EFFECTS OF A DECREASE IN MECHANICAL STRESS ON FEMORAL REGIONAL BONE MINERAL DENSITY AND OSTEOLAST MICROSTRUCTURE: COMPARISON IN A MODEL OF FREELY MOBILE AND CAST IMMOBILIZED RATS

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Abstract

Purpose: Decreased mechanical stress causes disuse bone atrophy characterized by reduced bone mass and weakened bone. However, few studies have measured the mechanism behind such changes in different areas of bone. The present morphological study investigated the effects of decreased mechanical stress on bone mineral density in different areas of the femur by measuring bone mineral density and assessing the microstructure of osteoblasts.

Methods: Twenty-one 9-week-old male Sprague-Dawley rats were acclimatized for one week, and then were divided into control, exercise, and cast immobilization groups. The study was conducted over an 8-week period, from age 10 weeks to 17 weeks in the rats. Bone mineral density was measured by dual energy X-ray absorption (QDR-2000) in the proximal epiphysis/metaphysis, diaphysis, and distal epiphysis/metaphysis of the femur. The microstructure of osseous cells was examined by Scanning Electron Microscopy (SEM).

Results: Bone mineral density of the distal epiphysis/metaphysis was significantly lower in the cast immobilization group than in the exercise or control groups (p<0.001). In the cast immobilization group, scanning electron microscopy of the distal epiphysis/metaphysis revealed no boundary between small osteoblast and smooth neighboring cells; however, in the exercise group, actively osteoblastic osteoblasts covered the bone surface, resembling a stone wall.

Conclusion: Decreased mechanical stress caused a decrease in bone mineral density that varied in different areas of the femur. The largest decrease in density occurred in the distal epiphysis/metaphysis. Osteoblast microstructure played an important role.


Key word: bone remodeling, femoral distal epiphysis/metaphysis, SEM, osteoblast

INTRODUCTION

Many studies have reported a relationship between osseous tissue and mechanical stress. Changes in bone weight and morphology in response to mechanical stress are important in bone remodeling to maintain bone density and are characterized by cortical and cancellous bone formation.

Decrease of bone mineral density is caused by aging, limitation of motion, and decreased physical activity, and this leads to increased fragility of the bone microstructure and a higher risk of fractures. Aging is associated with changes in gastrointestinal calcium absorption and endocrine disorders such as hyperparathyroidism with increased bone resorption. In addition, reduced mobility and physical activity due to cast immobilization or nerve resection decrease mechanical stress on bone, leading to a drastic decrease in bone mineral density. Therefore, mechanical stress on bone is essential for maintaining bone structure and bone mineral density.

Many studies have investigated the effects of increased and decreased mechanical stress on changes in bone mineral density, and basic research using DXA (dual energy X-ray absorption) is important.

In rats with hindlimb suspension, hindlimb bone mineral density decreases in response to a reduced mechanical load. But even with a reduced load on the hindlimbs of rats, muscle contraction and movement in midair is not restricted. In rats with resection of the sciatic nerve, nerve ligation or transection causes muscle paralysis, leading to
altered mechanical stress due to muscle and load changes. However, the decrease in bone mineral density, rather than due to the influence of mechanical load, has been largely attributed to blood flow and cytokine effects with changes in innervation. The results have been limited to quantification of total bone mineral density with disuse atrophy. To elucidate the mechanism of decreased mechanical stress on decreasing bone mineral density, bone mineral density must be analyzed at sites of predominantly cortical and cancellous bone. In addition, in order to clarify the mechanism by which bone mineral density is regulated, it is important to investigate osseous microstructure.

In this study, we investigated the effects of decreased mechanical stress on femoral regional bone mineral density and osteoblast microstructure, with comparison in a model of freely mobile and cast immobilized rats.

