How many days per week should rats undergo running exercise to increase BMD?

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Abstract The aim of the present study was to examine the effect of different frequencies of running exercise on increasing bone mineral density (BMD) and improving bone histomorphology at various sites of the skeleton (tibia, femur, and second lumbar vertebra) in young rats. Twenty-five female Wistar rats, 8 weeks old, were divided into five groups, of 5 animals each according to running load: control group, no running (A group); running load (RL), 4 days per week (d/w; B group); RL, 5 d/w (C group); RL, 6 d/w (D group); and RL, 7 d/w (E group). Rats ran on a treadmill at a speed of 15m/min for 30min per day over an 8-week period. The results indicated that the BMD of the tibia in the B, C, D, and E groups and that of the femur in the B and E groups increased significantly over that of the A group. However, the cortical BMD and trabecular BMD of the second lumbar vertebra did not change. In regard to bone histomorphometry of the tibia, a parameter of bone resorption (eroded surface/bone surface) was significantly lower in the B and D groups than in the A group. There were no differences in the parameters of bone formation. Tartrate acid-resistant acid phosphatase (TRACP) values were significantly lower in the B and C groups than in the A group. There were significant increases in body weight in the B group and in muscle weight in the C group. From the data obtained in this study, it was concluded that increases in BMD were obtained by a moderate running load at frequencies of 4 and 5 days per week.

Key words Running exercise · Rat · Treadmill · Bone mineral density · Bone resorption

Introduction

Although many investigators suggest that exercise is effective in preventing osteoporosis in humans, a definite conclusion has not yet been reached, because different kinds, periods, frequencies, and strengths of exercises have been recommended. From the clinical viewpoint, it is important to determine the optimal conditions of exercise both for increasing bone mineral density (BMD) before menopause begins, and for preventing a decrease in BMD after menopause.

Running exercise is recommended as one of the methods to prevent and treat osteoporosis, because of its effectiveness in maintaining and increasing BMD. Many studies concerning the relation between exercise and BMD in the premenopausal period, including puberty, have suggested that moderate exercise can increase BMD, while excessive exercise, as in marathon runners, induces a decrease of BMD [1–6]. Running exercise is one of the preventive methods that is accessible to all; however, it is difficult to determine what exactly are moderate conditions.

In addition, it remains problematic that, while running exercise is locally effective in bones of loaded sites, it has shown less, if any, efficacy on bones of the whole skeleton. Goto et al. [7] reported that moderate exercise for premenopausal women increased or maintained the BMD of the femoral neck, but the effect on the lumbar vertebrae was slight. Cavanaugh and Cann [8] also reported that light physical exercise, such as walking, in postmenopausal women could not prevent a decrease in BMD of the lumbar vertebrae. In fact, Goto et al. [7] observed some postmenopausal women in whom a long-term exercise program did not prevent the marked decrease of BMD in either the lumbar vertebrae or femoral neck.

Running conditions employed in animal experiments have varied widely. For example, Barengolts et al. [9–11]
imposed a running load of 4 days/week in 9 month-old ovariectomized rats, and found that moderate exercise could prevent a decrease in BMD. Bourrin et al. [12] determined that, in 9 week-old male rats, a running speed of 30m/min under a condition of 60% VO$_{2\text{max}}$ could increase BMD more than that of 80% VO$_{2\text{max}}$. However, Bourrin et al. [13] observed no increase in BMD from a running load in 5-week old male rats.

The purpose of the present study was to determine the frequency per week of running exercise in moderate conditions in rats that was effective in improving BMD and to discover whether the exercise had a demonstrable effect on bones other than those in the loaded limbs.

### Materials and methods

#### Animals and breeding conditions

Twenty-five female Wistar rats, 6 weeks old, with a mean weight of 120.6g, were purchased. Five animals per cage were kept in a room with a temperature of 22±2°C; relative humidity, 55±5%; and a 12-h dark and light cycle. They were allowed free access to commercial solid food (BM-1; Funabashi Farm, Funabashi, Japan), containing 1.2% calcium, and drinking water.

#### Running exercise and procedures

A treadmill device (Shinano, Tokyo, Japan) was used for providing a running load to the rats. Rats were preexercised for 2 weeks to habituate them to the device system and to determine suitable running conditions, as described later. The rats were then divided into the following groups: control (no running; A group); running load (RL) for 4 days/week (d/w; B group); RL, 5d/w (C group); RL, 6d/w (D group); and RL, 7 d/w (E group). Each group consisted of five animals (Table 1). Running load was initiated when the rats were 8 weeks old.

The running conditions were as follows. All rats ran at a treadmill speed of 15m/min, a speed at which they ran voluntarily and did not drop out. The running time was 30min once a day for a variable number of days per week, over a period of 8 weeks [14].

#### Measured items

Body weights and food intakes of the rats were measured every day before and after running to check for untoward findings arising from the load. To determine whether the treadmill load affected the 4-day sexual cycle of the rats, vaginal smears were collected at 11:00 a.m. daily for 5 days in the first and third weeks, and for 10 consecutive days from the sixth week of the experiment. The collected vaginal smears were stained with Giemsa, and cells were observed under a light microscope. The rats were injected intraperitoneally with tetracycline, at a dose of 2.5mg/100g body weight, at intervals of 7 days, before the completion of the experiment, to observe bone dynamics in bone histomorphometry.

The rats were killed under anesthesia using chloroform. The blood was collected from the posterior vena cava after celiotomy, and centrifuged at 3000rpm; serum was frozen and stored at minus 20°C. Concentrations of total serum calcium (Ca), phosphorus (P), and tartric acid-resistant acid phosphatase (TRACP) were determined by the O-CPC method, the molybdenum blue method, and the P-NPP substrate method, respectively.

Skeletal muscles (soleus and digitorum profundus extensor digitorum longus [EDL]) of the right hind limb and bones (tibia, femur, and second lumbar vertebra) were extracted. The Skeletal muscles were weighed to obtain the percentage of body weight (muscle weight / body weight at completion of the experiment × 100).

Bone samples; namely, the tibia, femur, and second lumbar vertebra, were fixed in 70% ethanol alcohol after muscles and tendons were completely removed. Measurements of BMD and the bone cross-sectional area were performed using a pQCT (voxel: 148μm, 18.9mm; XCT-960A) (Norland and Stratec, Germany). In this system, values of less than 395 and more than 690mg/cm$^2$ are regarded as trabecular BMD and cortical BMD, respectively. The trabecular BMD was measured at a position 3mm from the center of the cartilage plate image in the distal epiphysis of the femur and in the proximal epiphysis of the tibia; cortical BMD was measured 12mm away from the respective cartilage plate. Both the trabecular BMD and cortical BMD were measured at the center of the transverse cross-sectional direction of the second lumbar vertebra.

After the measurement of BMD, the proximal one-third of the tibia was immersed in Villanueva bone stain solution, dehydrated in alcohol and acetone, and then embedded in methylmethacrylate. The block was thin-cut with an inner blade cutter (MC-808D; Maruto, Tokyo, Japan) and ground

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**Table 1. Schedule of running exercise in each group**

<table>
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○, running; X, no running (resting)