The Effect of Eight Weeks of Moderate and High Intensity Endurance Training On Biomechanical Properties of Femur in Old Male Wistar Rats

Abstract

Background: Physical activity reduces the risk of bone fractures by decelerating the bone loss and enhancing muscle strength. Such activities promote the bone strength and bone density through bone stimulation. The present study aimed to compare the effect of endurance training with varying intensities on the selected biomechanical properties of femurs in old male Wistar rats.

Methods: 24 male Wistar rats (23 months old) with an average weight of 441.75 grams were randomly divided into 2 experimental and one control groups: Moderate intensity continuous training (MICT, n = 8), high intensity Interval training (HIIT, n = 8), and control group (CG, n = 8). Moderate and high intensity endurance training sessions were held five days a week with 60-70% and 80-110% of the maximum speed) and high (maximum speed) for eight weeks, respectively. In the MICT and HIET groups, the duration and distance (volume of training) were the same and only the intensity of training differed. Forty-eight hours after the last training session, the rats underwent surgery and their femur removed. Three–point bending flexural test was used to determine the effects of training on modulation, maximum endurance, fracture energy, and femur strength in the rats. The statistical analysis was performed using one-way ANOVA test at P <0.05.

Results: The study results indicated that eight weeks of endurance training with varying intensities had no significant effect on modulation (p=0.198), bone strength (p=0.24), fracture energy (p=0.204), deformation-to-maximum strength (p=0.89), and femur strength (p=0.31), and that no significant difference was observed among the three groups.

Conclusion: The biomechanical changes of the bone by such exercises require longer periods of training, which could be examined in future studies.

Keyword: Aging, Exercise, Bone, Biomechanical properties

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Introduction

Bone tissue is a dynamic and complex live organ in response to physical activity and mechanical loading such as training exercises\(^1\). The mechanisms associated with age-related acceleration in bone loss in older men seem to be different from those menopause-related factors in women\(^2\). The structural strength of the bone is largely maintained by a mechanical sensor feedback system, which senses changes in the mechanical load inside the bones and presents motor challenges through changes in bone size and shape\(^3\). New bones are formed in regions where the applied is greater than the usual mechanical load limit. On the other hand, the bones are reduced in regions where mechanical load is below the normal load range\(^3\). Thus a simple and effective way to increase bone mass and bone structure is the mechanical stimulation of bone cells\(^4\). In order for the bone be adapted to mechanical load, some changes in structural details (mineral mass, geometry, architecture, material properties) are observed\(^3\).
Exercise increases the bone mineral density (BMD) and bone mass, strength, and mechanical properties and it seems to have direct or indirect impact on almost all types of bone cells, thus influencing many aspects of bone reconstruction\(^5\). For example, Kohrt et al. reported that a 11-month sports program, including walking, running, and climbing upstairs, with a moderate intensity 60-70% of maximum heart rate at the initial stages and a high intensity of 80-85% in the final stages, resulted in a significant BMD increase in the entire body bones, lumbar spine, and femoral neck among women aged 60-74 years\(^6\). Another study found that walking for at least 4 hours a week compared with walking less than one hour per week reduced the risk of hip fractures by 41%, and those who walked regularly experienced increased balance, less risk of fracture\(^6,7\), decreased risk of falling, and increased BMD\(^8\). On the contrary, Maud et al. find no significant changes in the musculoskeletal system of runners who were aged above 70 and had the experience of more than 50 years of long-distance exercise\(^9\). In human studies, BMD is a representative of biomechanical properties of bone; however, accurate measurement of biomechanical properties of the bone, such as the use of three-point bone bending, which is better than non-invasive measurements such as bone mineral density, is possible in animal samples\(^10\).

A curve reflecting the biomechanical properties of the bone in the outer part of the bone is called a load-displacement curve\(^11\). On this curve, the left side of the yield point represents the elastic behavior of the bone. If the load on the bone measured by the three-point bending flexural test is released before reaching the yield point, the bone like an elastic band returns to its original form. After the yield point, there is the plastic part of the bone. If the load on the bone passes through the yield point, the bone will be permanently deformed (damaged)\(^11\). After the yield point, there is a maximum load. The final event of the test is the bone fracture. The curve also shows the bone stiffness, maximum load, load-to-yield point, work-to-fracture, and modulation. The gradient of this curve indicates the external stiffness of the bone, which used the amount of bone minerals and the surface below the chart to indicate the bone work-to-fracture\(^11\). Biomechanical properties of bones should not be interpreted on the basis of a parameter, since it leads to an inaccurate conclusion. For example, the bones in osteoporosis mice have high, but at the same time, extremely fragile minerals, which reduce the work-to-fracture and increase the risk of fracture. Conversely, although the young mice’s bones are poor in mineral content, they have a higher flexibility, which enhances their work-to-fracture (young animal’s bones can absorb more energy before fracture than adults)\(^12,13,14\). The mechanical stresses resulting from intense exercises are of paramount important for the structure and functional integrity of the skeletal system, including increased BMD and other biomechanical properties of the bone\(^15\). Huang et al. (2008) investigated the effect of eight weeks of continuous endurance training at a speed of 12-22 m/min and periodic endurance training at a speed of 12-30 m/min on biomechanical properties of the bone in growing rats (seven weeks. The results of their study showed that both types of endurance exercises improved the femoral features and had no effect on the stiffness and flexural strength of the femur\(^16\).

