

The Effect of 16 Weeks of Endurance Training on the Femur and Tibia Bones in Adult Male Wistar Rats: Biomechanical and Geometrical Parameters

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Abstract: Prediction and prevention of bone fractures is considered as one of the most important challenges of the world's health systems. The aim of this study was to evaluate the effect of 16 weeks endurance training on biomechanical and geometrical parameters of the femur and tibia bones in adult male Wistar rats. The 16 rats with an age range of 50-60 days and an average weight of 160 ± 10 g were randomly divided into two groups: training (progressive running protocol for 16 weeks at a speed of 18-36 m in minute for 20-60 min, 5 days a week) and control group (no activity). A day after the end of the training, the rats were sacrificed, the right femur and tibia bones were harvested, then by mechanical three-point bending test, the biomechanical parameters (stiffness, maximum mechanical strength and maximum energy to the point of maximum strength) and by digital caliper, geometric parameters (length and width) were measured. One way-ANOVA was used to compare biomechanical and geometrical parameters between groups ($p \leq 0/05$). The results demonstrated a significant increase in biomechanical parameters (stiffness (the femur and tibia), maximum mechanical strength (in the femur)) and geometric parameters (length (the tibia)) after 16 weeks endurance training. The 16 weeks of endurance training (used in this study) can be used to prevent fractures and osteoporosis and osteomalacia with improved biomechanical and geometry parameters used in the femur and tibia bones.

Key words: Endurance training, bone tissue, bone strength, bone health, tibia, femur

INTRODUCTION

Bone problems such as osteomalacia, osteoporosis and bone fractures are the major health issues in most developed communities and countries whose rate in men and women is 13-22 and 40-50%, respectively and is increasing as prosperity and welfare grow in such countries (Johnell and Kanis, 2005).

Given the importance of these fractures in increased mortality rate and reduced quality of patient's life and in order to decrease side effects of some unwanted medical interventions, it is of great significance to accurately predict the fractures and evaluate the effectiveness of various treatment methods in reducing the risk of fracture. According to medical findings, bone tissue regularly is modeled. Generally, this process starts with bone resorption by stimulating the osteoclast cells. Bone formation is done by osteoblasts (Albright and Skinner,

1979). Sensitivity to the mechanical environment is one of the bone's stimulating and anti-absorption factors which in fact represents the Wolff's law stating that bone's shape specifies its functional nature. Relationship between bone structure and forces acting on it is originated from healthy bone's ability to adopt constantly to the environment under load and thus the skeletal system will be adaptable to the existing environment. This ability is generally due to the modeling and remodeling properties of bone which removes the old bone tissue in the maximum and minimum loading environment and reforms new bone tissue in an environment with appropriate load. The adaptability of skeletal system to the environment creates significant changes in bone's mass, geometry and material properties (Frost, 1990). On the other hand, any kind of movement and activity on the ground also incur load on the weight-bearing bones and improves their structure. Bone formation and resorption

is sensitive to the external force caused by gravity and the internal force generated by muscle activity (Warner *et al.*, 2006).

Bone strength depends on various factors such as geometric features, non-uniformly distributed density, mechanical properties of its material, structural characteristics, loading conditions and stress distribution, so any solution that maintains and slows that the decreased bone strength process is very important as a useful strategy to reduce the risk of bone fractures (Renno *et al.*, 2007). Since, bone formation and resorption is sensitive to the external force caused by gravity and the internal force generated by muscle activity (Warner *et al.*, 2006), sports activities are considered as modifiable environmental factors which play a crucial role in improving and increasing bone strength (Renno *et al.*, 2007). However, numerous studies have suggested that bone strength depends on the type of body composition and amount of daily activities. Besides, scientific and empirical evidence has shown that people with reasonable and proper physical activities have more and better bone strength than people with sedentary lifestyle and that sports activities are essential for forming and maintaining strong bones in life.

According to the results of study done by Borer (2005), sports activities is the main factor in maintaining and stimulating bone formation which reduces the risk of bone fractures through mineral buildup, strengthening the muscles and improving individual's balance. These researchers have reported that sports activities in appropriate volume and intensity before puberty increases bone mineral content, strength and transverse growth.

