

The Progression of Bone and Muscle Atrophy in Mice Hind Limb with Immobilization

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ABSTRACT

This study investigated the time course of changes of bone and muscle atrophy in mice with immobilization by denervation and fixation. The animals were fifty-two male C57 BL/6J mice, aged 10 weeks old. Eight mice were used as the base line, and the remaining ones were cut at the sciatic nerve of the left hind limb and fixed with a plaster cast. At week 1, 2, 3, and 4 after the operation, a cross-sectional area of the rectus femoris muscles and bone mechanical strength with a three-point bending test of the femur and tibia were measured. The time course of changes of the bone mechanical strength and of the cross-sectional area of the rectus femoris muscles between the intact and experimental limbs in each period compared with the control limbs, was determined. The bone mechanical strength of the femur, tibia, and the cross-sectional area of the rectus femoris muscles of the experimental limbs significantly decreased compared with those of the intact limbs at week 4, 3, 2 and 1 after the operation ($p < 0.05$). Compared with the intact limbs, the bone mechanical strength and the cross-sectional area of the rectus femoris muscles of the experimental limbs declined approximately 10% and 30%, respectively, during the experiment ($p < 0.05$). It was demonstrated that bone and muscle atrophy occurred at an early stage after immobilization by denervation and fixation, and that both types of atrophy progressed simultaneously in the present study.

Key words: *Immobilization, Bone and muscle atrophy, Bone mechanical strength, Muscle cross-sectional area*

Disuse affects various physical and psychological functions. It is important to prevent disuse because the deterioration of these functions can become an obstruction to rehabilitation, taking the form of pain, contracture, ankylosis, and dementia, etc. Immobilization is the most common reason for such disuse, causing the atrophy of bone and muscle. There are many reports of such atrophy resulting from paralysis, prolonged bed rest, and fixation^{7,13,16,30}. Bone mass decreases 10–20% over the course of several years²⁰, and muscle strength, accompanied by the loss of muscle mass, decreases 6–40% in several weeks³ of bed rest. Thus bone and muscle atrophy occur rapidly. Since bone atrophy increases the risk of fracture and since muscle atrophy decreases muscle strength and physical activity, it is necessary to investigate when such atrophy occurs and how the different types of atrophy are related.

This study therefore investigated the time

course of changes of bone atrophy, with reference to its mechanical strength, and of muscle atrophy, with reference to its cross-sectional area, in sciatic denervated mice fixed with plaster casts.

METHODS

Animal Care

Fifty-two male C57 BL/6J mice aged 10 weeks old were used. Eight mice were sacrificed at the start of this study as the base line, while the remaining mice were cut at the sciatic nerve of the left hind limb under pentobarbital sodium anesthesia (50 mg/kg). Their left hind limbs were fixed with plaster casts and fixated constantly every day, and the contralateral limbs of each mouse were used as controls. They were kept in separate cages under the following conditions: $23 \pm 1^\circ\text{C}$ temperature, $50 \pm 5\%$ humidity, and a 12-hour day-night cycle. All mice were allowed ad libitum

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feeding and drinking water. This study was carried out in accordance with the Guide for Animal Experimentation of Hiroshima University and the Committee of Research Facilities of Laboratory Animal Science of the Hiroshima University School of Medicine.

Mechanical Testing

Animals were sacrificed at the end of weeks 1, 2, 3, and 4 after the operation. Femur and tibia bones were dissected out and soft tissue was removed. A materials testing machine (computer control system autograph AGS-1000A, Shimadzu Co., Kyoto, Japan) was used to measure the three-point bending strength of the bones (bone mechanical strength). The speed of the compression head was 0.5 mm/min, and it pushed the center of the specimens vertically. Calculated values were revised based on body weight. This test was to find the effect of the sciatic nerve denervation on the bone mechanical strength.

Measurement of Muscle Cross-Sectional Area

Both rectus femoris muscles were dissected out at the end of each experiment for morphological study. All rectus femoris muscles were fixed in 10% formalin and embedded in paraffin. After this, 3 sections (4–5 μm) from the center of the muscle toward proximal and distal were prepared and stained by Hematoxylin-Eosin. Photos of these sections with a microscope (E-600, Nikon Co., Japan) were inputted to a personal computer ($\times 100$), and 100 muscle fibers in one section of the cross-sectional area of both rectus femoris muscles were measured by NIH Image software on a Macintosh computer. This observation was to find the effect of fixation with a plaster cast on a cross-sectional area of the rectus femoris muscles, because this muscle is innervated by the femoral nerve.

Statistic Analysis

For statistic analysis, a paired-t test was used to determine the time course of changes of the bone mechanical strength and of a cross-sectional area of the rectus femoris muscles between the intact and experimental limbs in each period. In addition, a one-way ANOVA and Fisher's PLSD for finding the reduction rate of bone mechanical strength and of a cross-sectional area of the rectus femoris muscles of the experimental limbs was used for comparison with the control limbs. All values were expressed as means \pm standard deviation (SD). A P-value less than 0.05 was considered significant.

