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Research Article

The effect of endurance training and mesenchymal stem cells on ALP gene expression and osteopontin levels in rats with knee osteoarthritis

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Keywords:
Osteoarthritis, Mesenchymal stem cells, Endurance training, Osteopontin, Alkaline phosphatase.

Abstract

Background: Osteoarthritis (arthritis of the joints) is one of the most common metabolic disorders of bone tissue that reduces the process of absorption and reabsorption in bone. Exercise and stem cell injections can have beneficial effects in treating this disease. The enzymes alkaline phosphatase and osteopontin, as markers of bone formation, play an important role in diagnosing the progression or treatment of this disease. The aim of this study was to examine the effect of training, stem cells and hyaluronic acid on osteocalcin, ALP and osteopontin in the cartilage tissue of rats with osteoarthritis.

Materials and Methods: In this study, 25 rats were divided in 5 groups including: (1) healthy control, (2) patient control, (3) endurance training (3 days a week for one month), (4) recipients of mesenchymal stem cells (1 × 106 cells / Kg), and (5) simultaneous recipients of endurance training and mesenchymal stem cells. Alkaline phosphatase gene expression was assessed by RT PCR and the amount of osteopontin synthesis was measured by immunohistochemistry procedure.

Results: Training and mesenchymal stem cell injection had a significant effect on increasing alkaline phosphatase gene expression and osteopontin in patient rats compared to the patient control group ($P < 0.001$). Also, simultaneous endurance training and stem cell injection have interactive effects on increasing both factors ($P < 0.001$).

Conclusion: Based on the findings of this study, it seems that endurance training and injection of mesenchymal stem cells in the joints, either separately or simultaneously, can increase the expression of alkaline phosphatase gene and the amount of osteopontin.

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Journal of Sports Physiology and Athletic Conditioning

1. Introduction

Osteoarthritis is a common non-inflammatory disorder of the musculoskeletal system that presents with degenerative changes in the synovial joints, along with ossification. Symptoms of this disease are more common in the knee joints (1). The prevalence of this disease in the urban population of Iran is 16.6% and in the rural population is about 20%. About one-thirds of people over the age of 65 in the world have osteoarthritis of the knee (2). The disease can be divided into two groups: 1- Primary or idiopathic osteoarthritis in which the person has no underlying disease 2- Secondary osteoarthritis, which is a primary predisposing factor and is locally or systemically involved in the development of the disease (3). Numerous risk factors for the development or exacerbation of this disease have been identified, the most important of which are obesity, knee injury, gender, old age, muscle weakness, bone characteristics, and poor job-related status (4). In osteoarthritis, existing stem cells are depleted or their storage runs low, and on the other hand, their proliferating capacity and differentiating ability decrease; therefore, systemic or topical administration of stem cells to these individuals can lead to the repair of joint tissues (5).

Alkaline phosphatase is the most widely used biomarker of bone metabolism, which is involved in all stages of bone mineralization and is known as a specific indicator of osteoblast activity. The presence of calcium ions is essential for the activity of this enzyme (6). As alkaline phosphatase levels increase, the transfer of extracellular fluid ions to non-mineral osteoid increases and new cells are formed (7).

Osteopontin, formerly called bone sialoprotein, is an acidic glycoprotein and is an important factor in bone resorption, osteoclast calcification, cell adhesion, body chemical regulation, inflammation regulation, reproduction, and fetal growth (8). Physical activity is an important determinant of bone mass. Exercise has both a direct and indirect osteogenic effect on skeletal tissue. Aerobic exercise has been shown to help increase levels of bone detection biomarkers, including alkaline phosphatase (9,10). Reports of the effect of exercise are somewhat contradictory (11). Mesenchymal cells are pluripotent cells that have the ability to produce connective tissue such as cartilage, bone, tendons, ligaments, and stromal cords during histogenesis. In recent years, the use of mesenchymal stem cells in the treatment of cartilage damage has increased (12).

With the increasing number of surgeries for the treatment of osteoarthritis of the knee and due to the surgical risks, surgery can not be performed in all cases of osteoarthritis. In addition, few patients with osteoarthritis are willing to have surgery. On the other hand, drug interactions and side effects of these drugs make patients less inclined to undergo surgery and use anti-inflammatory and analgesic drugs, and hence, alternative therapies can become very important. For this reason, the results of this research can be a guide for many researchers in this regard.