METHODS

Subjects and methods

Twenty-one 9-week-old male Sprague-Dawley rats, purchased from Japan SLC (Hamamatsu) were divided into the following three groups: control group (n = 8), exercise group (n = 6) and cast immobilization group (n = 7). In the experiment, the rats were acclimatized for one week from the ages of 9 to 10 weeks and were used for 8 weeks from the ages of 10 to 17 weeks. Each rat was caged separately and placed in a room with a constant temperature of 24°C. Cages were illuminated from 08:00 to 20:00. Each rat was given 30 g of Rodent Laboratory Diet EQ (Japan SLC, Hamamatsu) daily and had free access to tap water. Rats were weighed once weekly from the start to the end of the experiment using a scale for small animals (HL-2000, AND, Korea). In the cast immobilization group, plaster casts were changed once weekly, and measurements were taken before cast immobilization.

All experimentation was carried out with the approval of Osaka University of Health and Sport Sciences Animal Ethics Committee and the “Basic guidelines for conducting animal studies in the field of physiology”, established by the Physiological Society of Japan.

Cast immobilization

Plaster was used to immobilize from the abdominal region to the tip of the left hindlimb with the hip and knee joints in the flexed position and the ankle in the dorsiflexed position.

Rat movements

In the exercise group, rats were allowed to run freely on a rotational wheel (Japan Cage, Tokyo). Running distance was measured daily using a counter (Fuji Kogyo CO. LTD, Kyoto, Japan) attached to the wheel.

Bone mineral density measurement

After the experiment, the rats were perfused using Karnovsky fixative under pentobarbital anesthesia, and the left and right femurs were removed and soaked in 4°C glutaraldehyde phosphate buffer (pH: 7.2).

The bone mineral density of the femur was measured by dual energy X-ray absorption (DXA: QDR-2000, Hologic, Inc., United States of America)6). Each specimen was scanned from the distal to proximal direction using the super high-resolution mode for analysis of small animal bones. In order to standardize the scanning direction, the center of the distal and proximal ends was placed along a line drawn on the outer bottom surface of a styrene case and was positioned so that the anterior surface of the femur could be scanned. Based on scans, overall area (cm²), bone mineral content (g) and bone mineral density (bone mineral content per area, g/cm²) were calculated. In addition, each specimen was divided into four areas. First, the femur was divided into two equal sections along the long axis, and the distance from the proximal head to the proximal metaphysis (or the distance from the distal epiphysis to the distal metaphysis) was then divided into two at a point equidistant from the head, to give 4 areas:
L1 (cancellous bone-dominant femoral head and proximal metaphysis: proximal epiphysis/metaphysis) L2 and L3 (cortical bone-dominant femoral diaphysis) and L4 (cancellous bone-dominant femoral condyle and distal metaphysis: distal epiphysis/metaphysis).

Bone mineral density was measured for the entire femur (total) and for each area (Figure 1).

**Scanning electron microscopy**

After soaking each specimen in glutaraldehyde phosphate buffer (pH 7.2, 4°C) for 4-5 days, the fixed specimen was split along its long axis under a stereomicroscope while still in the fixative, and then dehydrated using acetone. After subjecting the specimen to critical point drying and gold coating (SC7610, TOPCON, Japan), semi-elliptical bone fragments were analyzed by Scanning Electron Microscopy (SEM) (DS-600, TOPCON, Japan) and photographed.

**Analysis methods**

Bone mineral density of the left femur was compared among the control, exercise, and cast immobilization groups. In addition, in the cast immobilization group, the effects of cast immobilization on bone mineral density were analyzed by comparing the bone mineral density between the left femur (immobilized) and the right femur (not immobilized). Bone mineral density data were statistically analyzed using Macintosh Stat View (Fisher's probable least-squares difference test) to compare the bone mineral density of the left femur among the control, exercise, and cast immobilization groups. T-test (nonparametric) was used to compare bone mineral density between the left femur (immobilized) and the right femur (not immobilized) in the cast immobilization group. The level of significance was set at p<0.05.

