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The Effect of Eight Weeks of Endurance Training on Positive and Negative Slopes on the Selected Biomechanical Parameters of the Femur Bone in Male Wistar Rats

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Abstract

Introduction: Regular exercise and physical activity is one way to promote health and prevent physical and mental diseases. This study aimed to investigate the effect of endurance training (ET) on positive and negative slopes (ETPS and ETNS, ±15°) on the biomechanical properties of the femur bone in male Wistar rats. **Materials and Methods:** This experimental study was performed on 15 male Wistar rats with an approximate age of eight weeks and a weight range of 180-200 g. For this purpose, the rats were divided into three groups, namely, the control (without activity), ETPS, and ETNS groups. The ET program was performed five days a week for eight weeks. The obtained data were statistically analyzed using the one-way ANOVA test at $P<0.05$. **Results:** Significant improvement was observed in biomechanical variables including maximum force, strength, deformation, and maximum absorbed energy of the femur bone in the ETPS group compared with the other two groups. However, the ETNS reduced all biomechanical variables of the femur bone. **Conclusion**: The results indicated that ETPS is effective in improving bone indices. Given the significant difference between the ETPS and ETNS groups in the biomechanical parameters of the bone, it is suggested that a training program with different intensity and duration levels be implemented in other age groups on different slopes.

Keywords: Animal mode; Biomechanical testing; Endurance training; Femur bone; Three-point bending

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Introduction

Bone is a dynamic and living connective tissue (1). This hard, dense, and rigid tissue, which makes up the vertebrate skeleton (2), protects the internal organs (1,2). The bones and skeleton of the body play an important functional-mechanical role in the body because of their strength (3) for help to body movement(2). The most important function of the bone tissue is to withstand and transmit forces without fractures. Bone strength depends on the amount of tissue, the composition of its materials, and the organization of the bone material in terms of microarchitecture and geometry, namely, the shape and size (4-6). Bone is a vital element for exercise. The presence and structure of bone entirely rely on the shape, amount, and activity of bone cells. Exercise or mechanical stimulation is one of the main factors affecting bone mass (7).

Exercise promotes physical and bone health and is the first choice for non-pharmacological strategies that can help improve and prevent the complications of many chronic diseases (8); it is recommended as a low-cost and safe intervention strategy to maintain musculoskeletal health (9). In different types of exercise activities, endurance training (ET) includes exercises in which the body uses oxygen to provide energy. These exercises use the largest muscle group in the body with intensity, repetition, and sufficient time (10). Although the specific mechanisms by which exercise improves bone health have not yet been specified completely, it is widely accepted that the mechanical load resulting from exercise could increase muscle mass, create mechanical stress on the skeleton, and increase osteoblast activity (11-13).

Snyder *et al.* (14) investigated the effect of two ET programs of equal intensity and time on 38 female Wistar rats in three running, swimming, and control groups. The results showed that exercise had a lesser effect on bone growth in the animals in the running group. However, it stimulated bone growth in the group that received swimming training. humerus bone in The

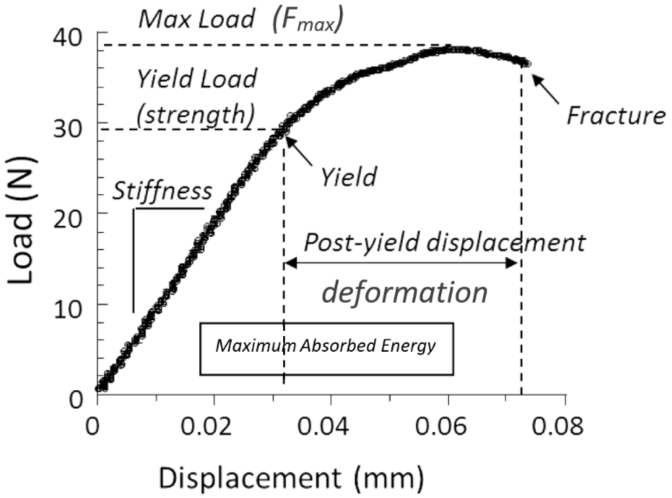

Figure 1. Example load-displacement plot from a mouse femur bending test

the swimming group was significantly larger than in the running and control groups ($P<0.05$) and had greater bone mineral content (BMC). In addition, the muscular forces exerted by the swimming training protocol produced greater bone adaptation compared to forces exerted by the running training protocol with the same intensity and time. Experimental studies have been performed on long bone diaphysis, but evaluating the biomechanical effects of bone involves the metaphysis of the femur or tibia because long bone diaphysis consists only of bone marrow; however, major changes caused by bone-related diseases in the trabecular bone, which exists in the metaphysis of bone (15,16).

