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EFFECTS OF RUN TRAINING ON BONE DEVELOPMENT AND BONE MINERALIZATION IN GROWING MICE

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ABSTRACT: We planned to study the body weights, bone sizes and bone mineral (Ca, Mg, Zn) contents of growing mice subjected to treadmill training. Twelve 4-week-old male Swiss Albino mice were divided into sedentary and exercise groups. The mice were trained by running exercise on a flat bed treadmill with 15 m/min, 30 min/day motion, throughout 5 days per week, for 12 weeks. The body weight of animals, and length, fat-free dry weight and Ca, Mg, and Zn contents of bones were measured in both groups. Body weights of animals, and lengths and wet and dry weights of the femur and the tibia were significantly higher in the exercised group. Also, the Zn, Mg and Ca mineral contents of bones in the group that underwent exercise were higher than in the other group. Running exercise with a flat bed treadmill performed by the growing mice is an effective exercise mode, especially for bone morphology.

KEY WORDS: exercise, bone Ca, Mg and Zn, bone size, total weight

INTRODUCTION

Although the optimal training mode has not been established, the weight training type of physical activity causes bone hypertrophy [1,16]. Weight training is an important modality to preserve, or increase bone mass [2]. Several researchers have studied the effects of artificial loading imposed by external stimuli on the bone. However, limited data exist so far concerning the energy of the whole body at rest and during the exercise of young mice [12,17].

To date, there have been a number of reports describing the effects of running, jumping and swimming on the bone mass and morphology for adult and elderly rats (up to 18 months of age) in the literature [6,20]. The intensity of training undoubtedly correlated with the amount of bone hypertrophy, but the frequency of training was not the major determinant as far as bone response is concerned. There are also few studies regarding the effects of physical training on the bone mineral density in young mice. The studies on bone minerals were focused on Ca, due to the intensified interest on osteoporosis in aging, especially for the aged animals. There are a few studies concerning the Mg content of bone, as mentioned above, but none of them addresses Zn content for the growing mice under exercise [10]. However, magnesium and zinc play an important role in physical performance since the cofactors of enzymes are involved in both energy production (magnesium and zinc) and the antioxidant defence system (zinc) [5]. Different exercise modes have different effects on growth and bone mass [4]. The aim of this study was to evaluate the effects of running on body weight, bone size and bone Ca, Mg and Zn contents applied in growing young mice and to show the benefits of treadmill training to augment bone mineral content and other parameters as well.

MATERIALS AND METHODS

Animals and animal care. Twelve male Swiss Albino mice (4 weeks old, mean weight 16 g) were obtained from the Animal Care and Breeding unit of the Gazi University Medical Faculty, Ankara, Turkey. The animals were cared for in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (PSJ-5 December 2003). The mice were either assigned randomly to the exercise group (n=6) or the control (sedentary) group (n=6). The mice were housed individually in sedentary cages (15 x 30 x 50 cm), and they did not exercise until the physical training period. The training period lasted 12 weeks. These periods are equivalent to the early age of a human being who is nearly 2 years old and the suggested training period is nearly 6 years for a human who lives 70 years.

At the end of the training period, the mice were 16 weeks old. All the animals, throughout the experiment, were kept under controlled conditions. The ambient temperature was 20±2ºC, the mice were subjected to a 12-hour light and 12-hour dark cycle per day, and food and water were provided ad libitum to all mice throughout the study.

Training programme

The mice within their cage in the running training group were placed on the flat bed treadmill, the speed of which was constant at 15 m/min, on a plain floor. An electric grid at the rear of the belt was used to create a stimulus for the mice to run. All mice ran 5 days/week for 12 weeks. Each running training period lasted 30 minutes.

Data collection

After training, the mice were killed by decapitation under ether anaesthesia. The body weights of the mice in both groups were measured before and after the 12-week running training period. The left and right femur and tibia were removed. After careful removal of soft tissue, they were weighed (wet weight, WW) and the lengths of the femur and the tibia were measured using a sliding calliper. After the lengths were measured, the bones were immersed in solvent (2 volumes of chloroform were combined with 1 volume of methanol) for 1 week and they were dried at 100^0C for 2 hours. Then their fat-free dry weights were measured with a balance (Mettler Inc.).

The control group was kept at rest throughout the 12 weeks. Body weights, fat-free dry and wet bone weights and lengths of the femur and tibia of the control group were also measured.

Determination of bone mineral levels

After the fat-free dry weights of samples had been determined, each sample was then dissolved in a perchloric acid-hydrogen peroxide mixture (1:2) for Ca, Mg and Zn level determination. The minerals were measured with a Perkin-Elmer 103 Atomic Absorption Spectrophotometer by using different dilutions, such as 1/25 for Ca and Mg, directly for Zn and flames; 442 nm for Ca, 285 nm for Mg and 307 nm for Zn [5].