Nickander *et al.* (2009) mentioned that the best type of activity to stimulate the bone to increase its density is weight-bearing exercises which is effective on most bones. Research on investigation of the effect of exercise on human's bone is mainly limited to radiography or evaluation of bone markers which has mostly conducted aiming to assess bone density and mineral content (Lawson *et al.*, 2004; Nordstrom *et al.*, 2005; Munoz *et al.*, 2004). However, many studies in the past decade have suggested that bone density cannot be an accurate predictor of bone health or strength (Frost and Jee, 1992). Therefore, given that research on human have its own limitations and clarifying how endurance training can lead to bone health through strategies besides increasing density mineral content is of great significance using animal samples to measure the effect of exercise on bone condition can be very practical (Iwamoto *et al.*, 2005; Iwamoto *et al.*, 1999). When rats run on treadmill, more mechanical load is imposed on its lateral organs than axial

skeleton hence, Tibia and Femur bones are placed under external load more than lumbar vertebrae (Iwamoto *et al.*, 1999; Mozayyany *et al.*, 2014). Using animals and performing direct biomechanical tests will represent a better reflection of bone mechanical properties than bone density measurements (Huang *et al.*, 2008; Cullen *et al.*, 2001).

Research has shown that with regard to the type of activity and load applied to the bone, exercising creates an osteogenic effect (Marques *et al.*, 2011; Umemura *et al.*, 2002; Johannsen *et al.*, 2003; Bergstrom *et al.*, 2008; Maddalozzo and Snow, 2000). Type (weight bearing exercise), intensity and duration of various sports activities creates different adaptations associated with bone features (Magkos *et al.*, 2007; Wolman *et al.*, 1991; Bayramoglu *et al.*, 2005). At present, there is little consensus about which types of exercise (with that duration and intensity) in which parts of the body could be appropriate the most. For example, according to the reports, physical activities that increase the strength of thigh and hip are different from exercises affecting bone strength in the spine zone (Kelley *et al.*, 2013). So, taking into account the effect of endurance training on the prevention and non-pharmacological treatment of some diseases (osteoporosis, osteomalacia, etc.) and bone fractures in puberty and given that many people tend to do exercises such as hiking and running with a steady rhythm as a non-pharmacological intervention to improve bone structure and prevent possible fractures in different stages of life and considering the contradiction between the results of studies conducted on the effect of endurance training on bone (some research indicate positive effects (Iwamoto *et al.*, 2005, Marques *et al.*, 2011; Umemura *et al.*, 2002; Johannsen *et al.*, 2003; Bergstrom *et al.*, 2008; Maddalozzo and Snow, 2000; Magkos *et al.*, 2007; Wolman *et al.*, 1991; Bayramoglu *et al.*, 2005; Fernada *et al.*, 2005; Kelley *et al.*, 2013; Nikander *et al.*, 2009; Cavanaugh and Can, 1988; Burrows *et al.*, 2003; Cooper and Joy, 2005; Fredericson and Kent, 2005; Prather and Hunt, 2005; Habibzade *et al.*, 2011; Sakamoto and Grunewald, 1987; Dabidi roshan and Nobahar, 2013) some reflect the negative effects (Cavanaugh and Can, 1988; Prather and Hunt, 2005) and some suggest no effect (Habibzade *et al.*, 2011; Sakamoto and Grunewald, 1987) and that the effect of long-term endurance training (16 weeks) with the intensity used in this study on treadmill on bone's biomechanical behavior and geometric parameters have not completely specified, the present article aimed to evaluate 16 weeks of endurance training on biomechanical parameters (stiffness, energy absorbed to

the point of maximum strength and maximum mechanical strength) and geometric parameters (length and width) of femur and tibia bones in male Wistar rats.

MATERIALS AND METHODS

First, a number of matched male Wistar rats (born from parents of the same race and lifestyle) with an age range of 50-60 days old were purchased from Razi Vaccine Serum Research Institute of Iran. After the weight of 16 rats with an average of 10 ± 160 g is matched, they were entered into the study. In line with the policy of the Iranians Association of Laboratory Animal Protection used for scientific and laboratory purposes, rats studied here were kept in Animal House of Physical Education Faculty, Tehran University in four cages (4 rats per cage) under dark lighting cycle (12 h of light and 12 h of darkness) and humidity of $5 \pm 60\%$ and temperature of $22 \pm 2^\circ\text{C}$ and free access to food and water. Standard food in the form of pellets produced by Iran's Razi Institute was used to feed the rats. The rats were randomly and evenly divided into two groups: the training group (16 weeks of endurance training) and control group (no exercise).