**RESULTS**

**Body weight comparison, and running distance for the exercise group**

Figure 2 shows body weight at the end of the experiment for the exercise, control, and cast immobi-
lization groups. Body weight for the cast immobilization group (331.14 ± 39.12 g) was significantly lower than that for the exercise and control groups (433.50 ± 25.8 and 462.00 ± 12.92, respectively, p<0.05).

The mean running distance for the exercise group was 2808.98 ± 1571.46 m/day.

**Comparison of femoral bone mineral density among the three groups**

Figure 3 compares the bone mineral density of the left femur among the exercise, control, and cast immobilization groups. Femoral bone mineral density for the cast immobilization group (0.202 ± 0.012 g/cm²) was significantly lower than that for the exercise (0.25 ± 0.008) and control groups (0.243 ± 0.013, p<0.01).

In addition, a significant relationship was detected between average bone mineral density of the left and right femurs and average daily running distance for the exercise group (r = 0.93, p<0.01): the longer the running distance, the greater the bone mineral density (Figure 4).

**Comparison of bone mineral density among the four areas of the femur in the cast immobilization group**

The effects of cast immobilization on the bone mineral density of different areas of the bone were ascertained by measuring the bone mineral density of the four areas of the left and right femurs in the cast immobilization group. Table 1 shows the results. The total bone mineral density of the left and right femurs in the cast immobilization group was 0.202 ± 0.012 and 0.210 ± 0.006 g/cm², respectively; the difference was 0.009 ± 0.010 g/cm². Hence, the bone mineral density of the left femur (immobilized side) was lower, but no significant difference was noted,
Figure 3. Comparison of femoral bone mineral density among the exercise, control, and cast immobilization groups.

Femoral bone mineral density was significantly lower for the cast immobilization group (0.202 ± 0.012 g/cm²) than for the exercise (0.250 ± 0.008) and control groups (0.243 ± 0.013) (p < 0.01).

Figure 4. Relationship between running distance and bone mineral density in the exercise group.

The average daily running distance for the exercise group was 2806.98 ± 1571.46 m/day. A significant correlation was observed between average daily running distance and average bone mineral density of the femur (r = 0.93, p < 0.01).
Table 1. Comparison of bone mineral density among the different areas of the left and right femurs in the cast immobilization group

<table>
<thead>
<tr>
<th>Bone mineral density</th>
<th>Right femur (non-immobilized side)</th>
<th>Left femur (immobilized side)</th>
<th>Left/right difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bone mineral density (g/cm²)</td>
<td>0.210±0.006</td>
<td>0.202±0.012</td>
<td>0.009±0.010</td>
</tr>
<tr>
<td>Proximal epiphysis/metaphysis (L1) (g/cm²)</td>
<td>0.207±0.011</td>
<td>0.193±0.012 *</td>
<td>0.014±0.016</td>
</tr>
<tr>
<td>Femoral diaphysis (L2-L3) (g/cm²)</td>
<td>0.197±0.010</td>
<td>0.195±0.012</td>
<td>0.002±0.006</td>
</tr>
<tr>
<td>Distal epiphysis/metaphysis (L4) (g/cm²)</td>
<td>0.252±0.009</td>
<td>0.225±0.020 **</td>
<td>0.028±0.023</td>
</tr>
</tbody>
</table>

Mean ± S.D., *: p < 0.05, **: p < 0.01

Bone mineral density of the proximal epiphysis/metaphysis (L1) was significantly lower in the immobilized left femur (0.193±0.012 g/cm²) than in the right femur (0.207±0.011) (p<0.05). Bone mineral density of the diaphysis (L2/3) was lower in the left femur (0.195±0.012 g/cm²) than in the right femur (0.197±0.010) (N.S.). Bone mineral density of the distal epiphysis/metaphysis (L4) was significantly lower in the left femur (0.225±0.020 g/cm²) than in the right femur (0.252±0.009 X p<0.01).

