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EFFECTS OF DIFFERENT DURATIONS OF TREADMILL TRAINING EXERCISE ON BONE MINERAL DENSITY IN GROWING RATS

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Abstract: In this study, we aimed to investigate the effects of different durations of treadmill training exercise (daily for 30 min and 60 min) on bone mineral density (BMD) in young growing rats. Training consisted of treadmill running at 5 days per week during a period of 13 weeks. The rats in 30 min and 60 min exercise groups began to training on day 63 of life and had maintained for at least a week, with a minimal progression as a guide to the rats' training and adaptation to the treadmill. Running time was gradually increased from 15 min to 30 and 60 min per session for two exercise groups respectively. Control rats were kept in the cages at the same environmental conditions and daily inspected to control their health. At the end of 13 weeks, bone mineral densities of the bilateral tibia of all rats were measured .with dual-energy X-ray absorptiometry (DEXA) (QDR 4500/W, Hologic Inc., Bedford, MA, USA) and results were evaluated. There were significantly increases in BMD of right and left tibia of rats in 30 min exercise group at post-exercise period ($p<0.01$ for both sides) when compared to the control group. BMD of right and left tibia of rats were also correlated with each other ($r=0.556$ and $p=0.003$). Otherwise, there is a positive correlation between pre- and post-exercise body weights of rats ($r=0.588$ and $p=0.002$). From our results, we concluded that subjects should perform moderate running exercise for development of bone mass and its protection during the lifelong. However, intensity and duration of performing exercise are required to put in order for every ages or actual physical conditions.

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Key words: Bone mineral density - Training - Exercise - Treadmill - X-ray absorptiometry

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Introduction

Osteoporosis is a major public health problem that is predicted to worsen over the next decade and preventative strategies that increase bone strength have become the focus of substantial research [1]. Exercise is usually beneficial for the skeleton, but strenuous overtraining may be harmful [2]. Appropriate endurance exercise is capable of increasing bone mass and strength in both animals and human [3]. Short-term resistance exercise increases bone turnover but not bone mass in humans, while long-term exercise increases both bone strength and mass [4]. Iwamato *et al.* demonstrated that treadmill exercise stimulates bone formation and suppresses bone resorption, increases the serum 1,25-dihydroxyvitamin D [3] level, and decreases the serum parathyroid hormone level, resulting in an increase in bone mass with stimulation of longitudinal bone growth in young growing rats [5]. They also suggested that cancellous bone adaptation to treadmill exercise is site specific, and the effect may be influenced by factors such as mechanical loading and metaphyseal bone architecture in the young growing rat [6]. It was reported that age does not influence the bone response to treadmill exercise in female rats [7].

Unlike from previous studies, we aimed to investigate the effects of different durations of treadmill training exercise (daily for 30 min and 60 min) on bone mineral density (BMD) in young growing rats.

Materials and Methods

Subjects: Twenty-six Wistar-Albino adult male rats were used. Principles of laboratory animal care (NIH publication) were regarded, and Inonu University Ethic Comity of Experimental Animals approved this study protocol. The control (n=10), 30 min exercise (n=8) and 60 min exercise (n=8) groups of rats were housed in 3 groups in colony cages, at ambient temperature of 23^{o}C with a 12h/12h dark cycle. The animals had free access to water and pellet chow containing 1.8-2.2% of calcium, 1.1% of phosphorus and 2650 kcal/kg energy.

Exercise procedure: As in a previous study [8], exercise was consisted of treadmill running. A steep grade treadmill inclination was used to stimulate high-intensity muscle activity in rats. Training consisted of treadmill running at 5 days per week during a period of 13 weeks. The rats in 30 min and 60 min exercise groups began to training on day 63 of life and had maintained for at least a week, with a minimal progression as a guide to the rats' training and adaptation to the treadmill. Running time was gradually increased from 15 min to 30 and 60 min per session for two exercise groups respectively, and treadmill speed was increased to 45 cm/second with increments of 2.5° in the grade incline to reach a final grade inclination of 17.5°. The chronological progression in the treadmill speed, grade inclination, and running time are shown on the Table 1 and Table 2. Control rats were kept in the cages at the same environmental conditions and daily inspected to control their health.

Table 1
Weekly variation in treadmill speed, inclination, and running time of 30 min exercise group

		Weeks							
Days		1	2	3	4	5	6	7	8-13
	cm/s	17	17	19	22	28	32	37	45
Monday	ºC	5	10	15	15	15	15	18	18
	min	15	15	30	30	30	30	30	30
	cm/s	17	17	19	22	28	32	37	45
Tuesday	ºC	5	13	15	15	15	15	18	18
	min	15	15	30	30	30	30	30	30
	cm/s	17	19	19	22	28	32	37	45
Wednesday	ºC	8	13	15	15	15	15.2	18	18
	min	15	15	30	30	30	530	30	30
	cm/s	17	19	19	22	28	32	37	45
Thursday	ºC	8	15	15	15	15	15.2	18	18
	min	15	15	30	30	30	530	30	30
	cm/s	17	19	22	28	32	37	45	45
Friday	ºC	10	15	15	15	15	15	18	18
	min	15	30	30	30	30	30	30	30

Osteodensitometry: In the all rats, bone mineral densities of the bilateral tibia were measured five times with dual-energy X-ray absorptiometry (DEXA) (QDR 4500/W, Hologic Inc., Bedford, MA, USA) and results were evaluated by the same examiner. The mean value of five measurements was used for statistical comparison. DEXA equipment uses switched pulsed stable dual-energy radiation with 70 kV and 140 kV. Subregion analysis software was used to analyze of the whole tibia. BMD value of the tibia was used, in g/cm^2. All tibia length was obtained as 2 pixel values by bone mineral densitometry [9,10].