Training protocol: Before getting started the main protocol and to familiarize the rats with the new environment and devices, rats in training group began to exercise on treadmill for 5 days, 15 min a day at a speed of 10-15 m/min with zero percent slope. The time of exercise in the original training protocol starts with 20 min in the 1st week which adding 5 min/week and by taking 7 min to warm up and 3 min to cool down it was raised up to 60 min of training a day with 0% slope on the treadmill during the 8th-16th week. The training first began with a speed of 16 m/min in the 1st week then 2 m/min was added to the speed of the treadmill every week until the 9th week ending, so, the speed in the 8th week reached 30 m/min. From the 9th week to the end of week 16, 2 m/min was added to the speed only every 2 weeks as rats were exercised at a speed of 38 m/min for 60 min in week 16 (Table 1). To warm up at the beginning of each training session, rats were run for 3 min at a speed of 10 m/min which was raised up 2 m/min. To cool the rats at the end of each training program, speed of treadmill was slowly reduced until it reached 10 m/min (for 3 min). Given the cost of energy, the intensity of training was 55% of maximum oxygen consumption in the 1st week and almost 88% in the last week (Dabidi Roshan and Nobahar, 2013; Bedford *et al.*, 1979). To stimulate the rats to keep running and not resting, an air pump was used during the exercise. Of course the rats over the stage where were familiarized

Table 1: Endurance training protocol

Weeks	1st	2nd	3rd	4th	5th	6th	7th	8th	9-12	11-12	13-14	15-16
Speed (m/min)	16	18	20	22	24	26	28	30	32	34	36	38
Time (min)	20	25	30	35	40	45	50	55	60	60	60	60

with activity on treadmill were taught to refrain from approaching to the ending part of the device and resting there.

Preparation of bone samples: At the end of 16 weeks and 1 day after training sessions were finished, the rats were anesthetized, operated and bone samples were collected and transferred to the Tissue Biomechanics Laboratory of the Department of Physiotherapy, Tarbiat Modarres University in Tehran. Initially, rats were anesthetized by injecting a mixture of ketamine/xylazine (80/10 mg ketamine to xylazine per kg of body weight) (Fuchs *et al.*, 2007). Then, the skin and muscles of the calf and thigh were cut and the femur and tibia bones of the right side of rat's paw were separated carefully and without damage to the periosteum which were immediately put into the gauze soaked in normal saline solution (sodium chloride 0.9%) to avoid dehydration. To transport to the laboratory, each bone was named and was placed in the container at the temperature of -20°C afterwards, so, we can accurately examine the information of each rat (Notomi *et al.*, 2002).

Measuring the mechanical strength of bone: The three-point bending test device (Zwick-material testing machine) was used to measure. Right after the bone samples were taken out of physiologic serum, the right-side femur and tibia bones of all rats were tested. First the device's jaw was set according to the three point bending test and bone was placed on two metal supports in an anterior-posterior position on the bottom jaws of device. The upper surface of bones was placed upwards and a sandpaper was used under two ends of bone and where it contacts the upper jaw of device to prevent bone slippage. The load placed on when bone tissue is breaking down as well as force-displacement curve were drawn automatically by device software and recorded in a computer monitor connected to the device. Next, biomechanical parameters studied including stiffness, maximum mechanical strength, the energy absorbed to the point of maximum strength were determined for each sample (Layne and Nelson, 1999) (Fig. 1).

