Detraining effects on the mechanical properties and morphology of rat tibiae

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Abstract. To study bone adaptation to detraining in growing rats, nine weeks-old immature female Wistar rats (n = 110) were subjected to treadmill running programs (30 or 60 minutes-a-day) for up to 15 weeks, followed by unrestricted cage activities for the subsequent 15 weeks. The results revealed that (1) the cross-sectional area and mechanical properties of the midshaft bone significantly increased in response to running exercise, (2) its structural properties remained unchanged after the cessation of exercise, whereas the material properties returned to control level at a relatively early stage, (3) in the metaphysis, cortical bone area remained unchanged but trabecular bone area decreased in response to running exercise, (4) both areas slightly increased after the cessation of exercise, and (5) the changes in the mechanical properties and morphology of bone depended upon the repetition number and/or the duration of exercise, and were larger with longer duration of exercise.

Keywords: Bone remodeling, cortical and trabecular bones, mechanical property, morphology, running exercise, detraining, rat

1. Introduction

The phenomena of bone remodeling which appear in response to the increase of load have been extensively studied in the field of biomechanics. For example, treadmill running exercise significantly increases bone mass and calcium content of long bones in the porcine [25] and in the rat [24]. It has been demonstrated that such low-frequency, high-load exercise as jump is more effective for bone remodeling than high-frequency, low-load exercise like running [20,23]. The use of implantable devices in experimental animals has shown that the remodeling of cortical bone is dependent on the magnitude of dynamic strain; bone formation is promoted when peak strain is larger than approximately 1000 micro strain [10,19]. Although these studies have supplied important and fundamental data for better understanding of bone remodeling, the loading conditions used were much simpler than those experienced during daily activities.

The bone often undergoes change in load from a high level to a normal level, for example, after the cessation of habitual exercise, i.e. detraining. Therefore, it is very important to know the response of

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bone to such a change in load. Although many studies have been done on the effects of detraining on bone mineral, the results obtained are contradictory and inconclusive, depending upon many factors like animal species, age, sex, and degree, duration, and kind of exercise. For example, LeBlanc et al. applied voluntary running exercise to mature Sprague-Dawley rats for 76 to 99 days [11]. They found that the rate of total body calcium gain was significantly greater in running rats than in control ones, and that the rate in the running rats returned to control level in 20 days after the cessation of exercise and became significantly smaller than control level thereafter. Vuori et al. also found in young women that bone mineral increased by exercise was decreased after the cessation of exercise [21]. In their study, the left limb of the subjects underwent one year of training followed by 3 month detraining. Bone mineral densities of the distal femur, patella, and proximal tibia showed insignificant but systematic increases during training, but declined towards baseline values in the detraining period. Similar results were also reported by Dalsky et al. [4]. In contrast, Karlsson et al. found that the effect of exercise on bone mineral content did not disappear for a long period after the cessation of exercise [6]. They determined bone mineral density in male weight lifters aged 64 years in average who had performed a training program of 10 hours-a-week for 1 to 34 years, and retired from the training 30 years ago in average. After the cessation of the training, increased bone mineral density in the spine was maintained in the subjects of 50 to 64 years of age, while it returned to control level in the subjects aged 65 to 79 years. Kiuchi et al. found that the length and bone mineral content of the femur and tibia of immature rats were increased by running exercise, and that the increases were maintained even after the cessation of the exercise [9]. They also found that bone mass acquired was also maintained for a while after the cessation of the exercise. These studies imply that, in younger subjects, exercise is more effective to increase bone mineral density, and the increased density remained unchanged after the cessation of exercise. However, the changes in the mechanical properties of bones due to detraining have not been fully determined in previous studies.

Besides bone mineral, the effects of detraining on the mechanical properties and morphology of bones have not been studied well. Such data are very important not only for the better understanding of bone physiology but also for the computational modeling of bone remodeling. Recently, computational techniques have often been used to simulate and analyze the phenomenon of bone remodeling [8]. Reliable information on the mechanical properties of bone and their changes caused by stress are prerequisite for the computational modeling of bone remodeling [18]. Therefore, the objective of the present study was to precisely examine the effects of detraining on the mechanical and morphological properties of bones using an immature rat model. Our hypothesis was that the changes in the morphology, material property and structural property are dependent on the duration of exercise, and that these parameters change differently in response to running exercise and its cessation.