Determining the effect of each treatment method during the process of repairing bone defects involves evaluating resistance to the point of fracture. Several structural parameters such as bone architecture, bone mineral content (BMC), and density are used to indirectly examine the biomechanical properties of the bone. However, bones ability to withstand fractures can only be evaluated via biomechanical strength tests (16,17).

Stiffness is defined as the slope ($\Delta y/\Delta x$) of the linear region of the load-displacement curve. It is a measure of the resistance offered by the whole bone to the applied displacement during the elastic region and is analogous to a simple spring constant (K) from physics. The stiffer a bone is, the more force is required to produce a given displacement, and thus the steeper the slope.. Yield load indicates the value of load at the yield point, where the load-displacement plot deviates from linearity. It is a measure of how much load the bone can sustain before it suffers permanent damage. It is one measure of bone strength. Maximum load (Fmax) (aka, Ultimate Force) is simply the maximum value of load attained during the test. It is the simplest measure of the whole bone's strength. The stronger a bone is, the higher its maximum load. Deformation is the displacement from the yield point to the fracture point, and is a measure of ductility. Maximum absorbed energy (aka, Energy-to-fracture) is defined as the total area under the curve. It represents the work that must be done to fracture the bone, sometimes called the whole-bone toughness. A tough bone requires more work to fracture (Figure 1) (18). A variety of biomechanical methods can be applied to identify the structural properties of the bone, including three or four-point tensile, compression, torsion, and bending tests (16,20). The three-point bending test device is the most common tool for mechanical experiments used to describe the biomechanical properties of long bone tissues in small animals (15,16,21).

Figure 2. Running and forced exercise test for measuring the biomechanical properties of the femur bone

Although the exact mechanisms of the beneficial effects of exercise on skeletal health have not so far been determined completely, it has been reported that exercise-induced changes are beneficial to bone health for many different aspects such as force stimuli, hormones, cytokines, and cells (19). Evidence indicates that not all exercise is bone-building, and different types of exercise with different intensities differently affect bones and muscles. Therefore, the results of this study can provide new knowledge about the effect of endurance training on positive slope (ETPS) and endurance training on negative slope (ETNS) on the biomechanical function of the femur bone.

Table 1. Endurance training on positive and negative slopes

Week	Activity repetition and recovery	Speed (m/min)	Intensity of activity	Intensity of recovery	Speed of recovery(m/min)	Total exercise time (min)
1	4.1	10	45%	25%	5	25
2	4.1	11	50%	30%	5	30
3	5.2	12	55%	30%	6	35
4	5.2	13	60%	35%	6	40
5	5.2	14	65%	40%	6	45
6	6.3	15	70%	45%	7	50
7	6.3	16	75%	50%	7	55
8	6.3	17	75%	50%	7	60

Materials and Methods

A variety of biomechanical methods can be applied to identify the structural properties of the bone, including three- or four-point tensile, compression, torsion, and bending tests (16,20). The three-point bending test device is the most common tool for mechanical experiments used to describe the biomechanical properties of long bone tissues in small animals (15,16,21).

Animals

In this experimental trial, 15 male rats with an approximate age of eight weeks in the weight range of 180-120 g were prepared from the Laboratory Animal Breeding and Reproduction Center of the Pasteur Institute of Iran and then transferred to the Animal House of the Faculty of Pharmacy of the University of Tehran (approval ethic: IR.SSRI.REC.1401.1510). The rats were kept for one week to adapt to the new environment under standard laboratory conditions. The subjects were then randomly divided into three groups, including the control (without activity), healthy (running on a positive slope), and healthy (running on a negative slope) groups. All the animals were kept under environmentally controlled conditions in standard rat storage boxes with an average temperature of 22±3°C and a humidity of 40% with a cycle of 12-12 hours of light and darkness in standard size cages. The subjects had free access to standard dry water and food (Behparvar Company).