Measuring the geometric parameters of bone: In order to measure the geometric parameters of femur and tibia bones, each bone was placed at room temperature. Then

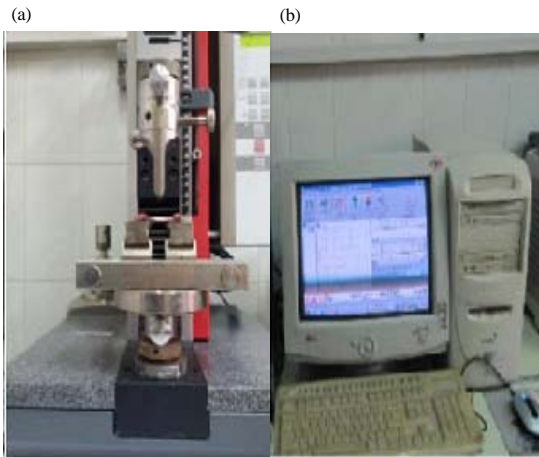


Fig. 1: a) Measuring biomechanical parameters by the three-point bending machine and b) simultaneous recording of information for each sample

the length and diameter of the bones was measured by a digital caliper (made in Japan, capable of measuring with an accuracy of hundredth of a mm). To get the length of the femur, it was measured from the medial condyle to the greater trochanter and tibia was measured from the upper epiphysis to the lower epiphysis. The diameter of femur was measured from the medial part of external split to the internal end of diaphysis and tibia diameter was measured from tibia-fibula joint in both internal-external and anterior-posterior directions by millimeters and up to two decimal figures.

Statistical analysis: After data was collected, descriptive statistics (mean and standard deviation) was used to describe the data before and after training. Before performing statistical analysis, Kolmogorov-Smirnov test was used to investigate the normal distribution of dependent variables scores on each level of factor variable. It was followed by the one-way ANOVA test ($p = 0.05$) to examine the hypotheses. All statistical analyses were carried out using SPSS-20 Software.

RESULTS AND DISCUSSION

There was no significant difference between weights of the groups studied. Mean and standard deviation values of mechanical strength parameters (stiffness, energy absorbed to the point of maximum strength and maximum mechanical strength) as well as geometric parameters (length and width) of femur and tibia bones related to two experimental and control groups are represented in Table 2. As it is shown, mean and standard

deviation of all mechanical strength parameters (apart from the energy absorbed to the point of maximum strength in tibia) and geometric parameters in both femur and tibia bones have increased in the training group compared to the control group.

According to the results of one-way ANOVA test, there was a significant difference in mechanical strength parameters (stiffness in femur and tibia, maximum mechanical strength in femur) as well as geometric parameters (length of femur and tibia) of rats after 16 weeks of endurance training (Table 3).

Bone tissue as a dynamic tissue responds to different types of mechanical stimuli. Physical activities preserve and stimulate bone formation and increase strength and reduce the risk of bone fractures through mineral buildup, strengthening the muscles and improving individual's balance (Layne and Nelson, 1999). The objective of this study was to assess the effect of 16 weeks of endurance training predetermined (training intensity in the 1st and last weeks were respectively 55 and 88% of maximum oxygen consumption) on biomechanical parameters (stiffness, energy absorbed to the point of maximum strength and maximum mechanical strength) as well as geometric parameters (length and width) in femur and tibia bones of male Wistar rats. The mechanical features of bone including stiffness, energy absorbed to the point of maximum strength and maximum mechanical strength and geometric parameters consisting of length and width were evaluated simultaneously in this research. The results showed that, 16 weeks of endurance training applied in this study has improved the mechanical strength of rats in the experimental group compared to the control group because the mean values of biomechanical parameters (stiffness, energy absorbed to the point of maximum strength and maximum mechanical strength) and geometric parameters (length and width) of femur and tibia bones apart from energy absorbed to the point of maximum strength in the tibia of rats in the experimental group were increased when comparing to the control group (Table 2). Although, this only represented a significant difference in mechanical strength parameters (stiffness in femur and tibia bones, maximum mechanical strength in femur) and geometric parameters (length of femur and tibia) of rats after 16 weeks of endurance training (Table 3). Various studies have addressed these parameters on a limited basis (2 or 3 variables) or after giving supplements, medicines or castration of the animals (mostly in female rats) (Iwamoto *et al.*, 2005; Kelley *et al.*, 2013; Dabidi roshan and Nobahar, 2013; Slovik, 1999; Joo *et al.*, 2003) mainly on Femur and Tibia bones for <16 weeks (Drummond *et al.*, 2013; Maddalozzo and Snow, 2000; Nikander *et al.*, 2009).

