every group, but phosphorus concentrations tended to be higher in the exercise-trained groups than in the C group. These observations suggest that exercise-training at every age suppresses age-associated bone loss, and that the effect of exercise during youth is greater than that during old age. The results of this study suggest that the effect of exercise on bone at an older age is different from that at other ages. —————— bone density; mechanical properties of the bone; Ca metabolism; voluntary exercise © 1998 Tohoku University Medical Press

Osteoporosis is one of the metabolic diseases in which reduction of bone mass is caused by resorption in excess of bone formation over a long period. Bone mass is reduced to a level at which the risk of fractures in increased. The incidence of senile osteoporosis has been rapidly increasing in Japan due to the increase in the

aged population (Orimo 1996). It is becoming a serious health problem facing the aged population and its prevention is an important issue today.

It is generally known that bone mass increases during the growth period, reaches a peak during the maturity period and then decreases with age (Matsushita et al. 1986; Mazess 1987). Bone mass in aged adults may decrease rapidly with unfit physical condition and/or life style. Exercise is reported to be effective for prevention of senile osteoporosis in humans (Aloia et al. 1978; Jacobson et al. 1984; Blalir et al. 1992) and in animals (Bourrin et al. 1992; Nishida et al. 1992), because it increases the bone mass or reduces its age-associated decrease. It has been reported that bone of aged animals responds to mechanical stimulation less efficiently than that of young animals (Rubin et al. 1992). However, we found that low-intensity exercise such as swimming was sufficient to improve bone density in aged mice, but that it had little effect on young mice (Hoshi et al. 1996, 1998). Therefore, it is considered that exercise in the maturity and in the aged period, as well as exercise during the growth period can contribute to prevention of senile osteoporosis. The optimal period of training age for prevention of senile osteoporosis is still not clear.

In this study we observed the effects of exercise at different ages on the bone density and mechanical properties of femoral bone of aged mice.

MATERIALS AND METHODS

Treatment of animals

Fifty 9-week-old female ICR mice were purchased from Nippon Biosupply Center (Tokyo) and were kept in a climatic chamber maintained at $23 \pm 0.5°C$ and $60 \pm 5\%$ relative humidity on 12-hour light and dark cycle with the light period starting at 7 : 00 a.m. In this study, after one week of habituation, the mice aged 10 weeks and weighing 29.5 ± 1.7 g (mean $\pm$ s.d.) were randomly divided into 5 groups: (1) no exercise for the entire experiment (control, C); (2) exercise-trained during the 10–70 weeks age period (entire experiment exercise group, EE); (3) exercise-trained during the 10–30 weeks age period (growth period exercise group, GPE); (4) exercise-trained during the 30–50 weeks age period (maturity period exercise group, MPE); and (5) exercise-trained during the 50–70 weeks age period (aged period exercise group, APE). Each group consisted of 10 mice. The animals in each exercise-trained group were subjected to voluntary running in a revolving wheel (Sinano, Tokyo). Circumference of the revolving wheel was 60 cm and the numbers of rotation were recorded. Running distances were calculated as m/day once a week. Some animals died during the experiment and the available numbers of animals for analyses were 7 mice in the APE group, 8 each in the GPE and MPE groups and 10 each in the EE and C groups. Mice were given standard laboratory chow (CE-2; Nippon Clea, Tokyo) and distilled water ad libitum throughout the experiment. All animal protocols were approved by the Institutional Animal Research Committee, Juntendo University

School of Medicine. One day after the end of the experiment, all animals were sacrificed after the age of 70 weeks by cervical dislocation after blood sampling under ether anesthesia.

