The influence of food restriction on bone in young female rats with voluntary wheel running over 5 weeks

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Abstract The aim of the present study was to report on the influence of food restriction on the bones of young female rats undergoing voluntary wheel running over 5 weeks. Seven-week-old female Sprague-Dawley rats were divided into three groups after a 1-week acclimatization period: a sedentary and ad libitum feeding group (SED), voluntary running exercise and ad libitum feeding group (EX), and voluntary running exercise and 30% food restriction group (EX-FR). The experiment lasted for 38 days. Results showed that the dry weight of the femur, length of the femur, bone mineral content (BMC) of the tibia, bone area of the tibia, bone mineral density (BMD) of the tibia, BMC of the lumbar, and bone area of the lumbar were significantly lower in the EX-FR group than in the SED and EX groups. BMD of the lumbar was not significantly different in each group. In conclusion, this study shows the possibility of constructing a rat model of a female athlete with low BMD in a short period of time.

Keywords: exercise, low food intake, bone mineral density, osteoporosis, short-term experiment

Introduction

Low bone strength is a significant problem in young female athletes⁷,². While exercise is an effective method for increasing bone strength by mechanical loading⁸, some studies reported that >15% adolescent female athletes have low bone mineral density (BMD)⁹,⁶. There is the concern that low BMD in female athletes is caused by strict dietary restriction or eating disorders¹⁰.

A rat model for female athletes with low BMD has been developed by other groups¹⁰ and by our group¹⁰. In a human study, it is difficult to study about low bone strength because of difficulties associated with collecting experimental subjects and regulating experimental conditions. Animal research solves these limitations and makes a detailed physiological examination possible within a short period. In mature female rats with voluntary wheel running, Dimarco et al.⁷ and Yanaka et al.¹⁰ reported that food restriction resulted in low bone mineral content (BMC) and BMD in the tibia and femur. In mature female rats undergoing treadmill running exercise, Swift et al.¹⁰ reported that food restriction caused low BMC of the whole body. In addition to these reports, our group reported that food restriction caused low breaking force and energy in the femur and low BMC and BMD in the tibia and lumbar in young female rats with voluntary running¹⁰. In these previous studies, the experimental period was over 12 weeks. We set our experimental period based on clear bone deterioration. The reason for selecting the experimental period in the other groups is unknown, but it may be similar to our rationale. Until now, there are no reports that selected an experimental period of less than 12 weeks for evaluating the influence of food restriction on bone in female rats with exercise. If clear changes in bone status can be observed in a short period, it will be beneficial in terms of labor, speed, and economics.

The aim of the present study was to report on the influence of food restriction on the bones of young female rats undergoing running exercise over 5 weeks. The selection period was accidental because the Great East Japan Earthquake cut short our 12-week experiment. Although our data were imperfect, we have reported our findings.

Materials and Methods

Experimental design. Female Sprague-Dawley rats (n = 25, 7 weeks old) were randomly divided into three experimental groups after a 1-week acclimatization period.
The groups included a sedentary and ad libitum feeding group (SED, n = 8), a voluntary running exercise and ad libitum feeding group (EX, n = 8), and a voluntary running exercise and 30% food-restricted group (EX-FR, n = 9). We had planned a 12-week experimental period; however, the actual experiment period was 38 days because the animal room became unusable due to the Great East Japan Earthquake. The earthquake hit this experiment on day 35, and it was considered that the rats might have suffered some stress from the earthquake. The rats were purchased from CLEA (Tokyo, Japan) and were fed a normal diet. Rats in the SED group were housed individually in regular cages (15 × 25 × 19.5 cm) whereas those in the EX and EX-FR groups were housed individually with free access to a wheel cage for voluntary running exercises (circumference, 1 m; 27 × 35 × 35 cm). The EX-FR group was fed a 30% restricted diet that contained 70% of the mean amount consumed in the previous week by the SED group. Therefore, the EX-FR group was fed a diet with a mean 37% reduction compared to the EX group. The room was maintained at 22°C ± 1°C under a constant 12:12 h (hour) light-dark cycle (light 8:00-20:00 h). Animal care and experimental procedures were approved by the Animal Experimental Committee of the University of Tsukuba (approval number: 11-377).

