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Original Article

The effect of eight weeks of high intensity interval training on osteoponetin and some bone mineral indices in young women

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Abstract
The purpose of this study was to investigate the effect of eight weeks of high intensity interval training (hiit) on osteopontin and some bone mineral indexes (calcium, phosphorus, magnesium, iron) on active young women. Materials and methods: in this semi-experimental study, 16 active women were selected and divided into two groups: hiit with mean age 22.2±1.2 years, height 162.8±6 cm, weight 57.6± 9 kg, and control with average age of 22.3±4 years, height 156.2±4 cm, and weight 54.6±7 kg. Running- based anaerobic sprint test (rast) with a 3-minute break-in interval, which was added every two weeks. Blood samples of participants were taken before and after the protocol, data were analyzed using independent t-test ($p \leq 0.05$) was analyzed. Results: the results showed a statistically significant difference between the two groups in the osteopontin index ($p = 0.0001$). The results also showed that there was no significant difference between calcium ($p = 0.259$), magnesium ($p = 0.19$), fe ($p = 0.883$) and phosphorus ($p = 0.153$) in the young women of the two research groups. Conclusion: the results of this study showed that the implementation of 8 weeks hiit exercises improved bone mineral density.
Key words: high intensity interval training, osteopontin, bone mineral indices, active young women

Introduction

The bone is a transplanted tissue that together with the cartilage forms the body's skeleton. This tissue is composed of osteoblastic cells and bone matrix. Unlike other transplanted tissues, the bone matrix has the unique ability to calcify. One of the major components of bone marrow is osteopontin. Osteopontin which was formerly called bone sialoprotein, secreted phospho protein-1, and activator-1 t cells, is an acidic glycoprotein and an important factor in bone turnover and osteoclast tightness, cell adhesion, body chemistry control, cell retention, tissue alteration, and inflammation regulation (kiefer, zeyda et al. 2008; de fusco, messina et al. 2017). Osteopontin is a protein at very low levels in normal skeletal muscle. However, the induction of muscle damage has been reported in the increase of 100 equalities of the opn transcription site (hoffman, gordish-dressman et al. 2013; yang, ramachandran et al. 2014).

On the other hand, the bone is interacting with many external and internal factors that affect calcium homeostasis, half of the bone tissue is formed by hydroxyapatite, carbonate, citrate, magnesium, sodium, and steronium crystals. Give that there are many factors affecting the production of osteoblast cells and restoration of osteopontin of a person whose genetics and race are the most important, but nutrition and exercise are among the most important factors that one can control them (menuki, mori et al. 2008). The results show that performing appropriate sporting movements before reaching the maximum bone density can produce 2 to 20% difference in bone density (nordström, karlsson et al. 2005).

Sport activity is a preservative and stimulant of bone formation, which strengthens muscles and improving individual balance through the accumulation of minerals, and also can reduce the risk of bone fractures. In fact, exercise activities in two ways lead to the transfer of force to the bone: muscle tension and gravity. These forces can increase bone density; however, people who have active life, have a significantly higher bone mass compared to their non-aged people, and their pre-eminence is preserved until the seventh and eighth decades of their lives. Appropriate intensity, regular exercise and balanced exercise, along with proper nutrition, have a beneficial effect on bone density (ginty, rennie et al. 2005; zernicke, mackay et al. 2006; barry and kohrt 2008; moayyeri 2008; langsetmo, hitchcock et al. 2012; alghadir, gabr et al. 2015).

Still, no studies have been conducted on the effects of intense periodic exercises on osteopontin and bone mineral density, and because of the limited studies in the field of the effect of these exercises on the performance indicators of athletes, according to if the positive effects of such exercises are confirmed to the athletes, it can be used as an appropriate training protocol to improve the performance and physiological indices of athletes. However, the difference in the effect of these exercises on osteopontin and bone mineral density (calcium, phosphorus, magnesium, iron) is not yet clear in people who are on a regular basis. Therefore, the

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present study intends to investigate the effect of eight weeks of hiit on osteoponetin and some bone mineral indices in active young women.

Materials and methods

The present study was semi-experimental and conducted as a pre-test and post-test design. The study was approved by alzahra university faculty exercise physiology research ethics committee and enrolled participants gave their written informed consent. The statistical population of this study was the young female activists of alzahra university in the age range of 20 to 24 years old, among them 16 female students volunteered to participate in this study. Individual data, their medical and sport records were collected. Also, in an individual session, the anthropometric and physiological characteristics of the subjects were also measured. Subjects were randomly divided into two groups of hiit and control with 8 people due to the criteria of no drug and sports supplements usage, no history of physical and metabolic disease and non-smoking.

Exercise protocol

The exercise program included 8 weeks of 3 sessions per week for 20-30 minutes at alzahra university under the supervision of a trained specialist and supervised by the researcher. The protocol was started with 3 sets consisting of 6 repetitions of 35-meter distance (80% of maximum heart rate), while in the second week, 3 sets were maintained, but the intensity increased to 85%. The rest was 10 seconds between repetitions and 3 minutes between sets. The third and fourth weeks there were 4th sets, and at the fifth and the sixth week, there were 5 sets with full power.