Table 2
Weekly variation in treadmill speed, inclination, and running time of 60 min exercise group

		Weeks							
Days		1	2	3	4	5	6	7	8-13
	cm/s	17	17	19	22	28	32	37	45
Monday	ºC	5	10	15	15	15	15	18	18
	min	15	15	60	60	60	60	60	60
	cm/s	17	17	19	22	28	32	37	45
Tuesday	ºC	5	13	15	15	15	15	18	18
	min	15	15	60	60	60	60	60	60
	cm/s	17	19	19	22	28	32	37	45
Wednesday	ºC	8	13	15	15	15	15.25	18	18
	min	15	15	60	60	60	60	60	60
	cm/s	17	19	19	22	28	32	37	45
Thursday	ºC	8	15	15	15	15	15.25	18	18
	min	15	15	60	60	60	60	60	60
	cm/s	17	19	22	28	32	37	45	45
Friday	ºC	10	15	15	15	15	15	18	18
	min	15	45	60	60	60	60	60	60

Data analysis: Wilcoxon and Mann-Whitney U tests were used to compare pre- and post exercise body weights of rats and for the comparisons of BMD values among the groups, respectively. Pearson Correlation was also used to correlate variables and $p<0.05$ was considered to be statistically significant.

Results

Body weights of rats and their alterations at post-exercise period in control, 30 min and 60 min exercise groups are shown on the table 3. Increase in body weight at post-exercise period was found to be statistically significant only in 30 min exercise group when compared to pre-exercise period ($p<0.01$). There were also significantly increases in BMD of right and left tibia of rats in this group at post- exercise period ($p<0.01$ for both sides) when compared to the control group (Table 4). BMD of right and left tibia of rats were correlated with each other ($r=0.556$ and $p=0.003$).

Otherwise, there is a positive correlation between pre- and post-exercise body weights of rats ($r=0.588$ and $p=0.002$).

Table 3
Body weights of rats at pre- and post exercise periods (X±SD, grams)

	Pre-exercise	Post-exercise
Control (n=10)	155.00±5.70	232.80±20.59
30 min (n=8)	164.00±4.11	295.75±16.71*
60 min (n=8)	160.13±7.88	269.38±15.49

*difference from control group and $p<0.01$

Table 4
Bone mineral densities of right and left tibia of rats in control and exercise groups at post-exercise period (X±SD, g/cm^2)

	Right side	Left side
Control (n=10)	0.1584±0.0217	0.1584±0.0171
30 min (n=8)	0.1895±0.0182*	0.1788±0.0623*
60 min (n=8)	0.1744±0.0142	0.1665±0.0243

*difference from control group and $p<0.01$

Discussion

Studies of rats have evaluated the osteogenic responses to several types of unique (i.e., not usual cage activity) exercise interventions, including running (treadmill and voluntary), swimming, jumping, standing, climbing, and resistance training. Results have been equivocal, demonstrating that mechanical stress can enhance or compromise bone mass, formation, and/or mechanical properties [11]. Strains on bone greater than needed for steady state remodeling will cause a modeling response that increases bone mass to meet the increasing load requirement. This adaptive response occurs primarily during periods of growth and development, suggesting the importance of understanding the effects of habitual physical activity on bone accretion during childhood and adolescence [12].

It is clear that the chemical composition of bone varies with the degree of exercise, and that the bone becomes strengthened at the same time [13]. Joo *et al*. showed that running exercise significantly increased BMD, bone volume, bone volume fraction, trabecular thickness, and trabecular number, whereas trabecular bone pattern factor [3]. Although many studies have shown that running exercise has beneficial effects on BMD, in an experimental model, the adverse effects of long-term strenuous exercise on bone tissue have been reported in rats [8].

Our results showed that daily 30 min treadmill exercise increases BMD and body weight in growing rats, whereas 60 min treadmill exercise does not significantly alters these parameters. In view of the fact that daily 60 min treadmill exercise has not an adverse effect on BMD and body weight gain at the growing period because it elevates both of them. It was reported that in general, running and swimming of moderate intensity have been found to have positive effects on bone mass and material properties in the cortical and trabecular regions of the tibia and femur in growing and mature rats. However, decreases in bone mass, trabecular thinning, and structural properties have been observed in response to exercise that is very intense and/or excessive, particularly in growing animals [11]. In the present study, it appears that daily 30 min exercise is more effective than the other in that BMD elevation. This finding may help to optimize daily exercise time for humans that aim to improve and protect BMD and body weight. But, it is essential that intensity and duration of performing exercise be required to put in order for every ages or actual physical conditions.

Conclusions

From our results, we concluded that subjects should perform moderate running exercise for development of bone mass and its protection during the lifelong. However, intensity and duration of performing exercise are required to put in order for every ages or actual physical conditions.

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