Suspension “Hypokinesia/Hypodynamia” May Decrease Bone Mass by Stimulating Osteoclast Production in Ovariectomized Mice

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Summary This study was conducted to examine, in detail, the histological changes in the femurs of suspended ovariectomized (OVX) mice to assess the role of mechanical stress on bone remodeling. Suspended-OVX, suspended-sham-ope, nonsuspended-OVX, and nonsuspended-sham-operated mice underwent operations 8 weeks after birth. Immediately after operation, hypokinesia/hypodynamia was created by a suspension harness for one week. Five specimens in each group were sacrificed 9 weeks after birth. The trabecular bone of the femurs in the suspended-OVX mice was removed and replaced extensively by bone marrow. The number of tartrate-resistant acid phosphatase (TRAP)-positive cells was larger in the suspended-OVX mice than in the remaining three groups. No significant differences in the number of TRAP positive cells were found between the suspended-sham-ope, nonsuspended-OVX and nonsuspended-sham-ope mice. The femurs of the OVX mouse with suspension “hypokinesia/hypodynamia” thus exhibits extensive trabecular bone loss in association with an increase of osteoclasts.

Key Words bone morphology, hypokinesia/hypodynamia, osteoclast, OVX, suspension

Muscle atrophy is a characteristic phenomenon of prolonged hypokinesia resulting from restricted movement, prolonged bed rest, limited muscle activity, or immobilization (1–6). The causes may be disuse, inadequate functional loading, insufficient food intake, or lack of exercise. Moreover, bone decalcification is regarded as a typical symptom of the same conditions as mentioned above (1–3, 4). With reduced loading to the musculoskeletal system from weightlessness in rats,
trabecular bone loss has been demonstrated (7). Prolonged immobilization also leads to a negative trabecular bone balance (8). The related weight loss may also be attributed to dietary effects. Given these previous findings, it is of great significance to study the mechanisms of hypokinesia/hypodynamia to find solutions to the various physiological problems resulting from prolonged bed rest (9).

To this end, short-term hypokinesia was induced in an osteoporosis mouse with ovariectomy (OVX), and the present study was thus conducted to examine, in detail, the histological changes in the femurs of suspended-OVX mice to assess the effects of mechanical loading on bone remodeling in terms of osteoclast expression.

MATERIALS AND METHODS

C57BL male and female mice were imported from Jackson Laboratory (Bar Harbor, ME, USA). The mice were kept in metal cages (22 × 32 × 11 cm) with autoclaved wood chips for bedding in an animal room (temperature 24±2°C; relative humidity 50±5%). The study was approved by Hiroshima University of Animal Use Committee, and the animals were maintained in accordance with the guidelines issued by Hiroshima University for the care and use of laboratory animals. The 20 mice were divided equally into four groups of suspended-ovariectomized (OVX), suspended-sham-ope, nonsuspended-OVX, and nonsuspended-sham-operated mice. They were fed a solid diet (CE2: Clea, Tokyo, Japan), individually supported or housed, and weighed every day during the experimental period. All animals underwent operations or sham operations 8 weeks after birth. The suspended group was placed in a suspension harness made of denim material and fastening tape for one week immediately after the surgery (Fig. 1). The mice were sacrificed 9 weeks after birth.

Morphological changes of the femurs were examined for suspended-OVX, suspended-sham-ope, nonsuspended-OVX, and nonsuspended-sham-operated mice by use of soft X-ray imaging (RM-60: Asahi Roentgen, Kyoto, Japan) and histological techniques. Radiographs were taken immediately before the sacrifice.
Suspension “Hypokinesia/Hypodynamia” on Bone Remodeling in OVX Mice

Fig. 2. Changes in body weight for five mice in each group.

with an accelerating voltage of 20 kV and exposures of 3.0 s.

For light microscopic observation, the femurs removed from the mice were fixed with 4% formaldehyde for 12 h at 4°C, decalcified in 5% ethylenediamine tetra-acetic acid (EDTA, pH 7.4) for one week, embedded in paraffin, and cut into longitudinal sections of 7 μm thickness. The sections were stained with hematoxylin and eosin (HE) and for tartrate-resistant acid phosphatase (TRAP), which is generally acknowledged as a cytochemical marker for osteoclasts, and finally counterstained with hematoxylin. ANOVA was carried out, followed by Scheffé tests to detect pairwise differences among the four groups.

RESULTS

1. Body weight

The body weight of the suspended-OVX and suspended-sham-ope mice decreased up to 3 d, then exhibited a slight increase from 3 to 7 d. Suspended-OVX and suspended-sham-ope mice thus showed no detectable differences in body weight. The body weight of nonsuspended-OVX mice was not substantially different from that of nonsuspended-sham-operated mice (Fig. 2).

2. Radiographic findings

Soft X-ray imaging revealed that the trabecular bone of the femurs in the suspended-OVX mice was massively removed and replaced by bone marrow (Fig. 3A). In the suspended-sham-ope and nonsuspended-OVX mice, no major differences in bone structure were observed in comparison with the nonsuspended-sham-operated mice (Figs. 4A, 5A, and 6A).