Blood samples were taken from nurse's sitting position for 48 hours before and after the exercise period at a rate of 5 milliliters of blood from the right hand vein of the subjects. Blood samples were immediately placed in a centrifuge machine at a rate of 3000 rpm for 20 minutes and frozen at -70 ° c.

serum osteopontin levels were measured using osteopntin kit (opn, zellbio, ulm, germany), with a coefficient of variation of 4.9% and a sensitivity of 0.2 ng / ml, serum calcium levels was measured using pars-chlorometric enzymatic test kit with a coefficient of variation of 1.9% and a sensitivity of 0.04 mg / dl, serum levels of phosphorus using the pars- calorimetric enzymatic test kit with a coefficient of variation of 0.8% and a sensitivity of 0.3 mg deciliters, serum levels of magnesium were measured using the pars- calorimetric enzymatic test kit with a coefficient of variation of 0.9% and a sensitivity of 0.05 mg / dl and serum levels iron was measured and analyzed using pars- calorimetric enzymatic test kit with a coefficient of variation of 3.2% and a sensitivity of 5 milligrams per deciliter. All stages related to the measurement of relevant factors were performed at the laboratory of endocrine sciences and research institute of endocrine sciences in tehran province. Using the kolmogorov-smirnov test, the natural distribution of data was analyzed. Then independent t-test was used to check the changes between the two groups. All statistical research was performed using spss software version 22 and significance level of $p < 0.05$.

Results

The descriptive characteristics of the subjects are presented in table 1. The results showed that there was a significant difference between the amount of osteopontin in the two research groups ($p = 0.0001$). But, the results also showed that there was no significant difference between calcium ($p = 0.259$), magnesium ($p = 0.19$), fe ($p = 0.883$) and phosphorus ($p = 0.153$) in the young women of the two research groups.

Table1. Descriptive characteristics of subjects (mean ± sd)

Variable	Groups	Mean ± sd
Age (yaer)	Hiit	22.2 ± 1.2
	Control	22.3 ± 4
Height (cm)	Hiit	162.8 ± 6
	Control	156.2 ± 4
Weight (kg)	Hiit	57.6 ± 9
	Control	54.6 ± 7

Table2. Changes in levels of osteopontin, calcium, magnesium, iron and phosphorus of active young women after intervention

Variable	**Mean difference**	**T**	**Sig**
Osteopontin	8.92± 3.16	5.13	0.0001*
Calcium	0.058±0.38	1.17	0.259
Magnesium	0.19 ± 0.25	1.37	0.19
Fe	-8 ±11.3	-0.15	0.883
Phosphorus	-0.012±0.22	-1.15	0.153

*indicates a significant difference between the two groups

Discussion

The results of this study showed that eight weeks of hiit caused a significant increase in serum osteopontin in active women. This result of the present study is consistent with the findings of some of the previous findings (hoffman, gordish-dressman et al. 2013; barfield, uaesoontrachoon et al. 2014; marędziak, śmieszek et al. 2015). The amount of osteopontin is altered by various conditions such as the type of exercise,

the intensity of exercise, and how to practice based on the type of genotype of individuals (barfield, uaesoontrachoon et al. 2014). Increasing the amount of osteopontin in the present study indicates that the exercise protocols used in this study have increased the density and bone mineralization of the subjects. However, the results of the present study are contrary to the findings of duggan et al. Probably the reason for inconsistent of the results of this study are related to the type of subjects and the type of protocols. In a study by duggan et al., the effect of aerobic exercise on serum osteopontin was evaluated for 12 months as an inflammatory factor in obese people. Their results showed that serum osteopontin in the experimental group did not have a significant difference with the control group (duggan, xiao et al. 2014). The type of subjects in this study was young women. Differences in the age, type, intensity and duration of exercise protocols used in this study and most importantly, the subjects in this study were not obese and had good fitness can be the reasons for difference between the results of the research and the results of the present study. Several stimuli coexist during exercise and provide the background of the skeletal muscle to be vascular while the most important of which are skeletal muscle hypoxia, increased blood flow, or shear stress, vascular expansion due to increased lacrimal gland, the mechanical pulling of the tissue, the contraction of the skeletal muscle and the resulting metabolites (brown and hudlicka 2003). In the present study, given that the subjects performed hiit protocol, this type of activity caused a general increase in the blood flow of the skeletal muscle, which leads to increased tensile forces and shear stress. These reasons for the intensity of exercise can be attributed to the increase in the mean of the current study variable, regardless of hypoxia conditions. Other possible reasons for increase in osteopontin following severe activity include secretion of important interleukins such as il-1, il-6, il-10, and tnf-α (lund, giachelli et al. 2009). However, in the present study, the values of immune factors were not measured. During the intensive exercise, the production of activated oxygen species increases and it is believed that the main source of these materials is the mitochondria of activated muscle cells. Two integrin receptors are involved in the angiogenesis of αvβ5 and αvβ3 (the ligand to osteopontin) expressed in endothelial cells. Based on this hypothesis, shear stress, by causing turbulence in the skeletal columns of the cell, changes the site of the endothelial cell in a local adhesive bond and activates the integrin, thereby producing an intracellular message that ultimately transpires the genes involved in the capillary growth process (jensen, schjerling et al. 2004).