2. Materials and methods

2.1. Experimental animal and design

One hundred and ten female Wistar rats aged 9 weeks (body weight = 220.4 ± 16.7 g, mean ± S.D.) were used for the experiment. They were randomly divided into Control group (n = 40), two training groups (T30 (n = 15) and T60 (n = 15)), and two training/detraining groups (T30/D (n = 20) and T60/D (n = 20)). In each group, five animals were housed in a cage with a light and dark cycle of 12 hours for each; they were given water and standard rat chow ad libitum. Their body weights were recorded every week throughout the experimental period. All experimental procedures were performed
under the Guideline for Animal Experiments, Graduate School of Engineering Science, Osaka University.

The rats of T30 group were subjected to a 1.6 km/h, 30 minutes-a-day, 6 days-a-week treadmill running program. The duration of running was 60 minutes-a-day in the rats of T60 group. The rats in both groups started running at 1.2 km/h, and the running speed was increased to 1.6 km/h within a week. The rats of T30/D and T60/D groups were subjected to the same programs as those for T30 and T60 groups, respectively, for the first 15 weeks and, then, the training programs were stopped. The rats of Control group were subjected to normal unrestricted activities in cages for up to 30 weeks. The rats were sacrificed at 0 (Control), 3, 7, 15 (T30, T60, and Control), 16, 18, 22, and 30 (T30/D, T60/D, and Control) weeks; five rats were sacrificed at each period in each group. Their tibiae were harvested, wrapped with gauze soaked in physiological saline solution, and frozen at \(-30^\circ\text{C}\).

2.2. Mechanical tests

Prior to mechanical testing, each left tibia was thawed at \(4^\circ\text{C}\) and dissected free of soft tissues. After tibial length was measured with a caliper, a 3 mm thick ring-like specimen was transversely cut out from the midshaft using a low-speed saw (Fig. 1). The proximal and distal sections of the specimen were microscopically observed via a CCD camera for the measurement of bone areas using an image analyzer (Pias-III, Pias, Osaka, Japan). The average of the areas at both sections was used as the cross-sectional area of the specimen (A). Thickness of the specimen was measured with the caliper used for the measurement of tibial length. The specimen was then, mounted on a conventional material tester (AG-5000E, Autograph, Shimadzu, Kyoto, Japan) having the load capacity of 50 kN. Compression testing was performed at a crosshead speed of 2 mm/min. During the test, the specimen was immersed in physiological saline solution of room temperature. Compressive load was measured with a load cell incorporated in the material testing machine, while deformation was measured with a laser displacement transducer (LC-2450, Keyence, Osaka, Japan) which has the resolution of 0.5 \(\mu\text{m}\). After both data were recorded on an X–T recorder, load at failure, elastic modulus, compressive strength, and strain at failure were obtained.

For hardness testing, the proximal cross-sectional surface of the remaining distal portion of the tibia (A-A section in Fig. 1) was used. The surface was polished with waterproof sand papers of decreasing grit size (800, 1200, 2000), and then with diamond particles (5 and 1 \(\mu\text{m}\) in size) embedded in polishing soft cloths. Hardness tests were performed at the anterior, posterior, medial, and lateral locations on the surface using a micro Vickers hardness tester (HM-102, AKASHI, Tokyo, Japan). At each location, the

![Fig. 1. Locations in the tibial bone used for compression and hardness tests, and morphological analysis.](image-url)
tests were carried out at five positions in each of periosteal (150 μm apart from the periosteal surface), endosteal (100 μm apart from the endosteal surface), and middle (between the periosteal and endosteal positions) areas. Indentation was performed with the load of 50 gf for 15 s.

2.3. Morphological analysis

Each right tibia was thawed at 4°C, and dissected free of soft tissues for a morphological analysis using a micro X-ray CT system (NX-HCP-C80-I, Nittetsu Elex, Tokyo, Japan). The system consists of a sealed micro X-ray tube (focus size = 6 × 8 μm, tube voltage = 20~80 kV, tube current = 0~100 μA, max power = 8 W), an image intensifier (input screen diameter = 4 or 6 inches), a high vision camera (number of pixels = 1920(H) × 1035(V)), and data processing and operating CPUs (Pentium processors). X-ray images were obtained on the transverse sections of the proximal metaphysis at 0.3 and 2.0 mm distal to the distal edge of the proximal growth plate, under the current of 100 μA and voltage of 40 kV supplied to the X-ray tube. Image matrix size and projection number, the latter of which is defined as the number of X-ray projection for each section, were set to 1024 × 1024 (high vision mode) and 600, respectively; these settings gave a resolution of 10 μm in reconstructed images. Slice thickness was approximately 10 μm.