3. Histological findings

The suspended-OVX, suspended-sham-ope, nonsuspended-OVX, and non-
Fig. 3. (A) Radiograph of the femur in a 9-week-old suspended OVX mouse. Arrowheads indicate bone marrow. Original magnification ×5.6. B, C, and D, longitudinal sections of the distal portion of the whole femur stained with TRAP activity and counterstained with hematoxylin. (B) Distal portion of femur. Original magnification ×28. (C) Secondary ossification of femur. (D) Epiphyseal growth plate of femur. Arrows indicate TRAP-positive osteoclasts. Original magnification ×70.

suspended-sham-operated mice showed no detectable differences in femur sizes and shapes (Figs. 3B, 4B, 5B, and 6B). Histologically, the cartilage growth plate in the suspended-OVX, suspended-sham-ope, nonsuspended-OVX, and nonsuspended-sham-operated mice showed a typical cartilaginous growth plate structure composed of multiplying cells (Figs. 3D, 4D, 5D, and 6D).
Fig. 4. (A) Radiograph of the femur in a 9-week-old suspended non-OVX mouse. Original magnification ×5.6. B, C, and D, longitudinal sections of the distal portion of the whole femur stained with TRAP activity and counterstained with hematoxylin. (B) Distal portion of femur. Original magnification ×28. (C) Secondary ossification of femur. (D) Epiphyseal growth plate of femur. Arrows indicate TRAP-positive osteoclasts. Original magnification ×70.

In contrast, the femurs of the suspended-OVX mice had a wide bone marrow cavity, and numerous TRAP-positive osteoclasts were observed on the trabecular bone surface (Figs. 3C and D). The bone marrow cavity with cell multiplication was larger in the suspended-OVX mice than in the suspended-sham-ope and nonsuspended-OVX mice (Figs. 3B, 4B, and 5B). Moreover, the bone marrow cavity...
was slightly larger in the nonsuspended-OVX mice than in the suspended-sham-ope mice (Figs. 4B and 5B). The trabecular bone was wider in the nonsuspended-sham-operated mice than in other three groups (Figs. 3B, 4B, 5B, and 6B).

The number of TRAP-positive cells on the trabecular bone surface was greater in the suspended-OVX mice than in the suspended-sham-ope, nonsuspended-OVX,
Fig. 6. (A) Radiograph of the femur in a 9-week-old sham-operated mouse. Original magnification × 5.6. B, C, and D, longitudinal sections of the distal portion of the whole femur stained with TRAP activity and counterstained with hematoxylin. (B) Distal portion of femur. Original magnification × 28. (C) Secondary ossification of femur. (D) Epiphyseal growth plate of femur. Arrows indicate TRAP-positive osteoclasts. Original magnification × 70.

and nonsuspended-sham-operated mice. TRAP-positive cells significantly decreased in the suspended-sham-ope, nonsuspended-OVX (p < 0.05), and nonsuspended-sham-operated groups (p < 0.01), compared with the suspended-OVX mice (Fig. 7). No differences in the number of the TRAP-positive cells were found between the suspended-OVX and nonsuspended-OVX mice. The number of TRAP-positive
cells was least in the nonsuspended-sham-operated mice, but not significantly different from those in the suspended-sham-ope and nonsuspended-OVX mice (Fig. 7). These findings may account for the extensive resorption of trabecular bone in the suspended-OVX mice (Fig. 3B).

**DISCUSSION**

Various hypokinesia conditions have been studied in association with the histological structures of the musculoskeletal system (1-6). Some previous studies have focused on the association of weightlessness with muscle atrophy because mineral loss, which often results in a negative mineral balance, is among the most consistent and repeatedly observed responses of bony structures to long-term bed life, immobilization, or weightlessness (1-4). It may thus be assumed that hypokinesia/hypodynamia substantially affects the growth and development of hard and soft tissues.

This study was designed to investigate the influences of hypokinesia/hypodynamia on bone remodeling, with special reference to osteoclast expression, in an experimental mouse model with artificial suspension and ovariectomy. In this study, the body weight was mainly loaded at the abdominal region. The weight loss observed for the suspended groups in this experiment may be due to reduced food intake compared with the nonsuspended mice. The number of TRAP-positive cells and extent of trabecular bone loss were greater in the suspended-OVX mice than in the suspended-sham-ope, nonsuspended-OVX, and nonsuspended-sham-operated mice. No significant differences in the number of TRAP positive cells were found between the suspended-sham-ope and nonsuspended-OVX mice.

Previous studies have demonstrated that OVX increases the number of colony-forming units of granulocytes and macrophages, enhances osteoclast development.
in in vivo cultures of bone marrow, and ultimately increases the number of osteoclasts in trabecular bone \((10, 11)\), which became most prominent 3 to 4 weeks after the operation. Moreover, TRAP-positive cells are increased by 3 weeks of space flight \((12)\). Weinreb et al also reported that adult rats exhibited a decrease in bone mass at 3 weeks during space flight \((8)\), and Globus et al pointed out that bone formation was inhibited in simulated suspended rats during a 5-d experiment and that bone mass was absorbed after 14 d \((13)\). From these findings, it is supposed that suspended-sham-ope and nonsuspended-OVX mice would exhibit a tendency to increased numbers of osteoclasts if the experiment were continued for more than three weeks. Mechanical stress regulates the production of transforming growth factors (TGF-beta 1 and 2) and bone morphogenetic protein (BMP), which initiate the activities of osteoblasts that are key to bone formation \((14)\). Thus the inhibition of new bone formation may be related in part to the decreases in bone mass resulting from reduced mechanical stress and ovariectomy.

In this experiment, the trabecular bone of the femurs in the suspended-OVX mice was removed and extensively replaced by bone marrow. Moreover, the femurs of OVX mice with suspension “hypokinesia/hypodynamia” exhibited an increase in osteoclasts, which was apparently due to the reduced mechanical stress associated with immobilization and/or weightlessness, although OVX showed a similar tendency to increase osteoclasts.

Although a primary cause of trabecular bone resorption in the suspended-OVX mice is estrogen loss from ovariectomy and an attendant lack of bone remodeling, the present findings suggest that mechanical stress also plays an important role in the histological structures of the femurs. Therefore it appears that long-term immobilization and the resultant deficiency of mechanical stress produce extensive bone resorption, which is accelerated when accompanied by estrogen loss.

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