Table 2: Mean and standard deviation values of mechanical strength parameters (stiffness, energy absorbed to the point of maximum strength and maximum mechanical strength) as well as geometric parameters (length and width) of femur and tibia bones in the experimental and control groups

Variables	Groups	Numbers	Femur Mean±SD	Tibia Mean±SD
Stiffness (N/mm)	16 weeks of training	8	117/951±10/246	52/218±3/148
	Control	8	97/131±10/592	40/255±4/161
Energy absorbed to the point strength (N/mm)	16 weeks of training	8	47/040±4/696	28/910±4/973
	Control	8	44/977±5/076	30/575±2/246
Maximum mechanical strength (N)	16 weeks of training	8	97/530±8/732	49/480±5/747
	Control	8	83/699±3/790	46/517±3/236
Geometric parameters				
Length (mm)	16 weeks of training	8	36/521±1/030	40/529±0/584
	Control	8	35/373±0/706	38/847±0/298
Diameter (mm)	16 weeks of training	8	2/970±0/074	2/603±0/347
	Control	8	2/852±0/148	2/417±0/091

Table 3: Results of one-way ANOVA test for mechanical strength parameters and geometric parameters of femur and tibia bones

Variables	Femur				Tibia			
	F-values	df	Sig.	Eta coefficient	F-values	df	Sig.	Eta coefficient
Stiffness (N/mm)	15/966	1	0/001*	0/533	42/045	1	0/000*	0/750
Energy absorbed to the point of maximum strength (N/mm)	0/712	1	0/413	0/048	0/745	1	0/403	0/051
Maximum mechanical strength (N)	16/886	1	0/001*	0/574	1/614	1	0/225	0/103
Diameter (mm)	4/041	1	0/064	0/224	2/140	1	0/166	0/133
Length (mm)	6/739	1	0/021*	0/325	52/612	1	0/000*	0/790

*Significance level (p = 0.05)

Some researchers performed in the field of the effect of long-term endurance training on humans by Burrows *et al.* (2003), Zanker and Torstoite who have studied bone features of professional endurance running reported low levels of bone mineral density and possible risk of reduced bone mass or osteopenia as well as ineffective endurance exercises on calcium level and ultimately bone strength. They even stated that endurance training can even be harmful (Huang *et al.*, 2008; Iwamoto *et al.*, 1999; Notomi *et al.*, 2002; Nordstrom and Karlsson, 2005) which is not consistent with the results of this research. The researchers revealed that lack of energy in short term may stop bone formation in endurance athletes and impair bone formation and reduce its strength (Notomi *et al.*, 2002). Furthermore, it looks like hard training doesn't place enough pressures and strikes on bones to stimulate bone cells because the minimum pressure and force needed to cause a minimum bone stimulation is 5.2 times body weight. However, the force exerted on bones through activities such as walking jogging is roughly equal to or slightly greater than body weight which is less than the threshold level for stimulating bone cells (Nordsletten *et al.*, 1993). Thus, the pressure caused by physical activity must be large enough to provide a favorable mechanical stimuli to the bones. In this regard, Kelly *et al.* (2013) and Karinkanta *et al.* (2007) stated that bone tissue as a dynamic tissue responds to different types of stimuli as minerals increase (Iwamoto *et al.*, 2004). Though, all types of sports programs do not positively affect bone strength

and reducing its vulnerability risk and that kind, intensity and duration of physical training are some of factors effective on bone strength. According to the investigation conducted on humans, endurance trainings are not generally ineffective. Given that long-distance (endurance) runners would run with the same volume of constant and mild load, after a while their bones will adopt to the load placed on them. It must be borne in mind that any increase in activity duration won't increase bone mass density and its strength. Instead we can have more effect on bone strength by changing exercise intensity (changing repeated rounds) than by altering exercise duration.

The findings of this study correspond with results by Warner *et al.* (2006), Iwamoto *et al.* (1998, 2004) and Barendolts *et al.* (1993) on improvement of some mechanical strength variables (maximum mechanical strength, stiffness) and geometric parameters (length of femur and tibia). It is noteworthy that results announced by these researchers were obtained from study on rats tested in a 10-12 weeks endurance training program.