Thus clarifying that decreased kinetic and mechanical stimulation due to cast immobilization lowered the bone mineral density of not only the immobilized side, but also the non-immobilized side. Bone mineral density of the left and right L1 areas (proximal epiphysis/metaphysis) was 0.193±0.012 and 0.207±0.011 g/cm², respectively; a significant left/right difference of 0.014±0.016 (p<0.05). Bone mineral density of the left and right L2 and L3 areas (femoral diaphysis) was 0.195±0.012 and 0.197±0.010 g/cm², respectively; a non-significant left/right difference of 0.002±0.006 g/cm². Bone mineral density of the left and right L4 areas (distal epiphysis/metaphysis) was 0.225±0.020 and 0.252±0.009 g/cm², respectively; a significant left/right difference of 0.028±0.023 g/cm² (p<0.01).

Comparison of bone mineral density of the distal epiphysis/metaphysis (L4) among the three groups

Comparison of the bone mineral density of the left and right femurs in the cast immobilization group showed that the degree of change in bone mineral density was marked for the distal epiphysis/metaphysis, and as a result, bone mineral density of the distal epiphysis/metaphysis was compared among the three groups (Figure 5). Bone mineral density of the distal epiphysis/metaphysis for the cast immobilization group (0.225±0.020 g/cm²) was significantly lower than that for the exercise group (0.295±0.007 g/cm²) and control group (0.283±0.018 g/cm²) (p<0.001). No significant difference was noted between the exercise and control groups.

Comparison of SEM images of the distal epiphysis/metaphysis among the three groups

Figures 6 to 8 show the SEM images of the distal epiphysis/metaphysis (L4) of the femur in the control, exercise, and cast immobilization groups.

In the control group, the osteoblasts had a diameter of 12.81 to 15.37 μm, were arranged as a monolayer on the endosteal surface, and formed an epithelial covering on the bone surface. The osteoblasts appeared as polygonal or round smooth cells in close contact with each other (Figure 6). In the exer-
Figure 5. Comparison of bone mineral density of the distal epiphysis/metaphysis (L4) among the exercise, control, and cast immobilization groups. Bone mineral density of the distal epiphysis/metaphysis (L4) for the cast immobilization group (0.225 ± 0.020 g/cm²) was significantly lower than that in the exercise (0.295 ± 0.007) and control (0.283 ± 0.018) groups (p<0.001).

Figure 6. SEM image of the distal epiphysis/metaphysis (L4) in the control group. In the control group, the osteoblasts had a diameter of 12.81 to 15.37 μm, were arranged as a monolayer on the endosteal surface, and formed an epithelial covering on the bone surface. The osteoblasts appeared as polygonal or round smooth cells in close contact with each other.

cise group, the osteoblasts had a diameter of 14.34 to 17.42 μm, were polygonal or round, had surface protrusions, were arranged on the bone matrix surface, and were actively forming bone. The osteoblasts covering the bone surface resembled a stone wall (Figure 7).

However, in the cast immobilization group, the osteoblasts had a diameter of 10.23 to 12.03 μm, and compared to the control and exercise groups, the cells were smaller and flatter. In addition, in the osteoblasts that covered the bone surface, disruption of the intercellular tight junctions was observed (Figure 8).

**DISCUSSION**

Decreased mechanical stress weakens osseous tissue by accelerating bone resorption and reducing bone mineral density. This reduced bone mineral density is characteristic of bones of the extremities
that support body weight, and imbalance in bone apposition and formation (osteoblasts and osteoclasts) plays a large role in such uncoupling. Moreover, long bones consist of areas that are mostly cortical bone and areas that are mostly cancellous bone, and as a result, the effects of mechanical stress on remodeling may differ in different areas. Analyzing the effects of increases and decreases in mechanical stress on the bone mineral density of different areas is meaningful in preventing disuse bone atrophy caused by a long-term reduction in mechanical stress.

