Effects of High-Intensity Interval Training and Moderate-Intensity Continuous Training on Bone Mineral Density in Rat

Abstract

Background: Moderate intensity continuous training (MICT) has been reported as an effective exercise on bone metabolism. However, very few studies have focused on high-intensity interval training (HIIT). The aim of this study was to investigate and compare the effects of HIIT and MICT training on bone density in middle-aged rats.

Methods: 30 male Wistar rats (16 weeks old) were randomly divided into three groups: control, MICT and HIIT. Both test groups completed 8 weeks (5 sessions per week) of treadmill training in which the HIIT group performed 10 sessions of running at a speed of 35-47 m/min with a 2-minute active recovery, and the HIIT group ran continuously for 10-45 minutes at a speed of 15-20 meters per minute. The body composition and bone mineral density in the whole body were evaluated through Dual Xray absorptiometry (DXA) at the beginning and after the intervention. The data was analyzed using SPSS software and one-way analysis of variance and Tukey's post hoc tests.

Results: After 8 weeks of intervention, BMD of the whole body and femur increased significantly in both groups (p≤٠0.05), although the observed change was greater in the HIIT group (p≤0.05). In addition, in the case of BMD of the lumbar vertebra, no significant difference was observed between all three groups after the intervention (p≥0.05).

Conclusion: These findings show that a period of HIIT and MICT training can improve bone density in middle-aged rats and compared to MICT, HIIT had more benefits on bone density.

Keywords: Wistar rat, Osteoporosis, Running, Bone mineral density

Received: 4 months before printing; Accepted: 2 months before printing

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Introduction

Fractures are a major problem for society. Treatment not only causes individual suffering but also incurs huge costs for society (1, 2). Many guidelines for osteoporosis prevention and fracture reduction recommend exercise and physical activity as the most effective non-pharmacological means to increase bone strength and reduce falls (3). Consequently, in multiple exercise studies (4) aimed at enhancing bone strength, primarily assessed through bone mineral density (BMD) in postmenopausal women, the most prominent and largest at-risk group for osteoporosis have been evaluated. Evidence regarding the beneficial effect of exercise on bone density in men is still limited (5, 6). In a meta-analytic review, data were reported on the positive effects of physical activity/exercise on bone density in men 18 years and above (7). Ash et al. also found that exercise has a pronounced effect on total hip BMD, though its impact on the femoral neck is negligible (8). While some evidence-based recommendations exist for exercise protocols, the optimal training for enhancing and improving bone density is still unestablished (9). Apart from training parameters and principles, even regarding the type of training to be implemented as a basic decision, there is no general consensus. Recently, Rahimi et al. reported a lack of effects of resistance exercise and negative effects of aerobic exercise with weight-bearing on lumbar spine and femoral neck BMD in postmenopausal women aged 60 and above (10). Unless these data are reliable and generalizable to the whole women's postmenopausal population, all current exercise recommendations and the effect of exercise on bone density, in general, are questionable [11]. Physiological adaptations to aerobic training and moderate-intensity continuous training (MICT) are specifically different: aerobic exercise improves cardiovascular adaptations that increase maximal oxygen consumption without significant change in power [12]. Interval training typically involves repeated bouts of relatively strenuous exercise interspersed with short recovery periods. A common classification scheme divides this method into high-intensity interval training (HIIT); "near maximal" efforts) and sprint interval training (SIT); "supramaximal" efforts). Both forms of interval training induce physiological adaptations like increased aerobic capacity (VO2max) and mitochondrial content [13]. But scientific evidence regarding bone tissue adaptations to interval training is very limited. Therefore, the aim of this study was to compare the effect of 8 weeks of moderate intensity continuous and high-intensity interval training on bone density in an animal model.

Methods

Animals:

The research method was experimental. In determining the sample volume, according to the formula for the volume of the sample for continuous scores, if the expected differences are equal to 1.1, with a test power of 80% at the significance level of α = 0.05, the number of samples in each group is 10 heads. In this research, 30 adult male Wistar rats weighing 250 to 300 grams and 16-weeks old were used, which were obtained from the Center for Laboratory Animal Sciences. The rats were kept at a temperature of 22 ± 2 degrees Celsius, a light-dark cycle of 12: 12 hours, and in polycarbonate cages (5 rats per cage). The rats were randomly divided into three groups' high-intensity interval training, moderate intensity continuous training, and control. Throughout the study, all rats had access to standard commercial food and free water. All rats were allowed to move freely in a standard cage. This study was conducted in accordance with the ethical criteria for working with animals at Arak University (IR.ARAKU.REC.1401.031).