2. Materials and Methods

Statistical sample of the study

The sample of this study included thirty 6 to 8 week-old healthy male Wistar rats with an average weight of 230 to 250 grams that were purchased from the Pasteur Institute of Iran and kept in the laboratory of Pasargad Tissue and Gene Research Center in Tehran and were then randomly divided into 5 groups (N = 6).

The standard conditions in terms of room temperature, dark-light cycle and water and food were fully observed. After one week of adaptation to the new environment and familiarity with the training protocol, the animals were divided into the following groups: 1) healthy control, 2) patient control, 3) training, 4) stem cells injection, and 5) training + stem cells injection.

The training protocol

In the first stage (preliminary stage), the rats worked on the treadmill with zero percent slope, 3 days a week, for 10 minutes at a speed of 16 meters per minute, about 60 to 70 percent of Vo_{2max}. The second stage (main training protocol) included 30 minutes of running on the treadmill without slope and at a speed of 16 meters per minute, with regard to the principle of overload progressively, so that the duration of training was 50 minutes in the eighth week.

Also, five minutes at 8 meters per minute were devoted to warming and cooling the animals before and after training. During the training protocol, the control group only stayed on the treadmill off.

Induction of osteoarthritis: In the present study, direct intervention method was used to induce osteoarthritis in rats. The rats were first anesthetized with the anesthetics ketamine and xylazine.

After shaving the right knee, a horizontal incision was made in the inside of the knee. After removing the skin, the lateral internal ligament of the knee was removed to show the internal meniscus. Then, by creating an incomplete incision, a tear and damage was created in the meniscus. Finally, the desired area was sutured with a sterile method.

Preparation and injection of mesenchymal stem cells: In order to use mesenchymal stem cells in bone marrow of adult male rats, after killing the animal under anesthesia with ketamine and xylazine, bone marrow cells were collected from the femur and tibia. After culturing these cells in DMEM culture medium, one million cells per kilogram of body weight were prepared for each rat and injected intra-articularly into the right knee joint during the recovery period.

RT PCR method for the detection of ALP: RNA related to alkaline phosphatase gene was extracted from cartilage tissue cells using RNX-Plus kit (SinaClon; RN7713C). The quantity and quality of RNA extracted was determined by ND-1000 nanodrop spectrophotometer.

CDNA synthesis was performed according to the instructions in the fermentase kit (K1622). The reverse transcription reaction was performed using the enzyme RevertAid ™ M-MuLV Reverse transcriptas. Table 1 presents the sequence of primers used in this study.

Table 1: Sequence of primers used in RT PCR related to ALP

Gene	Forward (5'-3')	Reverse (5'-3')
GAPDH	AAG TTC AAC GGC ACA GTC AAG G	CAT ACT CAG CAC CAG CAT CAC C
ALP	CTTTTGGACAGCAGGGTGGG	AAGGAGGGTTGGGTTGAGGGA

Evaluation of osteopontin synthesis by immunocytochemical method: First, 4,000 cells were inserted in each cell from the 12-cell plate, then the cells in the mentioned groups were treated for 21 days. After 21 days, the cells were fixed in paraformaldehyde and after washing with PBS, the cells were incubated with Triton for 10 minutes to increase permeability.

The cells were then incubated with bovine serum albumin and PBST buffer for 30 minutes. The polyclonal antibody osteopontin ab8448 from ABCAM company was placed on the cells. Hochst's staining was then performed on the cells and finally they were photographed with a fluorescence microscope.

To analyze the obtained data in this study, independent samples t-test and three-factor ANOVA statistical procedures were used.

3. Results

The results of independent samples t-test to compare osteopontin levels between the healthy control (58.72 ± 6.64) and patient (4.24 ± 0.96) groups showed that due to the induction of osteoarthritis, the levels of this protein was significantly reduced in the patient group (P <0.001).

Also, the results of ANOVA showed that training had a significant effect on osteopontin gene expression levels (F = 3187.326, P = 0.0001, μ = 0.990). Mesenchymal stem cells injection also had a significant effect on the increase of osteopontin (F = 1117.097, P = 0.0001, μ = 0.972). Besides, the interaction of training and mesenchymal stem cells injection (F = 494.224, P = 0.0001, μ = 0.939) had a significant effect on osteopontin levels.