It has been well documented that osteogenic responses from exercises are highly dependent on their intensity\(^18,17\). Hence it has been assumed that exercises with varying intensities results in different cellular responses and consequently different bone adaptations\(^19\). Scientific research suggests that all exercise protocols are not equally effective in improving bone mass. For example, there are methods that can significantly affect bone quality\(^20\). The American College of Sports Medicine (ACSM) states that individuals should
The Effect of Eight Weeks of moderate...

...take moderate to high intensity aerobic endurance activities (weight bearing exercises) to maintain and improve bone mass\(^{(21)}\). Moreover, the intensity of activity can affect the bone density. For example, some studies have found that only high intensity activities can affect metabolism and bone density\(^{(22)}\) because increased pressure on the bone through greater weight bearing, as well as increased pressure on the bone through increasing muscle contraction improves bone density\(^{(23)}\).

According to a thorough review of the methodologies used in recent studies on bone, the most common method for describing the biomechanical properties of long bones is the three-point bending flexural test\(^{(24)}\). Some advantages of this test include the use of proper bone size, proper access to bone during autopsy, documented data, validity, and reliability of the test\(^{(24)}\). There are few studies directly assessing the impact of different endurance training and changes in biomechanical properties of the bone. Although the impact is theoretically proved, to the best of our knowledge, it has never been investigated in response to the various intensities of training. There is still little knowledge of the moderated effects of various training protocols on biomechanical properties of the biomechanical parameters (bone stiffness, maximum endurance, and fracture energy), especially in the elderly. Accordingly, the present study aimed to evaluate the effect of endurance training with different intensities on changes in the biomechanical properties of the bone.

**Methods**

In this study, 24 male Wistar rats aged 23 months with the mean weight of 441.75 grams were prepared from Pasteur Institute of Iran and maintained at 22 ± 3 °C in 12: 12 hours of light and darkness. They were fed with rat foods and water. Moreover, all rule and ethics on how to behave animals (introduction, practice, anesthesia and killing of an animal) were observed according to the Association for Assessment and Accreditation of Laboratory Animal Care International approved by the Ethics Committee of the Research and Postgraduate Vice-Chancellor at Shahrekord University. After a week, the animals were acquainted with the laboratory environment and then they were weighed with a 2000 g scale SF-400A with accuracy of 0.1. Afterwards, based on homogeneity of their weights, they were randomly assigned into three C, MICT, and HIIT groups. The control group received no special intervention and only had three sessions per week for 15 minutes on treadmill at a speed of 2 m / min for the sake of further simulation.

**Test for determining the maximum oxygen consumption rate**

To determine the maximum oxygen consumption rate, Bidford et al.’s (1979) standardized test was used. This test was standardized by Leandro et al. (2007) for Wistar rats. The test consisted of 10 three-minute steps. The speed in the first stage was 0.3 km / h and 0.3 km / h was added in the next steps. Given that five exhaustive test methods were introduced by Reanoldo et al. to determine the maximum oxygen consumption with a different slope, this research used a zero gradient to determine the maximum oxygen consumption rate, and the speed at which the animal was unable to run was used as the maximum running speed of the animal\(^{(25)}\).

**Training protocol**

Mice were trained five sessions for eight weeks, with the same rate as the percentage of maximum oxygen consumption, which was converted to m/min. At the end of the fourth week, the animals were retested and the training speed was determined on the basis of the new test.

