Effect of decreased physical activity on bone mass in exercise-trained young rats

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Abstract The aim of this study was to determine whether decreased physical activity in exercise-trained young rats would result in a lower rate of bone gain or a reversal of the benefits of exercise. Thirty-five female Wistar rats, 6 weeks of age, were randomized into seven groups: 7 weeks of exercise (7EX), 7 weeks of sedentary control (7CN), 11 weeks of exercise (11EX), 7 weeks of exercise followed by 4 weeks of exercise cessation (7EX4C), 7 weeks of exercise followed by 4 weeks of decreased exercise frequency (7EX4F), 7 weeks of exercise followed by 4 weeks of decreased exercise intensity (7EX4I), and 11 weeks of sedentary control (11CN). The running intensity (speed) and duration were 25 m/min for 60 min/day at a frequency of 5 days/week. During the last 4 weeks, exercise frequency was reduced to 1 day/week in the 11EX4F group, and exercise intensity (speed) was reduced to 12 m/min in the 7EX4I group. After each period of exercise, the bone mineral content (BMC) of the proximal, middle, and distal tibiae, determined by dual-energy X-ray absorptiometry (DXA), was significantly greater in the 7EX and 11EX groups than in the 7CN and 11CN groups, respectively, but it was significantly lower in the 7EX4C group than in the 11EX group and did not differ significantly from the values of the 11CN group. Although the BMC of the proximal and middle tibiae did not differ significantly among the 7EX4F, 7EX4I, 7EX4C, and 11CN groups, the BMC of the distal tibia was significantly greater in the 7EX4F and 7EX4I groups than in the 11CN group and tended to be greater than in the 7EX4C group. The results of this study suggest that the effect of decreased exercise intensity and frequency on bone mass appears to be site specific in the tibia of the exercise-trained young rats. This study shows that exercise-trained young rats lose the benefits gained from exercise when exercise is completely ceased, resulting in the reduction of bone mass to levels that do not differ significantly from those of sedentary controls. At least, continuous exercise appears to be necessary for the maintenance of high bone mass.

Key words Exercise · Growing rats, Bone mineral content (BMC) · Physical activity

Introduction

Current strategies for the prevention of osteoporosis focus on maximizing bone mass early in life during growth and maturation and minimizing loss later in life.1,2 A maximal bone mass at skeletal maturity, in particular, is considered to be the best protection against age-related bone loss and the subsequent risk of bone fractures.17,18 Physical activity during childhood and adolescence may be one of the most important determinants of peak bone mass. The available evidence suggests that exercise during this period may promote gains in bone mass, leading to an increase in peak bone mass,6,7,13,15,19,21 and that the skeleton may be most responsive to exercise during this period.3,10,12,20,23

Several well-controlled studies have been reported on the effects of exercise cessation on bone mass. In human studies, Vuori22 showed that unilateral limb strength training increased the bone mineral density (BMD) in both the trained and untrained limbs of young women, but decreased toward its baseline value after training was terminated. Dalsky et al.8 also demonstrated that short- and long-term weight-bearing exercise led to significant increases above the baseline value in the lumbar bone mineral content (BMC) of postmenopausal women but that BMC reverted to baseline levels after exercise was ceased. On the other hand, Kontulainen et al.14 showed that an exercise-induced bone gain could be well maintained with decreased activity in female tennis and squash players and that maintenance of the bone gain was independent
of the starting age of activity. In animal studies, Le Blanc et al.\textsuperscript{16} showed that the increased rate of total body calcium gain produced by voluntary exercise was reversed following exercise cessation in adult rats. Yeh and Aloia\textsuperscript{24} also demonstrated that bone mass gained from treadmill exercise was lost during deconditioning in young female rats after the period of their growth spurt. All these studies suggest that bone mass gained from exercise during adulthood may be lost if the exercise program is stopped. Whether bone mass gained through exercise during the period of growth spurt is maintained, decreased, or entirely lost by the cessation of exercise during adulthood is unclear.