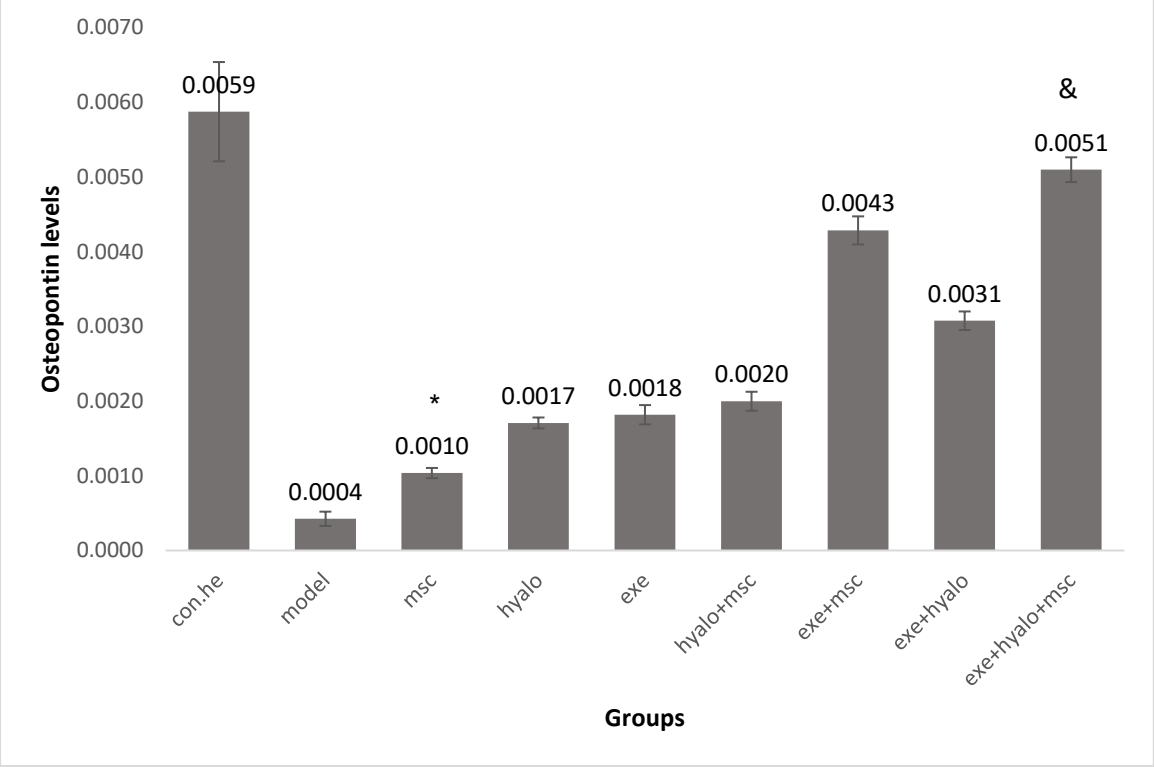

Figure 1: Osteopontin levels in the experimental groups. * Significant reduction of this protein can be seen in the patient group (model) compared to the healthy group (con.he). & Interaction of training and stem cells injection (exe + msc) had a synergistic and significant effect on osteopontin levels compared to other experimental groups.

The results of this test showed a significant difference in ALP gene expression levels between the osteoarthritis induction (3.94 × 10-4 ± 0.8 × 10 $^{-4}$) and healthy (1.6 × 10-2 ± 0.63 × 10-2) groups in male rats (P <0.001), so that due to the induction of osteoarthritis, the levels of this enzyme was significantly reduced in the patient group.

Based on the results of three-way analysis of variance, it was found that training had a significant effect on ALP gene expression levels (F = 97.745, P = 0.0001, μ = 0.849). Mesenchymal stem cells injection also had a significant effect on ALP gene expression levels (F = 21.697, P = 0.0001, μ = 0.753). Also, the interaction of training and stem cells injection (F = 20.976, P = 0.0001, μ = 0.001) had a significant effect on ALP gene expression levels.