Regarding studies investigating the effects of decreased mechanical stress on bone mineral density, Weinreb and colleagues\(^{15}\) severed the sciatic nerve in rats and reported that the bone mineral density of the femur decreased \(\geq 10\%\) only 10 days after immobilization. Eight weeks after the start of the present experiment, the degree of change in bone mineral density was calculated for the cast immobilization and exercise groups in comparison to that of the control group. Average bone mineral density for the cast immobilization group was 17% lower when compared to the control group. This reduction in bone mineral density was mostly due to bone resorption, and in the present study, although mechanical stress was decreased for a long period of time, the degree of decrease was comparable to the figure of 18% reported by Weinreb and colleagues. In our study, when compared to the control group, bone mineral density increased 3% for the exercise group. As to the effects of tail suspension on bone, reduced bone mineral density accompanied by decreased bone formation was seen 14 days after suspension\(^{15}\). Furthermore, Kodama and colleagues\(^{9}\) suspended rats by their tails and reported that the amount of force required to fracture the femur differed between the diaphysis and metaphysis. The reasons for this include atrophied cancellous bone, decreased number of trabecula, and advanced trabecular rupture. These findings suggest that decreased mechanical stress alters local bone metabolism; however, bone mineral density has not been thoroughly measured in different areas. In one study, bone mineral density was measured in seven areas of the rat femur to assess local bone metabolism\(^{16}\). However, scans were simply divided into seven sections to calculate bone mineral density. In another study, the
femur was divided into three sections (proximal 1/4, diaphysis, and distal 1/4)?, but when taking into account the cross-sectional properties of the femur and the internal structures of the cortical and cancellous components of the femur, the segmentation methods, reliability, and reproducibility of these previous studies were poor. Consequently, in the present study, the femur was divided into proximal and distal areas that are mostly cancellous bone and a diaphysis area that is mostly cortical bone, and the results showed that the bone mineral density of the proximal and distal ends for the cast immobilization group was significantly lower than that for the exercise or control group. Moreover, at the end of the experiment, body weight for the cast immobilization group was the lowest among the three groups, thus suggesting that progression in disuse atrophy due to cast immobilization involves atrophy of bone and connective tissue.

In the exercise group, running repeatedly impacted the ankle and knee. In particular, because the femur supports the body weight and transmits stimuli from the tibia, the effects of mechanical stress on the proximal end (hip joint) and the distal end (knee joint) should be high. Furthermore, mechanical stress applied to bone is transmitted to each constituent cell. The mechanical stress due to running is sensed by osteocytes in cortical bone and then transmitted to osteogenic cells and cell processes among osteocytes and osteoblasts, and communication with small vessels in bone is also believed to be an important pathway. In the exercise group, transmission of mechanical stress facilitated bone formation by osteoblasts, and this resulted in the difference in bone mineral density compared to the cast immobilization group. In addition, in the exercise group, continual running for 8 weeks resulted in a lower body weight than in the control group, and no direct correlation between hindlimb muscle weight and femoral bone mineral density was observed. However, femoral mineral bone density in the exercise group was correlated with running distance; thus, the increase in bone mineral density was related to amount of movement. The intensity of stress applied to bone by running is lower when compared to jumping or resistance movements, but the amount of exercise per day and the frequency of bone stimulation are greater. The greater the amount of exercise, the greater the mechanical signal transmitted to osteocytes, bone matrix, and osteoblasts. It is thought that bone formation and connective tissue strengthening are quickly adjusted based on the frequency of stimulation. In the cast immobilization group, decreased stimulation lowered the numbers of osteoblasts and osteocytes, thus advancing matrix decomposition. Therefore, in terms of the effects of exercise on bone, the frequency of stimulation appears to be more important than intensity.

However, as is the case with bone atrophy associated with excessive use (bone atrophy in marathon runners), excessive mechanical stress also causes bone atrophy, and there appears to be an optimal frequency for each individual. This issue needs to be investigated in the future.