RESULTS

The bone mechanical strength of the femur and tibia in the experimental limbs was significantly lower than that of the intact limbs at week 4 after the operation and, at week 3 and 4 after the operation, respectively (Table 1). The muscle cross-sectional area of the experimental limbs showed significantly lower values at all weeks after the operation when compared with the control group (Table 1).

Compared with the intact limbs, the bone mechanical strength of the femur in the experimental limbs declined approximately 3, 4, 5, and 10% at weeks 1, 2, 3, and 4 after the operation, respectively. The bone mechanical strength of the tibia declined about 3, 4, 7, and 12% at weeks 1, 2, 3, and 4 after the operation, respectively. The bone mechanical strength of the femur and tibia at week 4 after the operation was significantly decreased when compared with that of the control group (Fig. 1b and 1c). The muscle fiber cross-sectional area of the experimental limbs decreased approximately 4% and 30% at week 1 and 4 after the operation, respectively. The muscle fiber

Table 1. Time course of influences by sciatic denervation on bone and muscle

	Final Body weight (g)	Mechanical Strength (N/kg)		Muscle Cross-sectional Area(μm^2)
		Femur	Tibia	
Control Right	23.0 \pm 0.6	968.8 \pm 62.7	698.2 \pm 36.9	1297.5 \pm 518.6
Left		966.0 \pm 66.1	697.8 \pm 44.0	1301.5 \pm 521.5
Week 1 Right	23.1 \pm 1.0	972.6 \pm 66.4	718.5 \pm 40.9	1263.1 \pm 452.8
Left		943.7 \pm 48.8	698.3 \pm 51.3	1216.7 \pm 447.8*
Week 2 Right	23.4 \pm 1.1	970.2 \pm 49.1	711.2 \pm 54.4	1295.8 \pm 583.6
Left		931.5 \pm 48.3	677.1 \pm 45.7	1191.7 \pm 449.4*
Week 3 Right	24.3 \pm 1.6	970.3 \pm 73.8	704.5 \pm 68.4	1317.6 \pm 586.7
Left		919.4 \pm 72.2	652.3 \pm 62.8*	1093.1 \pm 412.3*
Week 4 Right	24.7 \pm 1.4	974.8 \pm 50.8	698.4 \pm 59.7	1396.4 \pm 537.9
Left		975.7 \pm 80.0*	611.7 \pm 54.5*	978.9 \pm 394.0*

*: Significantly different from the right (intact) limbs at the same stage ($p < 0.05$). (Mean \pm S.D.)

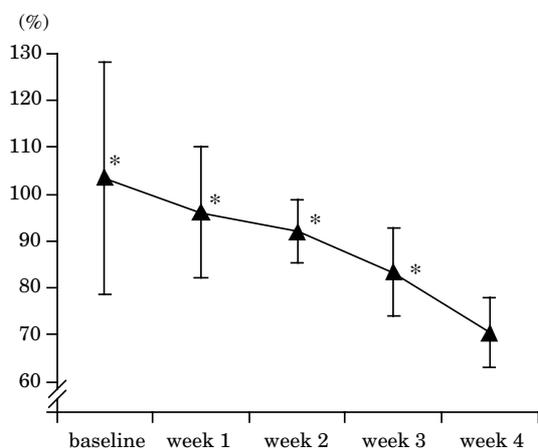


Fig. 1a. Changes in the cross-sectional area of rectus femoris muscles
*: Significantly different from that of week 4 ($p < 0.05$). (Mean \pm S.D.)

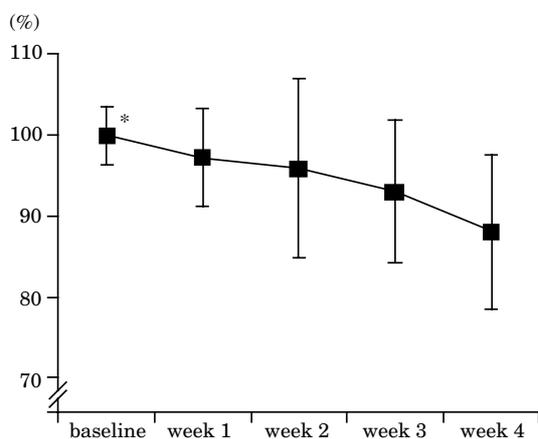


Fig. 1b. Changes in bone mechanical strength of femur
*: Significantly different from that of week 4 ($p < 0.05$). (Mean \pm S.D.)