Training protocol

For a week, the rats in the exercise groups were familiarized with how to run on a rat-specific electronic treadmill on both positive and negative slopes. The training groups performed the ET program on positive and negative slopes five days per week for eight weeks (Figure 2). In the first week of the exercises, the rats of both groups, running on positive and negative slopes, were warmed up by walking slowly for five minutes at a speed of 0.03 m/s (1.8 m/min), and then the speed was increased every three minutes until the rats were unable to walk and started running. The intensity of ET started from 45% of peak aerobic power with a speed of 10 m/min in the first week and continued with a speed of 17 m/min and 75% of peak aerobic power (all steps were applied in the table of ET on positive and negative slopes). The number of repetitions in ET on positive and negative slopes in the first week was five, including four repetitions of 45% intensity (10 m/min) and one recovery repetition of 25% intensity (5 m/min). The total time of one session started from 25 minutes and continued up to the eighth week with nine repetitions, including six repetitions with an intensity of 75% of peak aerobic power at a speed of 17 m/min and three repetitions with an intensity of 50% of peak aerobic power and 7 m/min and a total session time of 60 minutes. It should be noted that the time, speed, slope, and intensity of the shock were determined quite digitally when running on the animal treadmill (Model: Mahour, 5 lines, ±15 degree slope). The details of the ET program are presented in Table 1.

Biopsy and separation of rats

At the end of the eighth week and one day after the end of the exercise sessions, the rats were anesthetized (i.e., they were placed in a desiccator for 1-2 min to be anesthetized by inhaling ether gas; they easily died in this way with no damage to their bones) and then dissected (the skin and muscles of their legs and thighs were cut, and their bones were carefully removed without damage to their periosteum after the appearance of their right femur bone). They were immediately placed in Falcon test tubes (60 tubes, 50 cc) in a 10% normal saline solution to prevent hydration and stored at minus 70°C till the test time. For its transference to the laboratory, each bone was labeled and placed in Falcon tubes.