Bone density and cortical thickness index

Bone mass was assayed using the method described by Okumura et al. (1984). The right femoral bone was excised, and soft tissues were detached from the bone. Lateral soft x-ray images of the femoral bone were obtained by exposure for 1 minute, 40 kV, 5 mA and 45 cm film focus distance. A standard aluminum wedge was placed beside the bone specimen. The film was scanned at the midpoint of the femoral shaft using a microdensitometer (PDS-15; Konica, Tokyo). The width of slit was 0.01 mm × 1 mm, scan speed 0.02 mm/sec. The optical bone density was converted to the thickness of aluminum by pattern of the density of the standard aluminum wedge, and an M-shaped bone pattern was obtained. The integration of the pattern corresponded to the absolute bone density on the scanned plane. Cortical thickness index (CTI) was calculated from the diameters of the shaft and medullary cannel.

Mechanical properties of the bone

The maximum breaking force, ultimate stress, deformation and elasticity were measured by the three-point bending test of the right femoral bone based on the method by Aoki et al. (1993), although the equipment (Autograph, Shimadzu, Tokyo) and test conditions were slightly changed. The test was performed by adjusting the position of the femoral bone so that the plunger would work at the center of the anterior surface of the bone. The interfulcrum distance was 5 mm and the bending rate was 1 mm/min. The four mechanical parameters of the bone were determined from the force deformation curves; maximum breaking force was indicated by the maximum force on the force deformation curve, deformation was measured deflection at yield point, ultimate stress and elasticity were calculated using the method described by Miyanaga (1979).

Ca regulating hormones, Ca and P concentrations in blood

Plasma was separated immediately from fresh whole blood and stored at −80°C until measurement of C-terminal parathyroid hormone (PTH), calcitonin (CT), Ca and P levels. Plasma PTH and CT were measured by radioimmuno-assay kits (PTH-C Eiken, Calcitonin Kit Eiken; Eiken immunochemical Lab., Tokyo). Ca concentrations were determined by atomic absorption spectrometer. P concentrations were measured by colorimetric analysis.

Statistical analyses

Statistical analyses were performed using the Halbau statistical package (Gendaisuugakusha, Kyoto). Following one-way analysis of variance, inter-

group comparisons were made by Scheffe's methods.   Correlation coefficients were calculated between the bone density and the mechanical properties of bone. Level of significance was defined as $p < 0.05$.

<div align="center">RESULTS</div>

Body weight

Fig. 1 shows the mean values of body weights during the 60 week experimental period.   At the start of the experiment (10 weeks old), body weight was $29.5 \pm$ 1.7 g (mean $\pm$ s.d.) in all mice.   There was little difference in mean body weights in each group.   At 30 weeks old, the values were 37.8 ± 4.6 g in the C group, $34.7 \pm$ 2.4 g in the EE group, 35.2 ± 1.5 g in the GPE group, 37.0 ± 2.8 g in the MPE group and 39.2 ± 5.1 g in the APE group, with no significant difference among the groups. At 50 weeks old, the values were 44.5 ± 6.5 g, 37.8 ± 2.5 g, 51.7 ± 5.3 g, 36.1 ± 2.0 g, and 44.6 ± 8.8 g, respectively, with no significant difference between the C group and other groups, but with a significant difference between the APE group and the EE and MPE groups.   It was found that the animals of exercise-trained groups showed suppressed body weight gain during the exercise-training period, and the gain was accelerated during the maturity period in the GPE group and during the aged period in the MPE group.   At the end of the experiment (70 weeks), the values were 46.2 ± 5.8 g, 42.0 ± 2.2 g, 57.2 ± 6.2 g, 49.9 ± 7.2 g, and 40.7 ± 2.8 g, respectively; with a significantly higher weight in the GPE group than in the C group, and also significantly higher weight in the GPE group than in the EE and APE groups.
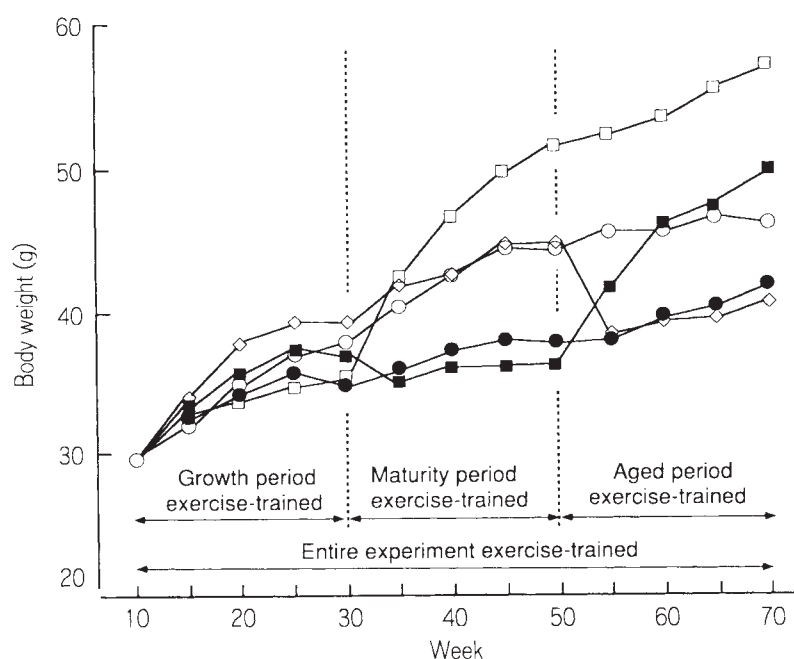