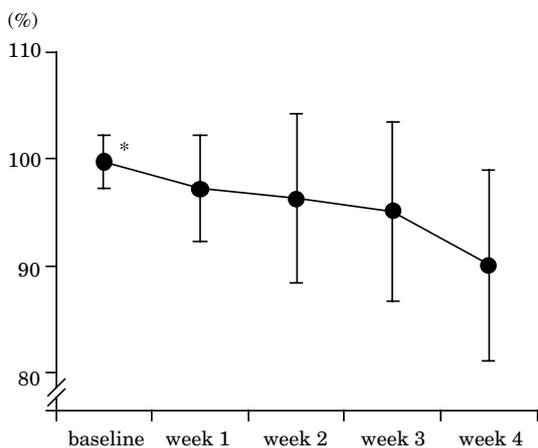


Fig. 1c. Changes in bone mechanical strength of tibia
*: Significantly different from that of week 4 ($p < 0.05$). (Mean \pm S.D.)

cross-sectional area at week 4 after the operation was significantly lower than that of all other groups (Fig. 1a).

DISCUSSION

Bone atrophy induced by denervation has been reported in previous animal studies^{17,28,29,31}. Bone loss induced by immobilization occurs mainly due to increased bone resorption and decreased bone formation^{17,28,29,31}, and bone loss mainly occurred between 1 to 4 weeks after immobilization^{21,31}. It has been reported that the mineral apposition area, the mineralizing surface, and the bone formation rate in immobilized rats significantly declines when compared to those of ovariectomized rats^{28,29,31}. Moreover, the number of osteoclasts has been found to increase in immobilized rats^{29,31}. These are all reasons for bone loss, and they affect bone mechanical strength. The bone mechanical strength of the femur and tibia has been found to significantly decline at two weeks after paralysis²¹. In a particular study²¹, a decline of calcified cartilage in the bone plate area and an increase of gaps in trabecular bone were observed. These changes of bone structure can weaken the bone mechanical strength, as the abilities of support and dispersion against compression force become low. These changes of trabecular and cortical bone occurred at an early stage after denervation.

In the present study, the bone mechanical strength values of the femur and tibia in experimental limbs were significantly lower than those of the control limbs at 4 and 3 weeks and 4 weeks after the operation, respectively. The decline of the bone mechanical strength in the femur and tibia progressed during the experiment, with the reduction rate of the bone mechanical strength of both bones at approximately 5% at 2 weeks and 10% or more at 4 weeks after the operation, compared with the control limbs. These results are similar to those of previous studies^{17,21,28,29,31}. Though these previous studies investigated the tibia, the present study investigated both the femur and the tibia, and it was found that changes of the reduction rate of the tibia differed from those of the femur. Significant differences in the bone mechanical strength between intact and experimental limbs in the tibia appeared earlier than those in the femur, and the bone mechanical strength of the tibia decreased more severely than that of the femur at the last stage. This difference was thought to be caused by the difference in load to the bones. The tibia responded to decreased loads more rapidly than the femur. This finding suggests that daily stimuli influence biomechanical thresholds.

Immobilization^{3,6,10,13,23,27}, unloading^{5,11,12,15,22}, and castration^{8,24} have all been found to induce

muscle atrophy. These kinds of atrophy have a common characteristic, which is that they all occur rapidly regardless of the situation. Muscle strength^{5,6,11,13}, size^{10,23,27}, number^{22,23}, type^{4,5,10-13,15,22}, and the biochemical properties^{4-6,8,11,12,24} of muscle fibers have been found to change immediately after the beginning of such situations. Stimulation to muscles such as gravity and contraction, however, has been found to lessen, or even prevent muscle atrophy^{1,9,26}. This finding suggests that muscles directly respond to decreased physical stimulation.

In this study, muscle atrophy in the muscle cross-sectional area was observed at 1 week after the operation and occurred prior to bone atrophy. The reduction of muscle cross-sectional area continued during the experiment, and a decline of approximately 30% was observed at 4 weeks after the plaster casting. These results indicate that muscle sensitively reacts to the cutting off of physiological activity by immobilization. Moreover, muscle atrophy progresses along with this decline in sensitivity.

The relationship between bone mineral density and muscle strength has also been studied, and the results have varied^{2,14,18,19,25}. It is unknown whether the atrophy of bone and muscle occur independently or serially. It is still uncertain whether bone and muscle atrophy are caused at the same time or bone atrophy is induced by muscle atrophy. It is generally thought, however, that the mechanisms of these types of atrophy take the former pattern, i.e., they atrophy simultaneously. Although muscle atrophy has been found to occur earlier than bone atrophy, morphological bone atrophy occurs rapidly after immobilization^{20,21,28,29}. This indicates that bone and muscle atrophy occur independently. The rehabilitation of disuse atrophy therefore needs to be carried out individually, as bone atrophy increases fracture risk and muscle atrophy decreases physical activity.

CONCLUSION

It was demonstrated in this study that bone and muscle atrophy occurred at an early stage after denervation and that bone and muscle atrophy progressed simultaneously.

(Received February 28, 2006)

(Accepted June 2, 2006)

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