High and moderate intensity endurance training protocol included three parts: 1) Warming, 2) Exercises including high and low intensity interval activities for the HIIT group...
and Moderate-Intensity Continuous Training for MICT group, 3) Cooling down (Table 1). High intensity endurance training was performed as follows: After warming, the rats first had high intensity exercises followed by low intensity ones. After performing the last high intensity activity, the rats, instead of exercising at speeds equal to 40% of the maximum speed for five minutes, continued cooling down with speed equal to 50% of the maximum speed rate. The number of high-intensity activities in the first, second, third, and fourth weeks was two, four, six, and eight repetitions, respectively. Thus the total time of the exercise including high intensity activity, low intensity activity along with warming and cooling down in the first, second, third, and fourth weeks was equal to 16, 24, 32, and 40 minutes, respectively. In the MICT group, the rats first ran on the treadmill to warm up for five minutes. Then, they did endurance training at a speed equivalent to 60% of the maximum speed rate in the first week, 65% of the maximum speed in the second week; 70% of the maximum speed from the third week to the end of the study period. The running distance for the rats in both groups was equal. Preparation of bone specimens

After eight weeks of training, the rats were anesthetized with ether in the desiccator container. Their right femur was placed in 10% formalin solution for morphometric study after removing soft tissue. The right femur was removed from the soft tissue for evaluation and kept in the freezer at -20 °C to be further tested. Three hours before the test, the specimens were placed at room temperature and wetted with saline. Then, they were transferred to the laboratory of mechanical properties and the three-point bending flexural test was run in the Korea SANTAM machine DBBP-50.

To run this test, after selecting the test indices in the machine software, the jaws of the machine were adjusted to the three-point bending flexural test. After removing the bone sample from the physiological serum, it immediately placed on the two anterior-posterior metal supports on the lower jaws of the device. The upper jaw then moved vertically on the longitudinal axis of the bone at a constant speed of 10 mm/min. To ensure the integrity of the force points in all the femoral samples and with the placement of the colitis in spine on the head far from the trunk (distal) and near the trunk (proximal) of the bone, the bone specimen was placed on the base of the machine with the force point of the machine at the mid-point of the bone tissue. The load applied during the bone tissue fracture as well as the force-displacement curve was automatically plotted by the machine software and recorded in the monitor of the computer connected to the machine. Statistical Analysis

In order to study the equality of variances, Levin’s test was used and the Kolmogorov-Smirnov test was used to check the normal distribution of data. The mean and standard deviation were also used to describe individual features, and one-way analysis of variance (ANOVA) was run to analyze the effectiveness of the interventions. Statistical analysis was performed using SPSS software version 20. The significance of data differences was calculated at p≤0.05.

Results

The weights of the rats were measured in the first, and then in the fourth and eighth weeks (Table 2). The ANOVA test revealed that eight weeks of severe and moderate endurance training had no effect on the results of three points bending flexural tests in old male rats (p>0.05) (Table 3).
**Table 1. High and Moderate Intensity Endurance Training Protocol**

<table>
<thead>
<tr>
<th>Activity</th>
<th>High and Moderate Intensity Endurance Training Protocol based on the maximum oxygen consumption rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warming/5 min.</td>
<td>Moderate Intensity Endurance Training (maximum speed)</td>
</tr>
<tr>
<td>Max speed test (Average of 34.4 meters per minute)</td>
<td>Max speed test (Average of 33.8 meters per minute)</td>
</tr>
<tr>
<td>Cooling down/ 5 min.</td>
<td>High Intensity Endurance Training Protocol (2 min. for each repetition)</td>
</tr>
<tr>
<td>5 minutes with 40 to 50% of maximum speed</td>
<td>6 minutes with 40 to 60% of maximum speed</td>
</tr>
<tr>
<td>2 repetitions with 80% and 1 repetition with 40% of maximum speed</td>
<td>40 to 50% of maximum speed</td>
</tr>
<tr>
<td>Second week</td>
<td>24 min.</td>
</tr>
<tr>
<td>5 minutes with 40 to 50% of maximum speed</td>
<td>14 minutes with 65% of maximum speed</td>
</tr>
<tr>
<td>4 repetitions with 90% and 3 repetitions with 40% of maximum speed</td>
<td>40 to 50% of maximum speed</td>
</tr>
<tr>
<td>Third week</td>
<td>32 min.</td>
</tr>
<tr>
<td>5 minutes with 40 to 50% of maximum speed</td>
<td>22 minutes with 70% of maximum speed</td>
</tr>
<tr>
<td>6 repetitions with 100% and 5 repetitions with 40% of maximum speed</td>
<td>40 to 50% of maximum speed</td>
</tr>
<tr>
<td>Fourth week</td>
<td>40 min.</td>
</tr>
<tr>
<td>5 minutes with 40 to 50% of maximum speed</td>
<td>30 minutes with 70% of maximum speed</td>
</tr>
<tr>
<td>8 repetitions with 110% and 7 repetitions with 30% of maximum speed</td>
<td>40 to 50% of maximum speed</td>
</tr>
<tr>
<td>Fifth to eight week</td>
<td>40 min</td>
</tr>
<tr>
<td>5 minutes with 40 to 50% of maximum speed</td>
<td>30 minutes with 70% of maximum speed</td>
</tr>
<tr>
<td>8 repetitions with 110% and 7 repetitions with 30% of maximum speed</td>
<td>40 to 50% of maximum speed</td>
</tr>
</tbody>
</table>