Fig. 1.   Mean values of body weights during the 60 week experimental period.
        C, Control (○); EE: Entire experiment exercise group (●); GPE, Growth
        period exercise group (□); MPE, Maturity period exercise group (■); APE,
        Aged period exercise group (◇)

TABLE 1. *Voluntary running distance of each group (m/day)*

	Growth period	Maturity period	Aged period
EE ($n=10$)	12331.3±631.0	9427.7±2035.7[ab]	6698.0±1267.1[abcd]
GPE ($n=8$)	12042.5±938.5	—	—
MPE ($n=8$)	—	9018.3± 833.8[ab]	—
APE ($n=7$)	—	—	6779.5±1267.1[abcd]

Values are mean±S.D. EE, Entire experiment exercise group (10-70W); GPE, Growth period exercise group (10-30W); MPE, Maturity period exercise group (30-50W); APE, Aged period exercise group (50-70W)

[a]$p<0.01$, significant difference vs. growth period in the EE
[b]$p<0.01$, significant difference vs. growth period in the GPE
[c]$p<0.01$, significant difference vs. maturity period in the EE
[d]$p<0.01$, significant difference vs. maturity period in the MPE

Voluntary running distance

Table 1 shows the running distance of voluntary exercise for each of the exercise-trained groups. As the mice aged—through the growth, maturity and aged periods—their running distances were reduced progressively and significantly. There was no significant difference between the running distances of the EE group when compared with the same periods of the other exercise-trained groups.

Bone density and CTI

Fig. 2 shows the bone density and CTI measured by microdensitometry for each group. The mean±S.D. of bone densities were 0.81±0.08 in the EE group, 0.82±0.07 in the GPE group, 0.76±0.06 in the MPE group and 0.76±0.03 in the APE group, and 0.64±0.07 in the C group (Fig. 2A). In exercise-trained groups
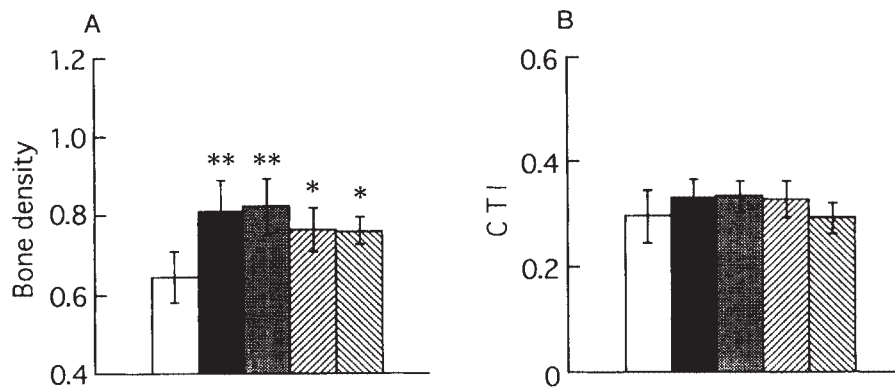