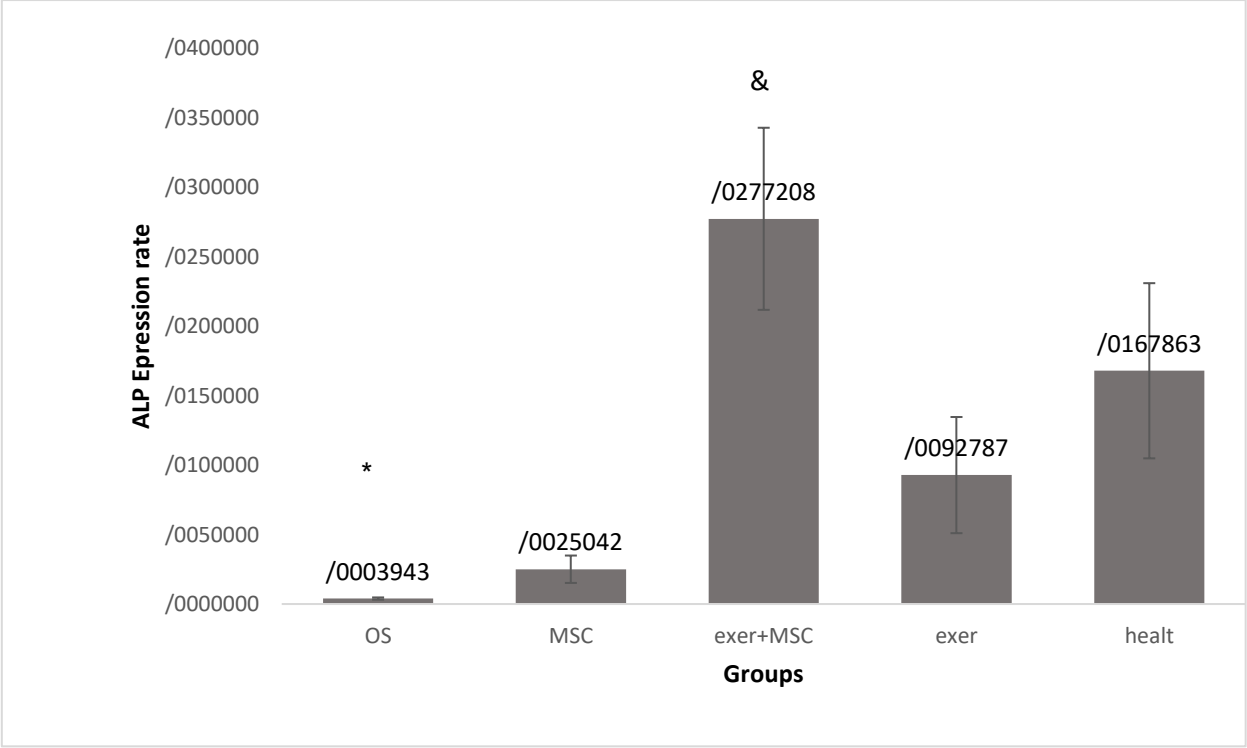

Figure 2: ALP gene expression levels in the experimental groups. * Significant reduction of this protein can be seen in the patient group (model) compared to the healthy group (con.he). & Interaction of training and stem cells injection (exe + msc) had a synergistic and significant effect on ALP gene expression levels compared to other experimental groups.

4. Discussion

The results of the present study showed that induction of osteoarthritis reduced ALP and osteopontin gene expression levels in bone tissue. Training and injection of mesenchymal stem cells alone had a significant effect on increasing ALP and osteopontin gene expression levels in rats with osteoarthritis. Still, interaction of training and stem cell injection had a significant synergistic effect on the dependent variables of this study. Exercise plays an important role in the HDAC3 / NF-KappaB signaling pathway in bone cells. According to research by Zhang et al. in 2019, exercise has a direct effect on inhibiting the transfer of the HDAC3 / NF-KappaB molecular complex into the nucleus, reducing the expression of inflammatory proteins such as MMP-13 and ADAMTS5. These proteins are a major cause of inflammatory disorders such as osteoarthritis (13).

Lubricin is produced as an anti-inflammatory agent in bone tissue cells, especially cartilage cells. Research by Blaney Davidson et al. in 2016 showed that exercise has a positive effect on gene expression of this protein. Lubricin binds to the TLR2 and TLR4 inflammatory receptors, blocking their function and ultimately reducing inflammatory processes. Increased lubricin levels can play an important role in the treatment of inflammatory diseases such as osteoarthritis. (14) It appears that inhibition of leukocyte-derived inflammatory factors, decreased inflammatory factors such as some interleukins (15), increased transcription of some bone growth and mineralization factors, and increased calcium deposition in bone tissue are some of the mechanisms of effect of exercise on bone tissue (16).