The results of this study showed that there was no significant effect on the amount of calcium in active women after 8 weeks of intensive exercise. The result of this study is consistent with the findings of gibson and michell. The researchers studied the impact of two-way sports in young girls and concluded that the higher the intensity of exercise, the greater the bone mass content would be compared to the lighter exercises in the entire body and pelvic bone (gibson, mitchell et al. 2004). Appropriate intensity, regular and balanced exercise, along with proper nutrition, has a beneficial effect on bone density. In contrast to the beneficial effects of exercise on bone density, severe physical activity may increase the risk of osteoporosis, especially in women, and the athlete's triple syndrome includes amenorrhea, malnutrition and osteoporosis. However, the results of this study were not consistent with the findings of some studies (florindo, latorre et al. 2002; vanaky, sadeghi et al. 2015). The contradiction of the present research with the above research can be due to the type of subjects. Subjects in this study were postmenopausal women with osteoporosis and elderly men whose exercise program was continuously and entertaining along with supplementation of calcium, vitamin d, and other inorganic minerals. Exercise in elderly people, along with the use of mineral supplements, leads to the improvement of structural and physiological variables. The subjects were active women aged 20-24 years and did not use any supplement before exercise. Also, the type of exercise program in the study was different from the present study. The effect of the training period, mostly on inactive people and non-athletes, especially postmenopausal women, and middle-aged men, make changes.

Also, the results of this study showed that eight weeks of hiit had no significant effect on the magnitude of magnesium in active women for eight weeks of hiit. The results of studies by kilic et al, brilla et al., are consistent with the results of the present study (brilla and conte 2000; kilic, baltaci et al. 2004). The results of the studies have shown that minerals, especially magnesium, may be excited during sweat during intensive exercises, and this decrease leads to occasional fatigue, with reduced endurance, and the performance of anabolic factors is not easily conducted. However, the results of sadeghi et al. Are not consistent with the results of the present study that may be due to the type of subjects who were women and men with osteoporosis and their sports activities may be associated with the administration of certain vitamins and minerals which is prescribed by a specialist physician are the reasons for the contradiction of the above study with this research. It has been shown that magnesium intake is lower than daily intake, and this shortage is more pronounced in athletes, and it may be necessary for athletes to use supplements to take advantage of their proper amount in their body (brilla and conte 2000). In addition, the results of the present study showed that there was no significant effect on the amount of active female iron in eight weeks of hiit. The results of this study are consistent with the findings of baker et al.(baker, de lisio et al. 2011). There is empirical evidence that during the tolerance exhausting exercise activities, moderate to severe ros production, as well as oxidative stress and tissue damage associated increase with physical activity. During the exhausting sporting activities, oxygen consumption is increased by 100 to 200 times the resting time. It has been shown that iron and the group of hemoglobin and myoglobin are potential sources of ros but it has not yet been determined how much they act in oxidative stress during or after the exhausting exercise(cooper and beasley 1999). The lack of reduction of iron in the subjects of the present study

could be due to the lack of continuity and long distance in the exercise protocol. Intensive exercises composed of a short period of time from several intermittent periods with both low and high intensity (boutcher 2010). Contrary to tolerance exercises with long distances, further sweating, along with a greater number of foot strokes, can lead to the destruction of red blood cells.

The results of this study showed that there was no significant effect on the amount of phosphorus in active women for 8 weeks of hiit. The results of sedegi et al are consistent with the results of the present study (vanaky, sadeghi et al. 2015). However, the results of duggan et al. And mason et al are not consistent with the results of this study (mason 2007; duggan, xiao et al. 2014). In the above studies, the elderly and postmenopausal women were at risk of osteoporosis, which had severe exercise for two years. Usually, people with lower levels of physiological characteristics have a better response to training, and older people can have such a feature. In a study by mason and colleagues in swimmers, women's parasols and runners who consumed 2 g sodium dihydrogen phosphate one hour before training all improved exercise performance and recorded better records. The contradictory reasons in this study can be difference in the intentional training protocols with these exercises as well as differences in the ability of individuals to perform these exercises. Another reason for not having a significant difference after the exercise period in this study may be due to the activity of these subjects, their compatibility with exercise. There is evidence of phosphate regulation of the gene expression, which may play a role in the mineralization of the bone. Phosphate expresses glycoprotein phosphorylation of osteopontin. Osteopontin plays a role in prolonging crystalline hydroxyapatite.

Conclusion

Regarding the results of this study, it can be said that the hiit program with the present training method makes it compatible with the biological ability and degree of mineralization of bone marrow cells in athletes. These results can be used to design a workout program by athletic instructors, especially coaches who lack the time to have athletes (for example, in academic teams).

Conflict of interest

The authors thus stipulate that there is no conflict of interest in the present research.

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