Furthermore, the bone mineral density of the different areas of the femur was measured for cast-immobilized limbs. The results showed significant decreases in the bone mineral density of areas mostly composed of cancellous bone, and the degree of decrease was the largest for the distal epiphysis/metaphysis. The reason for the decrease in bone mineral density is as follows. In the hindlimb in rats, the knee joint acts as a support against the load by receiving and dispersing the load due to movement and the impact transmitted from the tibia to the femur?5). In other words, this site is an attachment point between the quadriceps ligament and bone. Therefore, the distal epiphysis/metaphysis of the femur, as compared to the proximal epiphysis/metaphysis of the femur that forms the hip joint, is more prone to the effects of increased and decreased mechanical stress. In addition, the distal epiphysis/metaphysis of the femur is an area rich in cancellous bone. As compared to cortical bone, cancellous bone has a larger contact surface area with the bone marrow, with 8 to 10 times greater bone metabolic tur-
never\textsuperscript{4)}. Thus, the changes in bone mineral density are greater. Moreover, bone mineral density for the cast immobilization group was lower than that for the other groups, and as a result, long-term joint fixation may hinder local bone formation by restricting joint movement and muscle contraction and chronically decreasing mechanical stress. As to the effects of decreased mechanical stress on osteoblastic cells, Machwate and colleagues\textsuperscript{10)} suspended the tail of rats for 14 days and documented that proliferation of cells created by the bone marrow of the tibia was suppressed, thus suggesting hindered proliferation of osteoblast precursors, rather than hindered differentiation into osteoblasts. In addition, Kela and colleagues\textsuperscript{8)} severed the sciatic nerve and then compared immobilized and non-immobilized rats with regard to differentiation of undifferentiated mesenchymal cells in the femoral bone marrow into osteoblasts. While no change was seen in the rate of proliferation, the formation of calcified nodules and the expression of alkaline phosphatase in osteoblast were suppressed. Therefore, decreased mechanical stress may suppress differentiation of undifferentiated marrow cells into osteoblasts, and severing the sciatic nerve may bring about changes based on not only mechanical stress, but also based on humoral factors due to modification in innervation. Hence, the results of the present study support Machwate's findings.

In addition, Abe reported\textsuperscript{1)} SEM findings of enlarged osteoblasts actively forming bone. Also, during osteogenesis, the osteoblasts were polygonal or round, with surface protrusions. In our study, SEM showed that the osteoblasts arranged as a monolayer on the endosteal surface had a diameter of 14.34 to 17.42 µm in the exercise group and 12.81 to 15.37 µm in the control group. Morphologic observation of the osteoblasts showed polygonal or round cells with surface protrusion. Osteoblasts are involved in osteogenesis and present on the bone matrix surface, so most of the organic constituents in bone matrix are synthesized and secreted by osteoblasts. In addition to their osteogenic potential, osteoblasts play an important role in calcification of the bone matrix lacunae and differentiation and induction of osteoclasts. On transmission electron microscopic observation\textsuperscript{17)}, enlarged osteoblasts active in bone formation have abundant rough-surfaced endoplasmic reticulum and actively secrete collagen. The surface of these cells characteristically has alkaline phosphatase activity. Although enzyme-antibody staining was not performed, our findings showed active formation of bone, and as a result, an increase in bone mineral density.