After 12 weeks of endurance training of rats on treadmill, Warner *et al.* (2006) notified that applying mechanical load on bone deforms different types of bone tissues and stimulates it to adopt to the new requirements through remodeling. In the end, it increases bone strength and thus makes it resist against fractures. Joe reported that, an appropriate endurance exercise can increase bone mass and strength in animals and humans. They divided 14 male 4 weeks old rats equally into two exercise and

control groups. The exercise group was run on a treadmill at a speed of 30 m/min for an hour, 5 days a weeks for 10 weeks. The results indicated that stiffness was increased in the middle part of femur bone after training however no statistically significant difference was reported in relation to the geometric parameters of femur (Kariya *et al.*, 2014). Iwamoto *et al.* (2004) studied 20 young female growing Wistar rats and divided them into two control groups (7 and 11 weeks old) and two training groups (7 and 11 weeks old) and have them run on treadmill with a speed of 25 m/min for an hour a day, 5 days a week. They reported that exercise on treadmill stimulates bone formation and inhibits bone resorption. It also increases the length of femur and tibia by stimulating the growth of longitudinal bones (especially in weight bearing bones) which corresponds with results of the present article on increase in bone length after exercise though there was no significant difference between two groups in the strength of tibia bone.

Barengolts *et al.* (1993) studied healthy and castrated rats and have them exercise on a treadmill with 7% slope for 12 weeks at a speed of 21 m/min for 4 days a week and found that the exercise had no effect on bone mineral in healthy rats. In addition, the length of femur didn't change much which does not correspond with our results here. However, the maximum mechanical strength of femur in experimental group was significantly increased compared to the control group which is consistent with results obtained in the present study. In the end, they reported, although, endurance exercise improved minerals content and biomechanical properties (mostly in femur) in castrated rats and increased the strength of femur in healthy rats but the effect was not significant. In regard to the effectiveness of endurance trainings on geometric, researchers stated that 12 weeks of endurance training in ovariectomized rats maintained the length of femur bone. Iwamoto *et al.* (1998) examined the effect of 12 weeks of physical exercise on treadmill with different intensity and duration in 3 groups of rats with osteoporosis on femoral, tibial and fourth lumbar vertebrae within the following protocol. Exercise group 1: 5 days a week, 1 h a day at a speed of 12 m/min; exercise group 2: 5 days a week, 1 h a day at a speed of 18 m/min; exercise group 3: 5 days a week, 2 h a day at a speed of 12 m/min. According to the results, there was no significant increase in bone mass and mechanical strength of femur in the group with moderate intensity and a 2 h duration or the group with high intensity and an hour duration and the group with moderate intensity and an hour time. The maximum mechanical strength of femoral shaft in the group 1 was significantly more than control group which was

consistent with the results of the present study but the difference was not much in groups 2 and 3 compared to the control group. The results revealed that physical training on treadmill can only be effective in increasing bone mass and mechanical strength of femur and tibia (weight-bearing bones) under a standard diet with calcium and an optimal level (with appropriate intensity and duration) of physical training (where a little fatigue is observed in muscles).

It seems that multiple factors such as rats gender, training protocols (type, duration, intensity), methods of assessment and analysis of information or bones studied (weight-bearing or not) may affect response of bone metabolism parameters to physical activity intervention. Hence, the results of this study were not consistent with some research (Burrows and Bird, 2000). The physiology of the highly trained female endurance runner (Karinkanta *et al.*, 2007; Barengolts *et al.*, 1993; Bethany *et al.*, 2003; Nordsletten *et al.*, 1993; Hagihara *et al.*, 2005; Nozaki *et al.*, 2010). After 8 weeks of endurance training (Hagihara *et al.*, 2005; Bethany *et al.*, 2003; Sakamoto and Grunewald, 1987) stated that tibia strength of exercise group was not significantly different from control group (Robling *et al.*, 2006) and that 8 weeks of endurance training on treadmill did not have a significant impact on the length and diameter of femur and tibia of rats (Wang, 2000). Nordsletten *et al.* (1993) reported that even with regard to the applicability of endurance trainings, most people usually prefer exercises with moderate intensity. But bone strength of animals that have had moderate-intensity trainings had no improving changes (Xu *et al.*, 2012) through special test condition can also affect the results. The researchers calculated bone strength in living tissue. In such conditions, bone strength can be affected by muscle power resulted from exercise. Mechanical loads placed on bone at stimulation threshold level lead to a series of intracellular events such as increased intracellular calcium levels, the expression of growth factors, increased production of bone matrix and ultimately ossification. Animals given high-intensity exercise in some studies had higher bone strength indicating that high load-bearing capacity of the bones can be achieved by high intensity physical trainings which is useful to increase bone strength. In contrast, Nozaki *et al.* (2010) also suggested that high levels of tension or high load for a long time can result in osteoclastogenesis. Robeling *et al.* (2006) also reported that osteocytes cell death caused by overload will increase osteoclasts recalling for displacement of compromised tissue. Therefore, pressure of physical activity must be high enough to provide a good mechanical stimulus for bones.