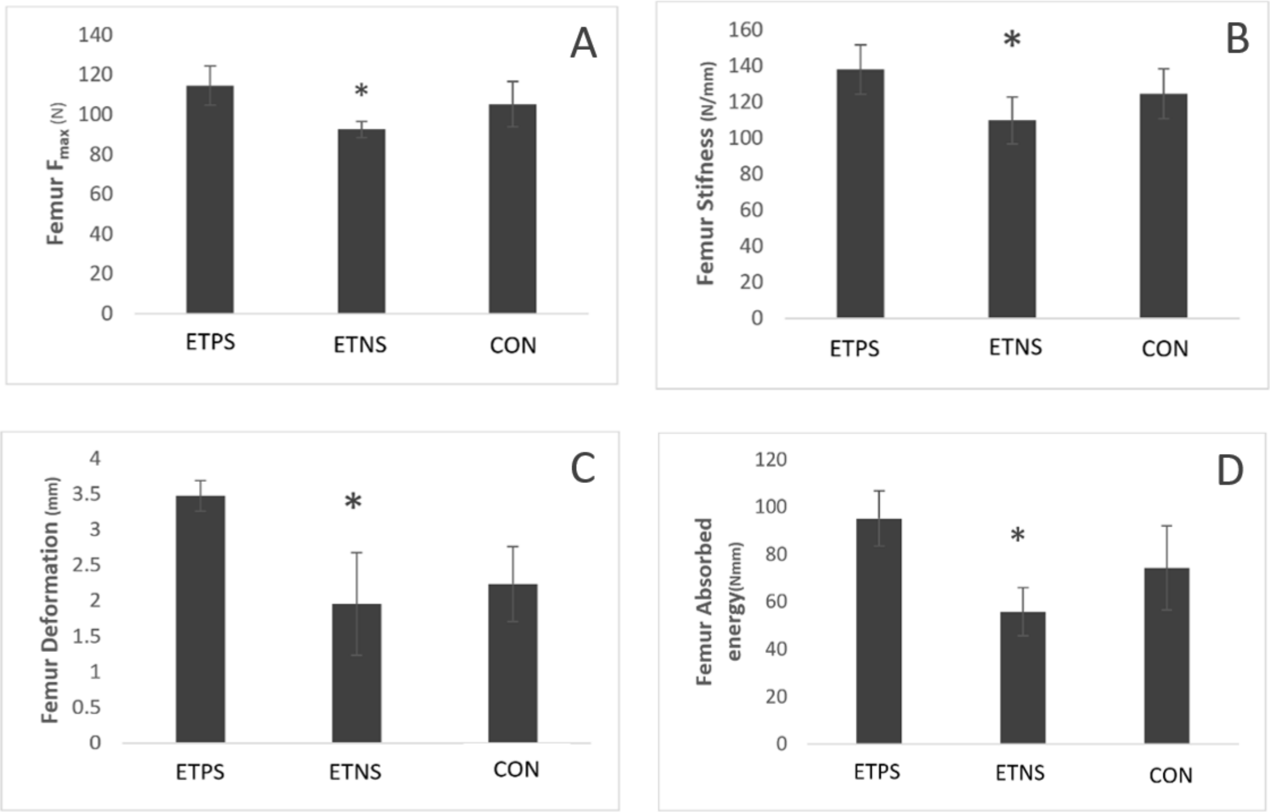

Figure 3. Changes of biomechanical variables (maximum force, strength, deformation, and maximum absorbed energy) of the femur bone among the groups; *Note*. * Indicates a significant difference compared with the positive slope running and control groups.

Then, one side of the femur bone was sent to the Biomechanics Laboratory of the Department of Physiotherapy of Tarbiat Modares University so that the biomechanical factors of their femur bones could be examined through the three-point bending test, while the other side of their femur bones was simultaneously transferred to the pathology laboratory of Imam Khomeini Hospital in Tehran for histological examinations.

Tensile test

The samples of the femur bone were inserted into the vertical arm of the tensile test device to determine their strength using the force exerted by the tensile device. The tensile property of the bone was obtained when force was directly and vertically exerted on the bone until fracture. The resulting curve showed the material's reaction to the exerted forces, and the maximum tension is highly important.

Protocol for measuring the mechanical strength of the bone

A Germany-made Zwick Roell testing machine (3-Point Bending) was used to measure the mechanical strength of the bone. Immediately after removing the bone sample from the physiological normal saline, the test was started on the femur bones of all rats. The jaws of the device were initially adjusted according to the three-point bending test, and the bone was anteriorly-posteriorly placed on two metal supports on the lower jaws of the device. The upper surface of the bones was placed upwards, and sandpaper was employed under the two ends of the bone and at the contact point of the upper jaw of the device to prevent the bone from slipping. Next, the applied load during the rupture of the bone tissue and the force-displacement curve were automatically recorded through the output of the relevant software application in the computer connected to the device, and the biomechanical parameters (stiffness coefficient (Newtons per millimeters), maximum force (Newtons), and maximum absorbed energy (Newtons per millimeters) were reported for each bone sample.

Statistical Analysis

All data were reported based on the mean and standard deviation. Data distribution normality and homogeneity of variance were tested using Shapiro-Wilk and Levene's tests, respectively. One-way analysis of variance (ANOVA) was applied to determine the effect of running on positive and negative slopes. If a significant difference was observed, the LSD post hoc test was used to determine the differences.

Table 2. Mean and standard deviation of biomechanical parameters of the femur bone

Variable Mean (SD)	F_{max} (N)	Stiffness (N/mm)	Deformation (mm)	Absorbed Energy (Nmm)
ETPS	111.28 (6.12)	133.38(8.61)	3.40 (0.13)	91.30(7.35)
ETNS	94.69 (1.31)	117.02 (4.21)	2.35 (0.23)	61.33(3.28)
Control	101.40 (3.40)	119.84 (4.16)	2.06 (0.15)	68.50(5.28)

Results

The results of the Shapiro-Wilk test revealed that the data were normally distributed. Levene's test results showed the homogeneity of variances, and the assumptions for the use of ANOVA were observed accordingly. Running on a positive slope had a significant effect on the Fmax of the femur bone (F=4.12, *P*=0.02). Running on a negative slope caused a significant decrease in Fmax compared with the positive slope running group (*P*=0.002) and the control group (*P*=0.05). As shown in Figure 3B, the stiffness degree of the femur bone was affected by running (*F*=4.29, *P*=0.009). Running on a negative slope, compared with running on a positive slope, resulted in a significant decrease in femur bone stiffness (*P*=0.008). Based on data in Figure 3C, the deformation degree of the femur bone was affected by running (*F*=15.50, *P*=0.001). The deformation rate was significantly higher in the positive slope group than in the negative slope group (*P*=0.001) and the control group (*P*=0.003). Figure 3D, illustrates that the amount of the energy absorption of the femur bone was influenced by running (*F*=7.91, *P*=0.005). According to Figure 3D, the energy absorption of the femur bone was significantly reduced as a result of running on a negative slope compared with the positive slope running group (*P*=0.001) and the control group (*P*=0.05). Table 2 provides the mean and standard deviation of biomechanical parameters (*i.e.*, Fmax, strength, deformation, and absorbed energy) of the femur bone for the positive slope running, negative slope running, and control groups.