Cortical bone area (Ac) and trabecular bone area (At) were calculated for each cross section. In addition, the thickness (Tb.Th) and number (Tb.N) of trabecular bones were obtained from the area of 1.5 × 1.5 mm² located at the center of the cross section (Fig. 2). These two values were obtained from the following calculations based on a parallel plate model [14]:

\[ \text{Tb.Th} = \frac{2 \times \text{BV}}{\text{BS}} \times 1000 \text{ (μm)} \]  

and

\[ \text{Tb.N} = \left[ \frac{\text{BV}}{\text{TV} \times \text{Tb.Th}} \right] \times 1000 \text{ (1/mm)}, \]  

where BV and TV are “bone volume” and “tissue volume” which represent the areas of bone and the areas of both bone and medullary canal, respectively, in a cross-sectional image. BS is “bone surface” which represents the peripheral length of bone in a cross-sectional image.

Fig. 2. An example of micro X-ray CT image and the square region of interest used for the analysis of trabecular bone. Angle, ω, represents the angle between the anterior direction of the tibia and the long axis of the fabric ellipse obtained from trabeculae.
Moreover, the fabric ellipse of mean intercept length (MIL) was determined from the bone structure in a cross section [2]. The aspect ratio of the ellipse \( R_A \) and the angle of the long axis of the ellipse from the anterior direction of bone (\( \omega \)) were obtained. For these morphological analyses, a software program for the analysis of bone structure incorporated in the CT system was used.

2.4. Statistical analysis

Results were expressed as means ± S.D. Student’s t-test was used to analyze differences among experimental groups at each time point. Significance was set at a level of \( p = 0.05 \) (5%).

3. Results

3.1. Body mass, and tibial length and cross-sectional area

The running groups showed significantly slower gain of body mass during the last 3 weeks of the training period (Fig. 3). After the cessation of running exercise, body mass returned to control level rapidly in T60/D group and slowly in T30/D group. Tibial length was 32.8 ± 0.4 mm (Control group) at 0 week and 40.6 ± 0.9 mm (average for all groups) at 30 weeks; no significant differences were observed among the experimental groups. The cross-sectional area of the tibial midshaft was significantly larger in T60 group than in Control and T30 groups at 7 and 15 weeks (Fig. 4). Significant area differences from Control group were observed after the cessation of running exercise except for 22 weeks in T60/D group.

Fig. 3. Body mass in all the experimental groups. The data obtained from all the rats alive at each period are shown.
3.2. Mechanical tests

Load at failure, compressive strength, and hardness in all the three groups (Control, T30 and T60) gradually increased until 15 weeks, although there were no significant differences in these parameters among the groups except for T60 group at 15 weeks (Figs 5–7). At 15 weeks, these parameters in T60 group were significantly higher than those not only in Control group but also in T30 group. The load at failure in T60/D group remained significantly higher than that in Control group up to 30 weeks (15 weeks after the activity change), except for 22 weeks. On the other hand, the compressive strength and hardness in T60/D group returned to control level at 18 weeks (3 weeks after the activity change). The mechanical parameters in T30 and T30/D groups were almost always somewhat larger than those in Control group; however, the differences were not statistically significant. Elastic modulus was 4.6 ± 1.3 and 4.9 ± 1.0 GPa in T30 and T60 groups, respectively, at 15 weeks, which were slightly higher than that in Control group (3.8 ± 0.9 GPa). The changes in the modulus after the cessation of running were similar to those in the compressive strength. In contrast, the strain at failure decreased with time, and became slightly lower in the training groups (3.1 ± 0.7 and 3.1 ± 1.2% in T30 and T60 groups, respectively) than in Control group (3.7 ± 0.8%) at 15 weeks. The strain did not change much after the cessation of running.

These results indicate that both material and structural strength of bone increase in response to running exercise, and that after the cessation of exercise material properties return to control level whereas structural properties remain unchanged.
Fig. 5. Compressive load at break of tibial midshaft. The load at failure was larger in the running groups than in Control group after 7 weeks, with significant differences observed at 15 weeks and thereafter except for 22 weeks in T60 and T60/D groups.