**Daily data collection and specimen harvesting.** Body weight and amount of dietary intake was measured every second day, and running distance was measured every day. The femurs, tibias and lumbar were collected from each rat two months after death. The adhering connective tissue was removed from the femur and the size was measured. The femur was then dried at 100°C for 24 h in an electric furnace, and the dry weight was measured. The tibias and lumbar were stored in 70% ethanol after being harvested and cleaned of soft tissue to measure BMC, bone area, and BMD.

**Calculation of energy availability.** Energy intake, exercise-induced energy expenditure, and energy availability were calculated as previously described. Briefly, energy intake was calculated by multiplying the amount of food intake in grams with the energy content of the food (3.73 kcal/g) (energy intake = food intake · 3.73 kcal/g). Exercise-induced energy expenditure due to daily wheel running was calculated by taking 5.0 kcal/kg body weight times the km run, as described previously (energy expenditure = wheel running distance · body weight · 5.0 kcal/kg body weight/km). Energy availability was calculated as energy intake subtracted by exercise energy expenditure, as previously described.

**Measurement of BMC, bone area, and BMD using dual-energy X-ray absorptiometry (DXA).** BMC, bone area, and BMD values of the tibia and L3-L6 of the lumbar spine were measured using DXA (Aloka, DCS-600R, Tokyo, Japan), as previously described.

**Statistical analysis.** All data are expressed as means ± standard error (SE). The statistical analysis was conducted using a one-way analysis of variance (ANOVA), and if a significant difference was observed, the variables in the three groups were analyzed using Tukey’s post-hoc comparison tests. Running distance and exercise-induced energy expenditure were analyzed using an unpaired t-test. For analysis of correlation between running distance and BMD in EX and EX-FR groups, Pearson correlation coefficient was used. The significance level was set at p < 0.05. All statistical analyses were performed using SPSS Ver. 19.0 (SPSS Inc., Chicago, USA).

**Results and Discussion**

**Food intake, running distance, and energy availability.** Table 1 presents the results of food intake, running distance, and related categories of energy availability. Food and energy intake were significantly higher in the EX group than in the SED group, and food and energy intake were significantly lower in the EX-FR group than in SED and EX groups. The wheel running distance in the EX-FR group was significantly higher than that in the EX group. Exercise-induced energy expenditure was not significantly different between EX and EX-FR groups. Energy availability was significantly lower in the EX-FR group than in SED and EX groups.

**Body weight and bone size.** Table 2 presents the results of the initial body weight, final body weight, dry weight of the femur, length of the femur, and major and minor axes of the femur. None of these parameters were significantly different between the SED and EX groups. The final body weight, dry weight of the femur, and length of the femur were significantly lower in the EX-FR group than in SED and EX groups. These results show that food restriction over 38 days resulted in growth inhibition of the whole body and bone size in young female rats undergoing running exercise. Major and minor axes of the femur were not significantly different between the groups.

**BMC, bone area, and BMD.** Fig. 1 presents BMC, bone area, and BMD results. None of these parameters were significantly different between SED and EX groups. In our previous study that had a 12-week experimental period, voluntary wheel running led to a high BMC and bone area of the tibia. The present results show that a 38-day experimental period did not show any positive effects of exercise on the bone because the experimental period was short.