Training program:

The program of moderate intensity continuous training (aerobic) was performed on a 5-channel treadmill due to easier control of speed and running time. The rats were trained for 8 weeks, five days a week. The entire training period was divided into three stages familiarization, overload, and maintenance of work intensity. During the familiarization stage (first week), rats walked on the treadmill for 10-15 minutes per day at a speed of 8 meters per minute. In the overload stage (second to fourth week), the rats initially ran for 20 minutes at a speed of 27 meters per minute, and gradually during three weeks, the exercise time increased (each session by 2 minutes) until reaching a final 60 minutes. Finally, in the maintenance stage, the work intensity reached 60 minutes of endurance training at 27 meters per minute for three weeks. During each training session, rats warmed up for 5 minutes (at a speed of 16 meters per minute) and, in the end, cooled down for 5 minutes (at a speed of 16 meters per minute with a gradual decrease in intensity to the lowest amount) was allocated [14] (Table 1). The high-intensity interval training program included treadmill running. The interval training group performed ten 1-minute bouts of high-intensity treadmill running with 2 minutes of rest for five days a week for eight weeks. The running speed was gradually increased to the maximum speed that each individual rat could continue running for 1 minute. The intensity of interval training started at 35 meters per minute. Walking was performed for 5 minutes at 16 meters per minute for warming up at the beginning of each session and for 5 minutes at 16 meters per minute for cooling down at the end of each session [15] (Table 2).

Table 1: Aerobic training program on the treadmill						
Weeks of training	**Acquaintance**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5 to 8**
Training duration (minutes)	10	10-20	20-30	30-35	35-40	45
Speed (meters per minute)	10	15	16	17	18	20

Table 2: Intermittent training program on the treadmill									
Weeks of training	**Acquaintance**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Number of runs per training session	2	10	10	10	10	10	10	10	10
Training sessions per week (number)	1	5	5	5	5	5	5	5	5
Average speed (m/min)	20	35	37	39	41	43	45	46	47
Average total running distance per week (meters)	50	1800	1870	1895	1950	1985	2050	2100	2170

Bone Density Assessment:
Bone mineral density of lumbar vertebrae (L4-L5), femur, and whole body in g/cm^2 was assessed by dual-energy x-ray absorptiometry (DEXA) (Discovery, Hologic, Bedford, USA). In all cases, imaging and data analysis were performed by one person, and the densitometer was calibrated weekly with suitable phantoms. Fat and fat-free mass were determined from whole-body images using the DEXA device and small animal-specific software. All these indices were assessed before and two days after applying sports interventions.

Statistical analysis:
The results are expressed as mean ± standard deviation for the samples present in each group. For statistical analysis, after ensuring the normality of the data using the Shapiro-Wilk normality test and examining the assumption of equality of variances from the Levene test. After the normality of data distribution and equality of variances were determined, a one-way analysis of variance and Tukey follow-up test was used at the significance level of $P \leq 0.05$ to analyze the data and compare between groups. All statistical calculations were performed using SPSS version 18 statistical software.

Findings
In Table 1, the statistical components of the mean and standard deviation of the body composition of the laboratory rat investigated are presented in grams. After eight weeks of sports intervention, it was observed that between the groups under study, there was no significant difference in terms of body weight ($P = 0.44$), fat mass ($P = 0.12$), fat-free mass ($P = 0.24$) and percentage of fat ($P = 0.19$) (Table 3).
By evaluating whole-body bone density, it was observed that there was a significant difference between the study groups ($P = 0.02$ and $F = 12.2$). The Tukey follow-up test showed that between the control group and the groups of continuous exercise with

moderate intensity (P = 0.02) and interval training (P = 0.01), there was a significant difference, and also the observed change in the interval training group was significantly greater (P = 0.04). In addition, when assessing lumbar vertebrae bone density, it was observed that there was no significant difference between the study groups (P = 0.25 and F = 3.1). On the other hand, when evaluating femur bone density in the study groups, a significant difference between the groups was observed (P = 0.001 and F = 48.3). The Tukey test showed that the femur bone density in the control group was significantly lower than the groups of continuous exercise with moderate intensity (P = 0.04) and interval training (P = 0.01), and also there was a significant difference between the training groups (P = 0.05) (Table 4).

Table 3: Body composition characteristics of the studied groups

	Control	Aerobic training	Interval training
Body weight (grams)	287.0±14.1	280.7±13.5	285.2± 12.4
Fat mass (grams)	35.6 ± 4.7	31.3 ± 3.8	32.4±4.1
Fat-free mass (grams)	240.2±2.9	237.3±8.1	238.9±8.3
Fat percentage	12.40 ± 1.3	11.15 ± 1.1	11.36±1.2

Table 4: Bone density in the groups under investigation

	Control	Aerobic training	Interval training
Whole body (g/cm2)	*0.16 ± 0.01	0.20±0.02	#0.22±0.03
Femur (g/cm2)	*0.16 ± 0.007	0.19±0.003	#0.20±0.002
Lumbar (g/cm2)	*0.19±0.005	0.18±0.006	0.19±0.02

The values are expressed as mean ± standard deviation.
***** indicates a significant difference (p<0.05) compared to other groups.
indicates a significant difference (p<0.05) compared to the moderate-intensity continuous training group.