Fig. 6. Compressive strength of tibial midshaft. The strength in the running groups was larger than that in Control group after 15 weeks, with significant differences observed at 1 week before and 1 week after the cessation of exercise in T60 and T60/D groups.
3.3. Morphological analyses

At 7 weeks, the cortical bone area in the tibial metaphysis was slightly smaller in the training groups than in Control group, with a significant difference observed between T30 and Control groups at 2.0 mm distal to the proximal growth plate (Fig. 8). At 15 weeks and after the cessation of exercise, the areas in T30 and T30/D groups were similar to those in Control group. However, the areas in T60 and T60/D groups became slightly larger than control level, although there were no significant differences. Trabecular bone area was smaller in the training groups than in Control group at 7 and 15 weeks, with a significant difference observed at 7 weeks between T30 and Control groups at 0.3 mm distal to the growth plate (Fig. 9). At 1 week after the cessation of training, the area in the running groups returned to control level. Then, they had a tendency of increasing at 18 and 22 weeks, with a significant difference at 22 weeks at 2.0 mm in T60/D group. At 30 weeks, the area seemed to decrease to lower level than control.

Trabecular thickness and number in the training groups were smaller than those in Control group at 7 weeks, with a significant difference in trabecular number observed in T30 group at 0.3 mm (Fig. 10). However, they increased and returned to control level at 15 weeks. One week after the activity change, they showed almost no changes. Three and 7 weeks after (at 18 and 22 weeks, respectively), however, trabecular thickness and number increased in the training groups, with significantly larger thickness at 2.0 mm at 18 weeks and at 0.3 mm at 22 weeks and significantly larger number at both the weeks in T60/D group in comparison with Control group. Then, they returned to control level at 30 weeks, although those at 0.3 mm seemed to be slightly smaller than their control values.

The aspect ratio of the fabric ellipse obtained from metaphyseal trabecular structure at 0.3 mm distal to the growth plate was increased by the running of 15 weeks, with a significant difference observed in T60 group (Fig. 11). The increased ratios were remained unchanged after the cessation of running.
Fig. 8. Cortical bone area at 0.3 and 2.0 mm distal to the proximal growth plate. The areas in the training groups were smaller than those in Control group at 7 weeks, although the differences disappeared at 15 weeks. After the cessation of running, the areas in T60 and T60/D groups were slightly larger than those in Control group.

Fig. 9. Area of trabecular bone at 0.3 and 2.0 mm distal to the proximal growth plate. The area was smaller in the running groups than in Control group at 7 and 15 weeks. In T60/D group, it returned to control level at 1 week after the cessation of running, and then became larger than control level at 18 and 22 weeks and again smaller at 30 weeks.
Fig. 10. Thickness of trabecular bone at 0.3 and 2.0 mm distal to the proximal growth plate. Although trabecular thickness in the training groups was slightly smaller than that in Control group at 7 weeks, it became almost similar to that in Control group at 15 and 16 weeks. It became larger in the training groups than in Control group at 18 and 22 weeks, and returned to control level at 30 weeks.

Fig. 11. Aspect ratio of fabric ellipse obtained from trabecular bone at 0.3 and 2.0 mm distal to the proximal growth plate. After the cessation of running, the ratio in the training groups became larger than that in Control group at 0.3 mm distal to the growth plate.
exercise. These phenomena were also observed at 2.0 mm, but were much less clear compared to those at 0.3 mm. It was hard to see the effects of running and its cessation on the bone orientation (angle $\omega$ in Fig. 2) of the long axis of fabric ellipse because the results were very much variable (the data are not shown). However, the standard deviation of the orientation showed a tendency of decreasing due to 15 week running, and the decreased deviation seemed to be kept after the cessation of running. These results suggest that the trabecular bones close to the proximal growth plate tend to be oriented along the anteromedial direction by running exercise for 15 weeks, and that the orientation remains unchanged after the cessation of running.

4. Discussion

Load applied to the bone is often changed from a high level to a normal level, for example by the cessation of habitual exercise, i.e. detraining. However, the responses of bone to detraining have not been studied fully, as stated in Introduction. From our knowledge, the present study is the first one which dealt with the change in the mechanical properties of bones in response to detraining.