BMC of the tibia, bone area of the tibia, BMC of the lumbar, and bone area of the lumbar were significantly lower in the EX-FR group than in SED and EX groups (Fig. 1). BMC of the tibia was significantly lower in the
Table 1. Food intake, running distance, and energy availability.

|                        | SED     | EX      | EX-FR   |
|------------------------|---------|---------|---------|
| Food intake (g/day)    | 14.6 ± 0.2b | 16.7 ± 0.5a | 10.5 ± 0.0c |
| Energy intake1 (kcal/day) | 53.3 ± 0.8b | 60.0 ± 1.9a | 37.3 ± 0.2c |
| Wheel running distance (km/day) | -  | 6.9 ± 7.3b | 11.8 ± 9.3a |
| Exercise-induced energy expenditure2 (kcal/day) | -  | 7.3 ± 1.2  | 9.3 ± 0.8   |
| Energy availability3 (kcal/day) | 53.3 ± 0.8a | 52.7 ± 2.1a | 28.4 ± 1.0b |

SED: sedentary group. EX: exercise group. EX-FR: exercise + food restriction group. Values are expressed as means ± SE. Means with different letters are significantly different.

1 Energy intake was calculated by multiplying the amount of daily food intake in grams by the energy content of the food (3.73 kcal/g).

2 Exercise-induced energy expenditure from daily wheel running was calculated as 5.0 kcal/kg body weight times kilometers run11).

3 Energy availability was calculated as energy intake minus exercise energy expenditure.

Table 2. Body weight and bone size.

|                        | SED     | EX      | EX-FR   |
|------------------------|---------|---------|---------|
| Initial body weight (g) | 169 ± 2 | 172 ± 3 | 171 ± 3 |
| Final body weight (g)   | 258 ± 4a | 240 ± 8a | 167 ± 3b |
| Dry weight of femur (g) | 0.54 ± 0.01a | 0.55 ± 0.02a | 0.43 ± 0.01b |
| Length of femur (cm)    | 3.42 ± 0.01a | 3.40 ± 0.02a | 3.15 ± 0.02b |
| Major axis of femur (cm) | 0.381 ± 0.003 | 0.388 ± 0.005 | 0.369 ± 0.004 |
| Minor axis of femur (cm) | 0.310 ± 0.005 | 0.312 ± 0.004 | 0.299 ± 0.002 |

SED: sedentary group. EX: exercise group. EX-FR: exercise + food restriction group. Values are expressed as means ± SE. Means with different letters are significantly different.

EX-FR group than in the EX group. BMD of the lumbar was not significantly different between the groups. Food restriction while undergoing running exercises over 5 weeks caused low bone mass, and the degree of negative effect of bone mass was similar to the 12-week experimental period in our previous study10): BMC of tibia: -25% (5-week) vs. -25% (12-week), bone area of tibia: -15% vs. -15%, BMD of tibia: -12% vs. -13%, BMC of lumbar: -27% vs. -29%, bone area of lumbar: -21% vs. -22%, and BMD of lumbar: -7% vs. -10%.

Fig. 2 presents the relation between wheel running distance and BMD of tibia. There were no significant correlations between wheel running distance and BMD of tibia in EX group (p = 0.065) and EX-FR group (p = 0.052), however, these correlation coefficients indicated moderate correlations in EX group (r = -0.677) and EX-FR group (r = -0.662). These correlations were not significant, but p values were near the significance level. Excessive running may cause low bone strength in both adequate and inadequate food intake conditions. High running distance causes low energy availability, and it might also cause low bone strength by abnormal hormone secretion which affects bone turnover. Further investigation of the relation between wheel running distance and BMD is necessary.

Our results show that a 38-day experimental period was sufficient to observe low BMC, low bone area, and low
BMD by food restriction in young female rats undergoing running exercises. This study has the following limitation. We could not assess direct bone strength or metabolic markers. Because of the Great East Japan Earthquake, the end of this research was sudden, and dissection was performed without sufficient preparation and time, and we could not measure the materials immediately. However, we consider that the present report helps in developing a rat model for female athletes with low BMD.

In conclusion, a 5-week experimental period is sufficient to cause low dry weight and shorter femur length, low BMC, bone area, and BMD of the tibia, and low BMC and bone area of the lumbar by food restriction in young female rats undergoing running exercise. This
report shows the possibility of constructing a rat model for female athletes with low BMD even over a short period. In the future, it will be necessary to investigate bone parameters such as bone strength and bone metabolism markers.

Conflict of Interests
The authors declare that they have no conflict of interests.

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