In the cast immobilization group, however, the osteoblasts had a diameter of 10.23 to 12.03 µm and were smaller and flatter than in the control and exercise groups. Furthermore, disruption of the intercellular tight junctions was observed. The reason for appearance of the flat osteoblasts can be attributed to greater osteoclastic resorption in the femur without mechanical stress. Osteoclasts\textsuperscript{12)} are multinucleated giant cells that resorb bone and play an important role in bone modeling and remodeling. Osteoclasts are also released from the bone surface and can migrate by pseudopod extension. Their morphology and size vary greatly. Osteoclasts, sometimes even a single osteoclast, can resorb multiple bone surfaces, and the interaction between osteoclasts and osteoblasts is involved in bone maintenance. In other words, after old bone matrix is resorbed by osteoclasts, new bone matrix is formed by osteoblasts. The bone remodeling process is divided into phases: activation, resorption, reversal, and formation. The least understood phase is reversal. The reversal phase is particularly difficult to identify in small animals. In general, macrophage-like mononuclear phagocytes migrate from the bone marrow and blood vessels, degrade organic substances remaining in the resorption cavities, and prepare the environment for subsequent formation of bone\textsuperscript{12)}. The resorbed bone matrix, as compared to unresorbed matrix, is more susceptible to the effects of mechanical stress. Thus, the stress may have a greater effect on regulating osteoblast differentiation and osteogenesis. Bone maintenance by a balance
with surrounding matrix due to bone formation probably occurred in the exercise group. However, in the cast immobilization group, the loss in bone matrix due to resorption is also large, and with bone formation, a balance is achieved with the surrounding matrix. In other words, decreased mechanical stress can suppress osteogenesis. This may signify a decrease in osteoblast activation. In osteoclastic resorption cavities, osteoblasts differentiate, and osteoblast enlargement or migration is assumed to be involved in new bone formation. But there are no reports regarding this issue. In addition, osteoblast morphology and new bone formation near the resorption cavities varies with the extent of osteoclastic resorption. If the bone resorption cavity is small, osteoblasts enlarge and migrate, and new bone is formed. If the bone resorption cavity is large, osteoblasts enlarge (i.e. large cells), and there is covering by flat osteoblasts. Therefore, with decreased mechanical stress due to cast immobilization, the effects on bone resorption are greater than on bone formation. This accounts for the disruption in tight junctions between adjacent cells seen with flattened osteoblasts.

Furthermore, we reported\(^5\) that osteocytes form a mechanical network among cells, matrix, and osteoblasts, and that they express cell growth factors and bone formation factors in relation to the amount of stimulus transmission, thus closely adjusting bone formation. The results of the present study do not directly indicate whether or not cast immobilization caused the changes in bone mineral density and microstructure of osteoblasts via IGF-1 and TGF-\(\beta\) signals\(^{11}\). However, in the exercise group, the osteoblasts had surface protrusions and actively formed bone, thus suggesting that osteoblast morphology plays an important role in BMU (basic multilocular unit)\(^{35}\) remodeling in cancellous bone trabeculae and bone mineral density in the distal epiphysis/metaphysis of the femur.

The above findings suggest that, in the exercise group, repeated impact to the distal epiphysis/metaphysis facilitated the resorption of cancellous bone and the bone formation and calcification by osteoblasts. In contrast, in the cast immobilization group, immobilization suppressed stimulation and muscle contraction and weakened mechanical stimulation to osteocytes in the cancellous and cortical bone, thus suppressing bone formation.

Our findings suggest that a decrease in mechanical stress due to cast immobilization chronically suppresses bone formation and delays cancellous bone turnover. This has an effect on decreasing bone mineral density in the distal epiphysis/metaphysis of the femur.

Our study on the effects of decreased mechanical stress due to cast immobilization on regional bone mineral density and osseous microstructure in the rat femur showed decreased bone mineral density in the cancellous bone-predominant proximal epiphysis/metaphysis and distal epiphysis/metaphysis of the femur. The effect on decreasing bone mineral density was particularly marked in the distal epiphysis/metaphysis of the femur.

Long-term decrease in stress and movement stimulation due to cast immobilization alters the microstructure of osteoblasts and results in a persistent reduction in bone formation in the rat femur. However, the relationship between reduction of the bone mineral density of different areas of bone and the microstructure of osteoblasts remains to be clarified. Nonetheless, in the fields of sports medicine and orthopedics, the present findings might provide evidence that in order to avoid osteoporotic fracture, prevention is important for areas at greater risk of bone atrophy.

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