In the present study, body mass indicated slightly slower gain in the training groups than in Control group (Fig. 3). However, tibial length demonstrated no differences among the three groups. These results suggest that the running training program did not arrest bone growth. Since 9 weeks-old immature rats were used in the present study, the data obtained include the effects of both exercise and growth. However, by comparing experimental data to control data, we can discuss the effects of training and detraining. Moreover, it is important from clinical viewpoints to know the overall response of immature bone to training and detraining. Li et al. reported that exercise corresponding to more than 80% of aerobic capacity had an adverse effect on bone remodeling and induced bone resorption [12]. The strength of running exercise in the present study may not have been strenuous as that in the study by Li et al.

After 15 week running, the cross-sectional area of the tibial midshaft became significantly larger in T60 group than in Control and T30 groups (Fig. 4). The significant difference was remained for 15 weeks after the cessation of running. As mentioned in Introduction, Karlsson et al. found that spinal bone mineral increased by the long-lasting, intensive exercise of weight lifting remained unchanged after the cessation of exercise [6]. Kiuchi et al. applied a running exercise program to immature male rats; its protocol was 35 m/min, 60 minutes-a-day, and 5 days-a-week, which was similar to that of T60 used in the present study [9]. Their results showed significant increases in the length and bone mineral content of the femur and tibia. The acquired bone mass was retained for 10 weeks after the cessation of exercise. These previous results agree well with the present results, and all of these studies indicate that the effects of exercise on bone mass do not diminish after the cessation of exercise in the case of intensive and long-lasting exercise. In the present study, the cross-sectional areas in T30 and T30/D groups showed almost no differences from those in Control group throughout the experimental period, whereas those in T60 and T60/D groups were significantly larger than those in Control group. These results suggest that the magnitude, and the repetition number and/or duration of exercise affect bone mass. Cullen et al. reported that the formation of cortical bone in the rat tibia was enhanced as the repetition number of axial compressive strain (1000 $\mu$e) was increased [3]; the result essentially similar to that obtained from the present study. Rubin and Lanyon [16,17] and McLeod and Rubin [13] have performed a number of experiments using Rooster and turkey ulnae to study the effects of cyclic number of applied load on bone mass and they determined the site-specific relationship between the strain and bone change as a function of daily loading cycle. Based on those data, Qin et al. proposed a modified daily stress stimulus
theory. It was described by a nonlinear relationship between the strain magnitude and daily load cycle for maintaining bone mass as 

\[ Y = 10^{2.28(5.6 - \log_{10} X)^{1.5}}, \]

where \( Y \) represents the strain magnitude and \( X \) is the daily loading cycle number [15]. The theory implies that bone mass increases if the strain and the cycle number are larger than the above-described relationship. Keller and Spengler observed that surface strain in the tibia of running rats was approximately 500 \( \mu \varepsilon \), and therefore the mean strain in the tibial midshaft in the present study was considered to be less than this magnitude [7]. Using the modified daily stress stimulus theory, the loading cycle number to maintain bone mass is calculated to be more than 5,000 if the mean strain in the tibia is less than 500 \( \mu \varepsilon \). In the present study, the increase in the cross-sectional area of tibial midshaft in response to running training was insignificant in T30 group, but was significant (at 7 weeks) in T60 group. The cycle number of loading is considered to be approximately 5,000 a day (3 Hz for 30 minutes) in T30 group, but approximately 10,000 a day (3 Hz for 60 minutes) in T60 group. It is, therefore, suggested that the difference in bone area change in response to running exercise between T30 and T60 groups can be explained by the modified daily stress stimulus theory.

At 15 weeks, the load at failure, compressive strength, and hardness of the tibial midshaft in T60 group were significantly higher than those in Control group, whereas the results from T30 group were almost similar to Control data (Figs 5–7). Similarly to the cross-sectional area, these structural and material properties were dependent on the repetition number and/or duration of exercise. In T60 group, the load at failure started increasing at or before 7 weeks; on one hand, the increases in compressive strength and hardness were observed at 15 weeks. That is, the response of bone to increased load appeared earlier in structural properties than in material properties. After the cessation of running, the increased load at failure in T60/D group remained almost unchanged until 30 weeks (15 weeks after the activity change), whereas the compressive strength and hardness returned to control level at 18 weeks (3 weeks after the activity change). These results indicate that material properties more sensitively change in response to activity changes than morphological and structural properties.