**Table 2: Mean ± standard deviation of body weight and VO2max in male rats before and after four and eight weeks of endurance training**

<table>
<thead>
<tr>
<th>variable</th>
<th>Group</th>
<th>Before training</th>
<th>At the end of Week 4</th>
<th>At the end of Week 8</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>C</td>
<td>24.07±445.50</td>
<td>30.93±452.00</td>
<td>449.38±28.67</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>MICT</td>
<td>23.68±445.22</td>
<td>23.35±434.67</td>
<td>15.68±434.89</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>HIIT</td>
<td>23.36±436.14</td>
<td>49.43±426.75</td>
<td>42.25±416.12</td>
<td>8</td>
</tr>
<tr>
<td>VO2max</td>
<td>C</td>
<td>2.30±26.25</td>
<td>2.31±26.25</td>
<td>2.32±26.25</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>MICT</td>
<td>2.20±33.89</td>
<td>5.27±40.55</td>
<td>4.17±38.80</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>HIIT</td>
<td>2.58±33.13</td>
<td>5.82±38.75</td>
<td>3.72±46.87</td>
<td>8</td>
</tr>
</tbody>
</table>

**Table 3: One-way analysis of variance for comparing biomechanical properties of rat bones in three C, MICT, and HIIT groups using three-point bending flexural method**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>mean ±sd</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load or force (N)</td>
<td>C</td>
<td>142.12±42.4882</td>
<td>4.105</td>
<td>0.31</td>
</tr>
<tr>
<td>Stress or bone strength (MPa)</td>
<td>C</td>
<td>1.84±7.15</td>
<td>4.467</td>
<td>0.24</td>
</tr>
<tr>
<td>Energy (J)</td>
<td>C</td>
<td>59.75±36.05</td>
<td>1.712</td>
<td>0.204</td>
</tr>
<tr>
<td>Deformation to maximum strength point (mm)</td>
<td>C</td>
<td>0.112±0.0393</td>
<td>0.894</td>
<td></td>
</tr>
</tbody>
</table>
| C = Control group, MICT = Moderate-Intensity Continuous Training group, HIIT = High intensity Interval training group

**Discussion**

The results of this study indicated that eight weeks of endurance training with different intensities has no significant effect on modulation, maximum endurance, and fracture energy, and femoral strength of elderly male rats. These results are in accordance with Mohammad Ameli et al.’s (2016) study in which they examined the effect of eight weeks of endurance training on selected biomechanical properties in the femur of adult male rats. In their study, endurance exercise included climbing the ladder, which lasted five sessions per week for eight weeks. Their findings suggested that eight weeks of endurance training had no significant effect on stiffness, maximum endurance, and energy of femoral bone fracture in elderly male rats. Similarly, Rooney et al. (2014) found in that the protocol...
of training on a treadmill at a constant speed of 10 m/min for one hour per day, five days a week for 12 weeks, did not significantly change the maximum load, modulation, flexural strength, or maximum stress\(^{(29)}\).

On the other hand, the results of this study contradicted the findings of Singulani et al. In their study, the elderly mice who performed 16 weeks of endurance training enjoyed its beneficial effect on biomechanical parameters in bone tissue, compared to the control group\(^{(30)}\). The findings of the present study contradicted those obtained by Huang et al. in 2003. They studied the effect of 20-60 minutes of daily training and swimming at the speed of 12-22 m/min on bone growth among 29 male Wistar rats (aged seven weeks). Their findings showed that the mechanical properties of femur in rats of the training groups were significantly more than those in the control group\(^{(31)}\). Milliken et al. (2003) reported that 12 months of weight training and endurance training increase BMD and bone regeneration (bone formation and absorption)\(^{(32)}\).