Recently, Iwamoto et al.\textsuperscript{11} found that the response of cancellous bone to treadmill exercise in young growing rats differed among the proximal and distal tibiae and the lumbar vertebra. In particular, the bone gain from exercise was greater in the distal tibia than in the proximal tibia. Thus, we speculated that bone response to decreased physical activity in exercise-trained young rats might also differ according to the skeletal sites. In this study, we examined the effect of moderate treadmill running exercise on the BMC of the proximal, middle, and distal tibiae and the lumbar vertebra in young growing rats during the period of their growth spurt, and also examined whether decreased physical activity in the exercise-trained young rats resulted in a decrease in bone gain or a reversal of the benefits of exercise.

**Materials and methods**

**Animals and exercise program**

Thirty-five female Wistar rats, aged 3 weeks, were purchased from CLEA Japan (Tokyo, Japan) and housed in individual cages (25 × 18 × 34 cm\textsuperscript{3}) in a specific pathogen-free room with a temperature of 23°C ± 2°C, humidity of 55% ± 5%, and a 12-h on: off light cycle. The rats were allowed free access to water and a pelleted chow diet (CE-2; CLEA Japan). After 3 weeks of adaptation to this diet and new environment, the 6-week-old rats were randomized using a stratified weight method into seven groups of 5 animals each: 7 weeks of exercise (7EX), 7 weeks of sedentary control (7CN), 11 weeks of exercise (11EX), 7 weeks of exercise followed by 4 weeks of exercise cessation (7EX4C), 7 weeks of exercise followed by 4 weeks of decreased exercise frequency (7EX4F), 7 weeks of exercise followed by 4 weeks of decreased exercise intensity (7EX4I), and 11 weeks of sedentary control (11CN). The usual exercise regimen consisted of running on a flatbed treadmill (Shinano, Tokyo, Japan) for a specific period of time each day. During the first 2 weeks, the speed of the treadmill and the duration of each running session were gradually increased from 8 m/min for 5 min to 14 m/min for 45 min. The running speed and duration were then gradually increased to 25 m/min for 60 min/day in the third week, and this speed and duration were maintained at a frequency of 5 days/week for the rest of the exercise period. During the last 4 weeks, the exercise frequency was reduced to 1 day/week in the 11EX4F group, and the exercise intensity (speed) was reduced to 12 m/min in the 7EX4I group. This reduction of exercise speed by almost half may result in the reduction of the frequency of mechanical loading that the tibia and femur receive during 60 min of running exercise by almost half. The experiment was performed at Kitasato Institute Hospital, and the protocol was approved by the Research Animal Resource Committee of Keio University.

**Bone markers**

After the exercise regimen had been completed, the 24-h urine was collected using a metabolic cage. A blood sample was taken from the aorta under ether respiratory anesthesia. Urinary deoxypyridinoline levels (an indicator of bone resorption) were measured using enzyme immunoassay (Sumitomo, Osaka, Japan). Serum osteocalcin levels (an indicator of bone formation) were measured using a radioimmunoassay (BTI, Tokyo, Japan).

**Length of femur**

After the urine and blood samples had been collected, the animals were killed by exsanguination from the right axillary artery. After death, the right femur was dissected free of soft tissue. The length of the femur was then measured using a dial caliper (minimal scale, 0.01 mm; aberration, 0.03 mm).

**Bone densitometry**

The right tibia and the fifth lumbar (L5) vertebra were dissected free of soft tissue. Each bone was put on an acrylic plate (20 mm thick) in air and scanned by dual-energy X-ray absorptiometry (DXA) using a regular Lunar DPX-L instrument adapted for the measurement of small animals. A high-resolution mode (voltage, 76.0 kVp; current, 150 μA; collimation, fine; sample size, 0.15 × 0.3; sample interval, 1/64) was used with a scan width of 15 mm and scan lengths of 50 mm for the total tibia and 20 mm for the L5 vertebra. The BMC of the total tibia, proximal third tibia, middle third tibia, distal third tibia, and L5 vertebra was analyzed. The reproducibility of the data was evaluated by measuring