Statistical analysis

The mean values of the two legs of each animal were calculated before statistical testing. The data were analysed with Mann-Whitney U test by the StatView program to determine whether there were significant differences both owing to the effects of training and also by natural growth leading to increase in size or substance by assimilation of new matter into the living organism. Data reported in the results are expressed as means and standard deviations. $P<0.05$ was accepted as the significance level.

RESULTS

The data for the body weight, the wet and fat-free dry bone weight, the length of the femur and the tibia are presented in Table 1. The data on bone zinc, calcium and magnesium contents of sedentary and exercised groups are presented in Table 2. The differences between the exercised and sedentary group are presented as percentages in Table 3.

There were significant differences in both the initial and final body weights of the running training and the sedentary control groups. All the mice gained in weight ($p<0.05$) during the 12 weeks of study in accordance with their natural growth trend.

The wet weights of the femur and the tibia were significantly greater for mice in the running training group ($p<0.05$). The lengths

TABLE 1. BODY WEIGHTS, WET AND FAT-FREE DRY BONE WEIGHTS AND THE LENGTHS OF BONES IN SEDENTARY AND EXERCISED GROUP

Group	Body Weight (g)		Femur + Tibia Weight (mg)				Femur Length (cm)		Tibia Length (cm)	
			WW		FFDW					
	B	A	B	A	B	A	B	A	B	A
Sedentary (N=6)	18.1± 2	36.6±3*	216±5	337±6*	116±38	242±17*	1.5±0.1	1.7±0.1*	1.23±0.2	1.9±0.08*
Exercised (N=6)	18.0 ± 2	39.2±3*,**	217±6	401±5*,**	115±39	380±4*,**	1.5±0.1	1.8±0 *,**	1.2±0.2	2.1±0.08*,**

Note: B, initial (before exercise); A, final (after exercise); WW, wet weight; FFDW, fat-free dry weight. Body and bone weights and lengths compared with Mann-Whitney U test, U= 29 for $P<0.05$, * before and after, ** sedentary and exercised mice

TABLE 2. ZINC, CALCIUM AND MAGNESIUM CONTENTS OF FEMUR + TIBIA IN SEDENTARY AND EXERCISED GROUP

Groups	Zn content (µg/g dry W)		Mg content (mg/g dry W)		Ca content (mg/g dry W)	
	B	A	B	A	B	A
Sedentary mice (N=6)	175.0 ±12.5	207.7±25*	3.78±0.2	4.23±0.4*	253±31	323±45*
Exercised mice (N=6)	174.7±12.5	211.5±17.5*	3.70 ±0.3	4.34±0.5*	251±31	331±30*

Note: B, initial (before exercise); A, final (after exercise) period; W, weight. Before and after exercise period mineral levels compared with Mann-Whitney U test. *$P<0.05$ for U=29, * before and after in sedentary group or exercised group, of the femur and the tibia were also significantly greater for mice in the running training group ($p<0.05$). There was significant variance, with respect to the bone mineral contents, between the pre- and the post-exercise periods in both groups. The Ca, Zn and Mg contents of the femur and tibia increased significantly in both the exercised and the sedentary group during the 12-week study period. However, there was no significant difference between sedentary and exercised groups regarding the mineral contents, when compared using the Mann-Whitney U test. Nevertheless, those values obtained were significantly different, as a percentage.

DISCUSSION

The present study showed a significant difference in body weight between the control and training groups. Exercising animals gained more weight than the control group during the 12-week study period. These results confirm the earlier reports demonstrating that growing animals exposed to such strain which increase in size or substance by assimilation of new matter into the body can adjust their caloric intake to maintain their normal rate of body weight increase by consuming much more energy [15,20] than they expend. In addition, changes in body weight can influence the mechanical properties of the bone and gain in weight in the exercising animals that can be explained by increased lean body mass due to exercise.

The results show that running training for 30 minutes, 5 times a week, for a total period of 12 weeks, resulted in greater bone length than in the sedentary group in the growing mice.

Physical activity involving weight loading causes bone hypertrophy [8], but the most effective mode of training is not well documented. In animal bone studies, many researchers used only young and old rats [3,7,17,20]. Very few data exist concerning the effects of endurance running training of young mice [17]. Several researchers have observed the effects of an artificial load imposed by an external stimulus on the bone. They reported that, with respect to the type of loading, dynamic loading was more effective than a static one and the peak strain correlated with the amount of bone hypertrophy as well [14,21].