The inconsistencies between the findings of the present study and the above findings can be explained by some possible reasons, such as age of the rats\(^{(33)}\), short study time, and the type of applied mechanical load\(^{(34)}\). On the other hand, young subjects are more responsive to physical activity than older ones\(^{(35)}\). For example, Turner et al. showed that the osteogenic response of young mice is higher than that of older mice; even though, the older mice the same as the young mice had the ability to respond to mechanical load\(^{(33)}\). Some age-related changes in mechanical signals, hormones, growth factors, and cytokines seem to reduce the response of the elderly’s skeletal system to mechanical load\(^{(36)}\). Moreover, the ability of mechanical load to activate signaling pathways in the bone is also determined by the type of mechanical load applied to the bone\(^{(20)}\) since muscle strength is associated with bone density and its effect on the skeleton site\(^{(37)}\). The short duration of this study was also one of the reasons for the lack of effects of physical activity on bone metabolism. Since a majority of studies that had a significant effect on bone restoration lasted more than 150 to 200 days\(^{(38)}\), such a long time might be required to adapt to low intensity exercises. The study sought to investigate whether exercises with higher intensity and shorter time periods (like the current protocol) could have beneficial effects on osteoporosis.

Another reason for the ineffectiveness of these exercise protocols on factors associated with bone metabolism can be explained by Frost’s principle. Forest proposed two processes of bone erosion and bone formation in response to mechanical load\(^{(39)}\), in which bone structure is maintained through a feedback system as the increase in mechanical pressure causes bone stimulation and bone growth and formation. This theory is known as the theory of mechanical status. According to this theory, the mechanical pressure should be such that it can overcome the bone formation or restoration over the bone resorption process. This mechanical pressure is called the minimum effective pressure threshold\(^{(40)}\). In those studies, which there was no variation in bone density, and the present study, the lack of variation might be caused either by this issue indicating that the intensity and exercise load were not at the least effective level to improve bone density in femurs\(^{(40)}\) or by this principle indicating that if bone stress is above a certain level, osteoclasts are mobilized and restoration changes\(^{(41)}\). In short, we believe that the mechanical load level affecting the bone metabolism varies with age; however, we failed to determine the threshold value in this study.

In addition, the results of this study suggested that the mean weight of the control group after eight weeks of training was higher than that of the experimental group. On the other hand, the weight loss following the endurance training in this study can be one of the factors inhibiting the effect of endurance training on the biomechanical properties of femurs in the
old rats since the body weight imposed on the bone plays a critical role in the generation and maintenance of bone density and strength.\(^\text{[42]}\) Research has documented that weight loss in low-gravity environments leads to bone loss in weight bearing bones.\(^\text{[43]}\) Moreover, some research data has indicated that frequent activities of signaling pathways during repeated physical activities result in the synthesis of auto-feedback proteins, and these proteins prevent the activation of previous osteogenic pathways. This would lead to the saturation of the osteoblast function (and ultimately to the reduction of their function to the base level). Simply put, if the lack of sensitivity to sports training is obtained through negative feedback, then the resulting mechanical load has limited advantages and it may even have a negative effect.\(^\text{[44]}\) In order for the bone not to lose its sensitivity to mechanical load and for the mechanical load protocols to have a more osteogenic effect, mechanical load cycles should be performed with variability and no uniformity.\(^\text{[45]}\) Since the intensity of the tests was constant from week 4 to week 8 and the subjects experienced weight loss, the bone tissue sensitivity to this type of training might be reduced and, given that sports training requires a lot of energy, it might have disrupted the energy balance. These would lead to such an effect in the bone tissue of the old rats.\(^\text{[46]}\)

In general, it could be concluded that one of the possible reasons for not concluding the present study might be the beginning age of training since the mice were young in a majority of previous studies, in which the findings were promising. Another possible reason might be the exercise duration. The elderly may respond to longer training periods, and this issue needs further research. The elderly seems to need longer training periods than the youth in order for their bones to respond to the exercises in elderly years. In the present study, the concerned factors were only measured in the post-test phase and no information was available regarding the initial value of these factors before and during the intervention, which could overshadow the findings. Furthermore, the rats were used because of the need for elderly bones in this study; however, we failed to have a longer training period due to the rats’ short lifetime. As the number of the elderly is increasing in our country and other countries and given that the findings of most studies have confirmed the positive impact of activity on the physiological factors at this age, the future research is recommended to examine the effect of resistance and endurance training as well as combined training over a longer period at different intensities in old men and women.

**Highlights:** An examination of biomechanical variations in bones following such exercises over a longer period can provide a brighter picture of the positive or negative consequences for these types of exercises in the healthy and passive elderly.

**Conclusion**

### References

6. Kohrt WM, Ehsani AA, Birge SJ, Jr. Effects of exercise involving predominantly either joint-reaction or ground-
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