Fig. 2.   Bone density (A) and Cortical Thickness Index (CTI; B) in each group by microdensitometric examination
Control (□); Entire experiment exercise group (■); Growth period exercise group (▦); Maturity period exercise group (▨); Aged period exercise group (▧); Values are mean±S.D.
*$p<0.05$ **$p<0.01$, significant difference vs. C group

the bone densities were significantly greater than in the control group. The values of CTI were 0.30 ± 0.01 in the C group, 0.33 ± 0.03 in the EE group, 0.34 ± 0.03 in the GPE group, 0.33 ± 0.03 in the MPE group and 0.29 ± 0.03 in the APE group, with no significant difference among the groups, although they tended to be higher in the exercise-trained groups ($F = 3.007$, $p > 0.05$), except for the APE group (Fig. 2B).

Mechanical properties of the bone

Fig. 3 shows the mechanical properties of the bone. The values of maximum breaking force indicating the breaking point of bone due to pressure were 2.2 ± 0.4 kg in the C group, 3.5 ± 0.6 kg in the EE group, 4.2 ± 1.0 kg in the GPE group, 3.4 ± 0.7 kg in the MPE group and 2.6 ± 0.8 kg in the APE group. The mean values of the exercise-trained groups were significantly higher than that of the C group except for the APE group, which was significantly lower than that of the GPE group (Fig. 3A).
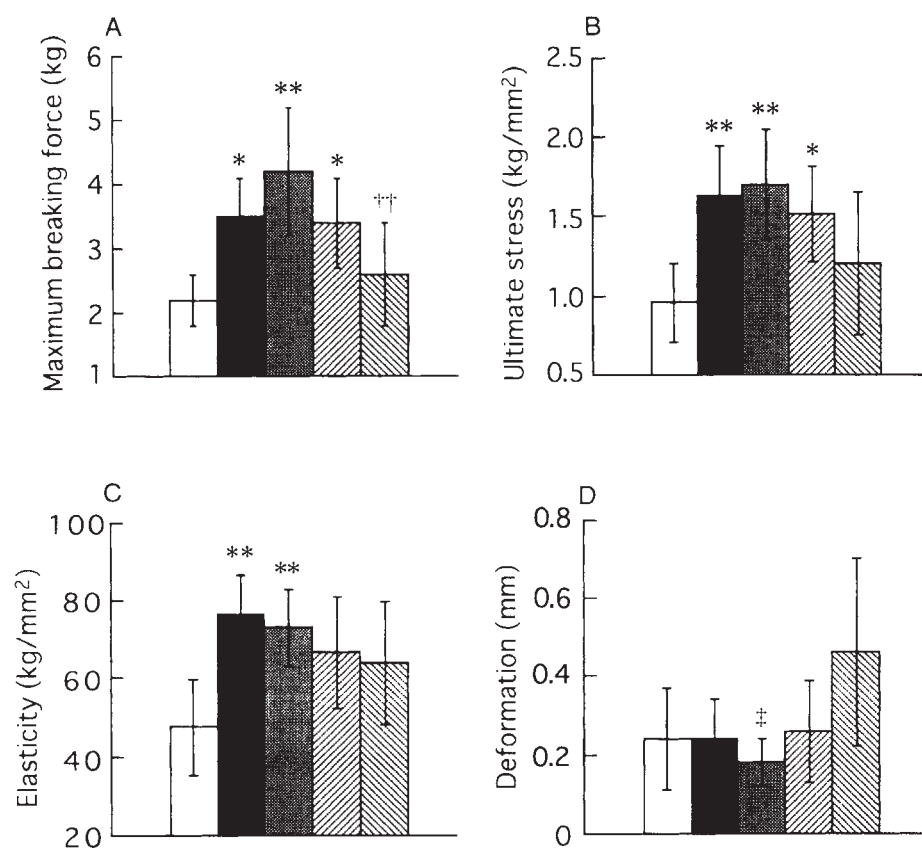

Fig. 3. Mechanical properties of femoral bone, maximum breaking force, ultimate stress, elasticity and deformation

Control (□); Entire experiment exercise group (■); Growth period exercise group (▨); Maturity period exercise group (▨); Aged period exercise group (▨); Values are mean ± s.d.