According to Wolff's law, bone is a living tissue which can adapt to the mechanical environment. In other words, it reacts to the amount and type of loading and unloading (Ryaby, 1998). Recent studies have shown that loading of tissue results in cell deformation, gene expression and construction operations in cell (Rojhan, 1994). Reformation is a major determinant of bone strength against pathological damages and atrophy and is originated from bone cells activity. When force placed on the bones is increased the activity of osteoblasts gets higher than osteoclasts so bone formation process gets started which raises bone density and strength (Draper *et al.*, 1995). In this process, after mechanical load is placed on bone, osteocytes play a key role in sensing the magnitude and extent of strains strategically and thus, response to changes in mechanical strains. Then they release fluid flow to transmit information to the surface cells of osteoblasts or canalicular network (Wiesel, 1988) which leads to a continuing adjustment of minerals and bone architecture. Osteoblasts are responsible for the formation of new bones and secreting its non mineral matrix (osteoid) which is used in calcification (calcium deposit) and regulation of calcium and phosphate flow into and out of cells. When acting, a number of osteoblasts are stored as bone progenitor cells. Some of osteoblasts are gradually surrounded by newly built matrix and turn into osteocytes (Ryaby, 1998). Physiologically, physical activity improves blood flow and nutrition of joints and bones. By increase in blood flow, more oxygen and nutrients will get to the bone cells and exercise with the right intensity will place enough pressure on bones accordingly bones response to the forces on them by getting larger and stronger and absorbing higher amounts of calcium (Barlte *et al.*, 1995). In addition, secreting of bone-building hormones such estrogen is increased by physical exercise. This hormone stimulates the production of collagen and thus increases bone strength. In fact, estrogen activates vitamin D and increases re-absorption of calcium from kidney and helps to absorption and maintenance of calcium in bones and their strength. Several possible factors are introduced as stimuli of bone remodeling including electrical potential of bone tension, direct effect of forces on cell membrane, changes in solubility of the minerals under load, heat generated during placing harmonic loads (Compston, 2001; Carter, 1984). With regard to the studies done on modeling and remodeling of bone, researchers concluded that bone resorption is a natural result of hormones activity in the body and that in contrast, mechanical stimuli are factors affecting the formation and prevention of bone resorption. So, preservation and maintenance of bones will only be possible by providing the right

balance between these two factors (Lanyon, 1984). Regarding all was explained in this study, a continuous endurance training protocol with 55-88% of maximum oxygen consumption was used. This amount of intensity at this age (24 weeks old) is likely to prevent and reduce cell death of osteocytes and osteoclastogenesis and its negative effect on bone quality and biomechanical strength of tibia and femur. It also can help to preserve and somewhat improve biomechanical strength and geometric parameters of tibia and femur bones.

CONCLUSION

In general, the results of this study indicated that long-term endurance training with appropriate intensity and frequency can be used to maintain and improve biomechanical and geometrical parameters and ultimately increase the health of femur and tibia bones and as an effective, reliable and inexpensive method to prevent fractures and some bone diseases (osteoporosis and osteomalacia).

RECOMMENDATIONS

Considering the importance of bone response to the loads applied, reducing the risk of bone's socially vulnerability, encouraging people to do endurance exercises in order to design more efficient training programs for all segments of society, conducting exclusive research (biomechanical, morphology and histology) on different bones considering various durations and intensities in young and elderly men can be helpful.

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