In a study conducted by Tung et al., the most important mechanisms of exercise training to increase osteoblast activity and increase calcium storage were known to be increased amounts of osteocalcin and osteopontin in the bone tissue matrix (17). The results of the present study were in line with some of the studies reported in this regard. Osteoblasts play a major role in the formation of bone tissue and originate from mesenchymal stem cells. The process of osteoblast genesis is controlled by several transcription factors. Macrophages play an important role in inducing osteoblast genesis through these transcription factors.Besides, by producing a variety of interleukins and macrophage colony-stimulating factors (M-CSF), macrophages play an important role in the process of osteoclast reabsorption and subsequent differentiation of osteoblast cells, resulting in the growth and differentiation of bone tissue (18).

In recent years, the use of mesenchymal stem cells has been considered as an anti-inflammatory factor in the treatment of osteoarthritis, because these cells are known to regulate the immune system. By acting on T-type immune cells, these stem cells transform them into regulatory type T cells; in other words, they modulate and regulate the expression of inflammatory factors in osteoarthritis (19). Research by Hanna et al. in 2018 showed that mesenchymal stem cells can increase the expression of CD105, CD90 and CD44 genes by increasing the expression and regulation of Ca^{2+} fluctuations; also, an increase in the amount of ascorbic acid can increase bone mass, the number of osteoblasts and finally, alkaline phosphatase activity in bone tissue (20).

The results of the present study on the effect of mesenchymal stem cells were completely in line with the related results reported on human and rat models.

Osteopontin produces proinflammatory cytokines and binds osteoclasts to the bone underlying substance. This protein is able to bind to CD44 and integrin receptors. On the other hand, CD44 and integrin are the main markers of mesenchymal cells.By binding to these receptors, osteopontin activates the TGF-β signaling pathway, thereby increasing the activity of proinflammatory cytokines such as IL-17, IL-6, and TNF-α. These proinflammatory cytokines destroy cartilage (21).

One of the most important findings of the present study was that the combination of endurance training with mesenchymal stem cell injection resulted in better effects on increasing bone metabolic markers. Given that endurance training has a direct effect on a wide range of signaling pathways and increasing osteopontin and alkaline phosphatase, and on the other hand, mesenchymal stem cells by reducing inflammation and increasing bone mass, play an important role in the treatment of osteoarthritis, applying these two independent variables simultaneously can have significant synergistic effects on the repair of osteoarthritis injuries in patients.

Conclusion

Based on the results obtained in this study, it was found that the use of endurance training and injection of mesenchymal stem cells, either separately or interactively, increases the levels of alkaline phosphatase and osteopontin in bone tissue and consequently repairs injuries in rats caused by osteoarthritis.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: M.H., M.A.A., M.P., S.A.H.; Methodology: M.H., M.A.A., M.P., S.A.H.; Software: M.H., M.A.A., M.P., S.A.H.; Validation: M.H., M.A.A., M.P.; Formal analysis: M.H., M.A.A., M.P., S.A.H.; Investigation: M.H., M.P., S.A.H.; Resources: M.H., M.A.A., M.P., S.A.H.; Data curation: M.P., S.A.H.; Writing - original draft: M.A.A., M.P., S.A.H.; Writing - review & editing: M.H., M.A.A., M.P., S.A.H.; Visualization: M.H., M.A.A., S.A.H.; Supervision: M.H., M.A.A., M.P., S.A.H.; Project administration: M.H., M.A.A., M.P., S.A.H.; Funding acquisition: M.P., S.A.H.

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Research Article

Relationship between erythropoietin and fasting glucose glucose after a resistance training program in male Wistar rats with type 2

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Resistance training, type 2 diabetes, erythropoietin, fasting blood glucose

Abstract

Background: Erythropoietin is known as a strong stimulant in the activation of satellite cells and increasing the regeneration function of muscle tissue. The purpose of this study was to investigate the relationship between erythropoietin concentration and fasting blood glucose after a resistance training program in male Wistar rats with type 2 diabetes.