In the tibial metaphysis, cortical bone area was smaller in the running groups than in Control group at 7 weeks; at 15 weeks, it returned to control level in T30 group, while it became slightly larger in T60 group than in Control group (Fig. 8). The cessation of running activity did not change the area in both groups until 30 weeks (15 weeks after activity change). Trabecular bone area in the training groups was smaller than that in Control group during exercise period (Fig. 9). One week after activity change (at 16 weeks), the area once returned to control level. However, the area in the T60/D group increased at 18 and 22 weeks, although it showed no change in T30/D group in these periods. Then the area became slightly smaller in both training groups than in Control group at 30 weeks. Similarly to the midshaft, the morphological response of the metaphysis to the running exercise and its cessation was dependent on the repetition number and/or duration of exercise. However, the tendency of morphological change in the metaphysis was different from that in the midshaft. As mentioned above, in the metaphysis, the cortical bone area in T60 group was once decreased by running for 7 weeks and, then, it was recovered to slightly higher level than control at 15 weeks; it remained almost unchanged after the cessation of exercise up to 30 weeks. In the midshaft, however, the cortical bone area in the group was significantly increased by 7 week running exercise (Fig. 4), which seemed to contribute to the increase in load at failure (Fig. 5). It showed no change at 15 weeks and also after the cessation of exercise. The reason for the difference of the change in cortical bone area between the metaphysis and the midshaft, which appeared relatively soon after starting exercise, is unknown. However, it may be explained from the view of functional adaptation; that is the metaphysial bone may play less important role in resisting to externally applied stress than the tibial midshaft. This explanation is also applicable to the result obtained by Westerling
et al., which demonstrated that the strain energy density of bone in the rat was larger in the tibial midshaft and epiphysis than in the tibial metaphysis [22]. Bone adaptation might be less active in the metaphyseal bone than in the midshaft having priority of bone formation.

Trabecular thickness and number in the running groups were smaller than those in Control group at 7 weeks, but returned to control level at 15 weeks (Fig. 10). Three to 7 weeks after the cessation of running (at 18 to 22 weeks), they became larger than those in Control group; thereafter, they returned to control level. These changes seem to be clearer in the longer exercise group; that is, the morphology and structure of trabecular bone depend upon the repetition number and/or duration of exercise. The changes in trabecular bone area in the training groups (Fig. 9) are attributable to those in trabecular thickness and number. Westerlind et al. also found that the number and thickness of trabecular bone in the rat tibia were increased by running exercise, which caused the increase in trabecular bone area [23]. The reason why the area, thickness, and number of trabecular bone once increased relatively soon after the cessation of running and then decreased is unknown. Frost suggested that the balance of bone resorption and formation is preserved by basic multicellular units [5]. In the present study, the balance in entire tibia may have been broken soon after the exercise cessation, and bone formation may have become predominant for a while as a transient effect.

Trabecular anisotropy was also somewhat affected by running exercise (Fig. 11). The aspect ratio of fabric ellipse was larger and the standard deviation of its orientation was smaller in the running groups than in Control group at 15 weeks, which did not change for 15 weeks after the cessation of exercise. These changes were clearer at 0.3 mm distal to the growth plate than at 2.0 mm, probably because bone resorption and formation were more active in the trabecular bone locating closer to the growth plate. The change in trabecular anisotropy was more dependent on the distance from the proximal growth plate, as compared with those in the area, thickness, and number of trabecular bone. These results suggest that special care would be needed for the computational modeling of trabecular bone remodeling.

5. Conclusions

Changes in the morphology, structural properties, and material properties of the rat tibia in response to running exercise and its cessation were determined in the present study, and the following conclusions were obtained:

(1) The cross-sectional area and mechanical properties of the midshaft bone change in response to running exercise. Although the structural properties remain unchanged after the cessation of exercise, the material properties return to control level at relatively early stage.

(2) The size and number of trabecular bone in the metaphysis once decrease in response to running exercise, although they return to control level. After the cessation of exercise, they show a biphasic change; that is, they increase several weeks after and, then, return to control level. Anisotropic changes occur in the trabecular bone near the growth plate in response to exercise, which remain unchanged after the cessation of exercise.

(3) These changes appear more clearly in the case of longer exercise.

It is known that bone remodeling is dependent on the degree of animal maturation [1]. Growing rats were used in the present study. Similar experiments using mature rats are being done, and their results will be reported elsewhere.
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