ETPS: endurance training on positive slope, ETNS: endurance training on negative slope

Discussion

The current study sought to investigate the effect of a period of ET on positive and negative slopes on the biomechanical parameters of the femur bone in 8-week-old male Wistar rats. The results demonstrated that the subjects in the ET group running on a rat-specific treadmill on a positive slope had a significant difference in biomechanical components at the end of eight weeks of exercise compared with the control (*i.e.*, the group with no activity) and negative slope running groups.

Based on the results of this study, the ET of running on a positive slope could significantly increase the biomechanical variables of the maximum force (Fmax), bone stiffness, deformation, and maximum absorbed energy of the femur bone in the rats. In a study on three-year-old female Sprague-Dawley rats, Huang *et al.* (22) reported that 12 weeks of ET (running on a treadmill) increased maximal thigh strength. Renno *et al.* (23) also evaluated the mechanical properties of the bone in 12-week-old female Wistar rats and found that physical exercise increased maximal bone strength in the rats. The findings of these studies are consistent with those of the present study. Similarly, Savage *et al.* (24)concluded that myostatin increased bone strength in rats that had exercised on a treadmill compared with those that had no exercise. However, Mohammad Amoli *et al.* (25) indicated that eight weeks of resistance training had no effect on the biomechanical variables of the femur bone in older male rats. This inconsistency seems to be directly related to the type of ET activity (running on a zero-degree slope). Moreover, the sample rats used in the mentioned study were very old (20 months). The response of the bone tissue to endurance activity may vary at different ages. In addition, the results of the present study contradict those of Hemmati Farsani *et al.* (26) They investigated the effect of eight weeks of high- and moderate-intensity ET on the biomechanical variables of the femur bone in 23-month-old male Wistar rats. Their findings showed that such training had no effect on the biomechanical changes of the rats' bones. To explain the inconsistency of these results, we can mention the role of factors such as the intensity of running on the treadmill, the age range of the rats, and exercise on a zero-degree slope.

The present study examined the biomechanical properties of the femur bone as a result of ET on a negative slope. The data revealed that ETNS significantly reduced the biomechanical parameters variables (maximum force, strength, deformation, and maximum absorbed energy) in the femur bone. The results of a study by Vernillo *et al.* (27) indicated that downhill running increased the impact force of the tibia, which is associated with injuries caused by overuse. Further, the muscle activity required to increase strength and absorb abnormal energy during downhill running puts more pressure on musculoskeletal tissues. The slope of the applied surface can also affect the ground reaction force (28). The findings of a study conducted in 2005 indicated that a negative slope of 9 degrees (downhill running) could increase the impact force by up to 54% compared with level (zero-degree) running (29). Researchers believe that downhill running can generally lead to significant neuromuscular fatigue due to peripheral and central mechanisms (30). These results support the findings of the present study. Considering the above-mentioned results and the findings of the current study, it seems that the duration of training on a negative slope was inappropriate in the present study. In this regard, it has been suggested to examine subjects in different time periods in order to accurately identify the time of the biomechanical changes in their bones. Therefore, better knowledge of this issue involves exploring the effect of different periods in ET on a negative slope.

Conclusion

Overall, the findings demonstrated that ETPS improved the biomechanical parameters of their femur bones. However, ETNS showed a reducing effect on the biomechanical parameters of the femur bones. Moreover, this study revealed some important questions about selected biomechanical parameters of skeletal system, there are still many unanswered questions in this regard. It seems that the reason for the inconsistency of research findings can be attributed to factors such as differences in the type of exercise, age, race, and duration of exercise. Therefore, more studies are needed in this area to more clearly evaluate the exercise intervention process on the bone. Thus, it is suggested to conduct further research in this field using different types of physical exercises with various intensity and volume levels on different slopes.

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Conflict of interest
None.

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Authors' contributions
All authors made substantial contributions to the conception, design, analysis, and interpretation of data.

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