TABLE 3. BODY WEIGHTS, WET AND FAT-FREE DRY BONE WEIGHTS AND THE LENGTHS OF BONES IN SEDENTARY AND EXERCISED GROUP

	Sedentary Group (% change)	Exercised Group (% change)	Differences between groups (%)
Body Weight	102.2	117.7	15.2
Bone WW	56	84.8	51.4
Bone FFDW	108.6	230.4	112.2
Femur length	13.3	20	50.4
Tibia length	54.5	75	37.6
Bone Zn content	18.7	21	12.3
Bone Mg content	11.9	17.3	45.4
Bone Ca content	27.7	31.9	15.2

Note: WW, wet weight; FFDW, fat-free dry weight

Our results concern male mice subjected to long-term (12-week) running exercise, aged 4 weeks, coupled with data for young rats subjected to long-term strenuous running exercise [20]. In our study, we have observed a similar tendency for the in vivo measurements of bone minerals and for the bone dimensions of young mice. However, some researchers have reported different results regarding old rats [9,17]. Raab and co-workers [16] made studies on the running training effects of old female Fischer-344 rats, and also observed that the femur weight of 25-month-old rats increased in the same manner as for 2.5-month-old rats. In their study, 2.5-month-old rats trained under a speed of 36 m/min with 15% grade for 1 hour and 25-month-old rats trained at a speed of 15 m/min for one hour [16]. The intensity applied to the old rats in their study was higher than the intensity applied in our study. The intensity of running training in our study was considerably smaller than in the preceding studies. We also observed significant bone hypertrophy of young mice. There have been reports that strenuous running exercise has no or few negative effects on bone [11,12,19,21]. Thus, intensity of application and age are important factors in choosing and establishing a running training mode.

In our study, the lengths of the tibia and femur were significantly greater in the running-trained mice than in the sedentary mice ($p<0.05$). The muscles attached to the femur developed and elongated on the bone during running. Also, subjecting the tibia to load bearing during physical activity causes bone hypertrophy. The load absorption and strength gaining character of physical activity increases bone mineral content of animals and humans. Most investigations have examined the effect of exercise or aging on the Ca content of long bones, such as the femur or humerus [14]. Our results describe the effects of flat bed running exercise on Ca, Zn and Mg mineral contents of the femur and tibia, in growing mice. Augmentations in both groups of the Ca, Mg and Zn contents of the femur and tibia were observed and the growing trend in these values was statistically significant ($p<0.05$). The percentage increases in the Ca, Mg and Zn contents of bones were different in the sedentary and exercised mice (15.2%, 45.4% and 12.2% respectively). However, these increases were not so significant by Mann-Whitney U test. We thought that reasonable statistical significance might have been observed if the test had been carried out with more animals. McDonald and co-workers [10] reported that femur Mg concentrations of young sedentary rats were higher than those of exercised rats while the femur Ca concentrations were higher in the exercised group. However, their exercise programme was different than ours. Newhall and co-workers [13] reported that voluntary running activity resulted in a marked increase in femur and tibia bone mineral contents in young male rats compared to the sedentary control group having the same weight. The Zn contents of the femur and tibia increased by 12.3% in the running-exercised group compared to the sedentary group (Table 3).

It is thought that tissue non-specific alkaline phosphatase (TNALP) plays an important role in mineralization processes. In Tesch and co-workers' [18] study, the null mutants (n = 7) and their wild-type littermates (n = 7) were bred and killed between 8 and 22 days after birth. The skeletal tissues were processed by small-angle X-ray scattering, quantitative backscattered electron imaging to assess mineral characteristics, and the bone was also analysed under light microscopy and immunoglobulin. The results showed reduced longitudinal growth and strongly delayed epiphysis ossification in the null mutants (TNALP-deficient mice) [18]. Alkaline phosphatases (ALPs) are zinc metalloenzymes [5]. Increased Zn levels in both sedentary and running groups may also be related to this enzyme's function. Recently Wu and co-workers reported the results of a study in which 4 weeks of exercise at a reduced level were followed by 4 weeks of running exercise in growing male mice. It was found that high bone mass and high ALP activity in bone marrow cultures were maintained at the end [22].

The criteria of percentage increases of sedentary and exercised growing mice (body weight, wet and fat-free dry weights and length of bones) in 12 weeks were significantly different in the two tested groups except for bone mineral contents (Table 3). It seems that bone mineral results would also have been significant in the Mann-Whitney U test if the number of animals had been increased.

CONCLUSIONS

The present study indicates that flatbed treadmill running training is an effective training mode for bone hypertrophy during the growing period. Thus, it appears that applying a temporary and minimal degree of activity to the sedentary animals would be sufficient to have an influence on bone size and mineral contents as well as other parameters during the growing period.

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