*$p < 0.05$ **$p < 0.01$, significant difference vs. C group

††$p < 0.01$, significant difference vs. GPE group

‡$p < 0.05$, significant difference vs. APE group

Values of ultimate stress indicating the bone strength were 0.96 ± 0.25 kg/ mm^2, 1.63 ± 0.31 kg/mm^2, 1.70 ± 0.35 kg/mm^2, 1.52 ± 0.30 kg/mm^2, and $1.20\pm$ 0.45 kg/mm^2, respectively. The mean values in the exercise-trained groups were high in comparison with the value in the C group. There were significant differences between the exercise-trained groups and the C group, except for the APE group.

Elasticity values indicating the degree of bending were 47.5 ± 12.1 kg/mm^2, 76.3 ± 10.1 kg/mm^2, 72.9 ± 10.0 kg/mm^2, 66.8 ± 14.3 kg/mm^2, and 64.0 ± 15.7 kg/ mm^2, respectively. The value of elasticity was significantly higher in the EE and GPE groups than in the C group. In the MPE and APE groups, the mean values were higher than that in the C group, but the differences from the controls were insignificant because the individual values were scattered widely.

Deformation values indicating the degree of bone destruction were 0.24 ± 0.13 mm, 0.24 ± 0.10 mm, 0.18 ± 0.06 mm, 0.26 ± 0.13 mm, and 0.46 ± 0.24 mm, respectively. A greater deformation in the APE group was observed rather than in the other groups. There was significant difference between the GPE and APE groups.

Ca regulating hormones, Ca and P concentrations in blood

The plasma PTH concentrations were 0.64 ± 0.15 ngEq/ml in the C group, 0.84 ± 0.29 ngEq/ml in the EE group, 0.67 ± 0.19 ngEq/ml in the GPE group, 0.63 ± 0.15 ngEq/ml in the MPE group and 0.73 ± 0.29 ngEq/ml in the APE group. The plasma CT concentrations were 55.6 ± 9.0 pg/ml, 52.9 ± 12.3 pg/ml, 64.7 ± 12.7 pg/ml, 56.6 ± 12.5 pg/ml and 66.9 ± 24.4 pg/ml, respectively. The plasma Ca concentrations were 86.2 ± 9.6 μg/ml, 88.6 ± 14.1 μg/ml, 80.0 ± 5.8 μg/ ml, 87.0 ± 7.7 μg/ml, and 81.0 ± 10.4 μg/ml, respectively. As to PTH, CT and Ca levels, no significant differences were observed among the groups. The plasma P levels were 116.2 ± 17.3 μg/ml in the C group, 146.4 ± 31.0 μg/ml in the EE group, 138.1 ± 6.7 μg/ml in the GPE group, 129.4 ± 23.7 μg/ml in the MPE group and 131.3 ± 8.0 μg/ml in the APE group. The plasma P concentrations in all exercise-training groups were higher than that in the C group, although the values were not significant ($F=2.885$, $p>0.05$).

DISCUSSION

A voluntary exercise model in laboratory animal studies has been widely used to investigate the relations between regular physical activities and health, because it involves little stress when compared with compulsory exercises such as treadmill or swimming, although it can not assess exercise intensity (Suzuki et al. 1994). In this study, animals in the exercise-trained groups performed voluntary running in a revolving wheel.