Discussion

The effects of interval training on bone parameters are largely unknown. The present study aimed to compare bone mineral density (BMD) in response to treadmill running exercise using moderate intensity continuous and high-intensity interval training protocols in middle-aged rats. To our knowledge, this is the first study to evaluate the effects of high-intensity interval exercise training on bone density in middle-aged rats. In summary, our results showed that an 8-weeks running exercise program based on high-intensity interval and moderate intensity continuous training improved BMD at several sites (whole body, femur), and these improvements were greater with interval versus aerobic training, which likely prevents the increased fracture risk typically observed in the elderly. The whole-body bone parameters measured by DXA are fully consistent with previous studies in comparable populations [16], especially Sato et al. [17], supporting that bone health declines with aging and fat accumulation compromises bone quality. The significant multi-site (whole body and femur) BMD improvements after the interval and moderate intensity training demonstrated that exercise interventions elicit bone adaptation to physiological (muscle contraction-induced) loading, consistent with previous studies showing exercise training can increase bone formation even in middle-aged rats [16,18]. Mechanical stimuli that deform bone induce dynamic pressure on the bone. Thus, the biological response of bone to this pressure is to stimulate osteoblastic activity and increase BMD. However, the mechanical stimuli that elicit changes in bone deformation are not well defined [19]. The greater improvement in BMD with high-intensity interval versus moderate intensity training may be due to the greater loads and intensity of the interval training sessions, leading to greater mechanical pressures and tensile forces. Indeed, the osteogenic index of an exercise depends on its intensity and can be calculated as the magnitude of the load (or stress) multiplied by the load frequency (20). This agrees with previous studies showing high resistance or high-impact loading exercises are critical for bone health maintenance in high fracture-risk populations (16,21). Moreover, the gradual increase in intensity throughout the high-intensity interval training protocol prevents bone cell accommodation to mechanical loading, which is a prevailing and governing principle in bone adaptation to exercise (22). Thus, bone cell accommodation to customary and uniform loading signals is a major limitation of some training regimens like aerobic exercise (18). Indeed, our results clearly demonstrate that interval training reduces fracture risk, as the increased BMD is consistent with the evidence in obese adolescents that weight loss exercise with high intensity decreases fracture risk with increased BMD (21). These interval training-induced improvements in bone sites are of clinical importance, as these skeletal regions are highly associated with hip fracture incidence (23). Therefore, intensity appears to be an important factor in bone exercise adaptations. Previous human and animal studies support a linear relationship between running speed and increased BMD (19, 24).

In line with some studies (25), no change in response to exercise training was observed at the lumbar vertebrae level. Based on previous research, it appears that changes in bone density and adaptation in the lumbar vertebrae occur later compared to other bones and thus require a longer duration of training (26). In studies where increased bone density in the lumbar vertebrae has been observed, subjects (postmenopausal women) had relatively low bone density, and the duration of exercise training was also long (10). On the other hand, in most studies like the present study, where no change in bone density of the lumbar vertebrae following exercise training was observed, imaging of the lumbar vertebrae 2 to 4 has been performed. Given that biomechanics of load distribution between vertebrae differs, including the smaller transverse cross-sectional area of lumbar vertebra one compared to lumbar vertebrae 2 to 4, therefore, it bears more loads, and lumbar vertebra 1 has lower bone density compared to lumbar vertebrae 2 to 4 (26,27). Therefore, the lack of change in bone density of the lumbar vertebrae may be related to these factors, which requires further research taking these considerations into account.

Although this is the first study examining the effects of HIIT training on bone response in aged rats, the results should be considered in light of some limitations. First, while the inclusion of a control group can be regarded as strength of the present analysis, the relatively small sample size should be noted. Second, the use of peripheral quantitative computed tomography would provide more information regarding bone size, geometry, and quality (28). While DXA cannot distinguish cortical and trabecular bone or determine volumetric bone density (23), it remains the most common non-invasive method for assessing bone health in studies. Moreover, a follow-up assessment to examine whether the observed changes are maintained over time post-intervention would have been ideal but was not feasible for practical reasons.

Conclusion

In summary, the present study demonstrated that an 8-weeks running interval training program elicited greater multi-site (whole body, femur) bone density increases compared to moderate intensity continuous training, likely reducing fracture risk. Indeed, these findings substantiate high-intensity interval training approaches as part of exercise interventions, even for the elderly. They support the notion that bone fragility may be ameliorated by the gradual increase in running exercise intensity based on an interval program.

Author Contributions

All authors met the standard criteria of authorship based on the recommendations of the International Committee of Medical Journal Editors.

Conflicts of Interest

There were no conflicts of interest in conducting this study.

Acknowledgments

The researchers hereby acknowledge all those who have assisted them in this endeavour.

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