It is known that physical exercise training reduces body weight gain and de-training accelerates the gain. In this study, we also observed that body weight

gain in the exercise-trained groups was suppressed during the exercise-training period. When the exercise-training was started, the body weight reached the level of EE within a week. When the exercise-training ceased, however, the mice gained weight very quickly, as shown in the GPE and MPE groups (Fig. 1). It has been reported that exercising animals consumed more food, but exhibited decreased body weight gain (Tokuyama et al. 1982). In this study, therefore, the weight loss was not considered to be due to little consumption of food, although food consumption was not determined. It is considered that reduction of body weight gain in the exercise-training period was caused by increases of energy expenditure over intake, and that acceleration of body weight gain in the de-training period was caused by reduced energy expenditure, but that appetite increase remained unchanged.

Physical training is reported to be effective for prevention of osteoporosis, because it increases the bone density or reduces its age-associated decrease (Blalir et al. 1992; Bourrin et al. 1992; Hoshi et al. 1996). It has been reported, however, that bone of aged animals responds to mechanical stimulation less efficiently than that of young animals (Rubin et al. 1992). Moreover, the bone density can be significantly altered by body weight (Koyama and Nishizawa 1993). In this study, bone densities in every exercise-trained group were significantly higher than those in the C group (Fig. 2A), although the body weights at the end of the experiment were higher in the GPE group in comparison with those in the C group. Therefore, the higher bone density in the GPE group may have been affected by extent of exercise as well as higher body weight. However, in the other exercise-trained groups, there were no significant differences in body weight between the exercise-trained groups and the C group. Therefore, extent of exercise is effective when the purpose is to reduce age-associated bone density decrease.

CTI values in the long tubular bones such as femur are reduced with aging. Age-associated CTI decrease is caused by increased bone absorption in the internal and external periosteum and reduced bone formation in the internal periosteum (Detenbeck and Jowsey 1969). The CTI levels had a tendency to be higher in the exercise-trained groups than in the C group ($p < 0.05$) except for the APE group. It is considered that the exercise-trained groups, except for the APE group, could have shown a suppression of age-associated CTI decrease. In the APE group, however, this value was similar to the C group. One of the reasons for the difference may be follows; effects of exercise on the CTI are considered to be derived from compression stimulation by exercise (Hoshi et al. 1996), but running distances in the exercise-trained during aged periods were reduced in comparison with the growth and maturity periods (Table 1). Also, it is considered that CTI was already lowered at the starting of the training of the APE group.

Mechanical properties of the bone, which reflect its supportive functions, are affected by bone mass and architecture (Burtstein et al. 1975). In the exercise-trained groups, except for the APE group, maximum breaking force, ultimate

stress and elasticity were higher than that in the C group. In the APE group, deformation was higher than that in the other groups ($p < 0.05$). In this study, significant positive correlations were found between bone density and maximum breaking force ($r = 0.809$, $p < 0.01$), ultimate stress ($r = 0.801$, $p < 0.01$) and elasticity ($r = 0.618$, $p < 0.01$), respectively, but no correlation was found between bone density and deformation. It is suggested that maximum breaking force, ultimate stress and elasticity could be improved by reductions in age-associated bone density decrease. In the APE group, however, exercise-training may have improved bone architecture in the aged mice.

PTH, CT and active vitamin D, which are Ca regulating hormones, stabilize blood Ca and P concentrations and regulate bone metabolism (Riggs and Melton 1986). In this study, blood PTH, CT and Ca concentrations were similar among all groups, but P concentrations tended to be higher in all exercise-trained groups than in the C group ($p < 0.05$). Active vitamin D was not measured in this study, although it is known to promote the reabsorption of P in the renal tubules and the increase of blood levels (Liang et al. 1982), and exercise-training has been shown to increase these hormone levels in humans (Hoshi et al. 1990). Therefore, the reabsorption of P in the renal tubules may have been more active in the exercise-trained groups than in the C group because of the increased active vitamin D levels in the exercise.

These observations suggest that exercise-training at every age suppresses age-associated bone loss, and that the effect of exercise during the young age period is greater than during old age. Also, the results of this study suggest that effects of exercise during old age are not always the same as during other years (younger periods).

Acknowledgments

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