Materials and Methods: Twenty-four male rats aged 6 weeks were divided into 3 groups: healthy control (n=8), diabetic control (n=8) and resistance training (n=8). Resistance exercises were performed for 8 weeks, 5 sessions per week, with an intensity of 100-30% of the weight of the rats in the resistance training group. In the last week of the training program, the maximum oxygen consumption of the rats was taken using the executive protocol on the rat treadmill. 48 hours after finishing the training program, blood samples were taken from the right ventricle of heart of the rats and erythropoietin and fasting blood glucose were evaluated. The data was statistically analyzed using Pearson's correlation and one-way analysis of variance at the alpha level of less than 0.05.

Results: The results showed that there is no significant relationship between erythropoietin and fasting blood glucose among any of the groups. Also, performing 8 weeks of resistance training in diabetic rats led to an increase in erythropoietin concentration ($P \leq 0.0001$) and a decrease in blood glucose ($P \leq 0.0001$).

Conclusion: It seems that more stimulation of EPO and regeneration of muscle tissue as well as increased energy consumption in muscle tissue is one of the possible mechanisms of blood glucose reduction caused by 8 weeks of resistance training in diabetic rats.

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1. Introduction

Erythropoietin (EPO) is a cytokine hormone that primarily activates the proliferation and growth of erythroid cells and is active in several types of non-hematopoietic cells through interaction with the EPO receptor (EPO-R). The erythropoietin receptor (EPO-R) is expressed in skeletal muscle cells, and EPO may promote myoblast differentiation and survival through activation of similar signaling cascades in hematopoietic cells (1). Expression of a specific erythropoietin receptor (EPO-R) that extends beyond hematopoietic cells defines pleiotropic functions for EPO. These functions are especially involved in the protection against oxidative stress in nerve cells, in the neovascularization and angiogenesis of the uterus, and in the maintenance and repair of the myocardium (2). Skeletal muscle exhibits a remarkable ability to regenerate, a process that has been shown to be dependent on satellite cells. Skeletal muscles at rest have quiescent satellite cells, whereas in response to growth or injury, satellite cells are activated, enter the cell cycle, and induce the proliferation of myogenic progenitor cells that either fuse with existing myofibers or form new myofibers. They are distinguished. This process is tightly regulated by the expression of key transcriptional regulators such as paired box transcription factor (Pax7) protein and myogenic regulatory factors (3). Studies have shown that EPO plays an essential role in the activation of satellite cell proliferation stimuli. Hence, EPO seems to help glucose metabolism in diabetics by repairing muscle tissue. Previous studies have implicated erythropoietin (EPO) signaling in the regulation of glucose metabolism. Whether EPO can be used to treat diabetes and its underlying mechanism are still not well understood (4). Clinical research in the field of diabetes has used erythropoietin injection as a fasting glucose reducer in diabetic samples.

But the increase in blood pressure and the risks of excessive angiogenesis caused by EPO injection limit its use as a drug, and it is recommended to use natural methods such as physical activity to increase EPO secretion (5). Exercise is mentioned as a factor to control diabetes and it has been one of the ways to control and prevent diabetes for many years (6). The effect of physical activity on the reduction of insulin resistance and the activation of some signaling pathways such as phosphoinositide 3-kinase (PI3K), AKt, AMP-activated protein kinas (AMPK) and Calcium/Calmodulin Stimulated Protein Kinase (CaMK) have been mentioned as pathways involved in the metabolism of glucose (7). Most of the studies conducted regarding the relationship between exercise and diabetes have tended to aerobic exercise as a type of exercises that creates the most metabolic adaptations in muscles. However, it has been shown that resistance training can also be effective as a non-pharmacological agent in controlling type 2 diabetes (8). In addition to exposure to altitude, exercise is one of the most important erythropoietin stimulants. Therefore, the increase of erythropoietin due to exercise can be considered as one of the possible mechanisms of the effectiveness of exercise for diabetic patients. However, what is the relationship between EPO changes and fasting blood glucose concentration in diabetic patients and to what extent is this relationship affected by resistance training, it is still not clear. This relationship can be changed through the effect of EPO on skeletal muscle function, facilitating the entry of glucose into skeletal muscles and the resulting molecular-cellular adaptations. Considering the role of resistance training on EPO changes, the present study seeks to answer the question of what is the relationship between these two variables and how does this relationship change with resistance training?

2. Materials and Methods

Subjects

The sample of the present study consisted of 24 male Wistar rats weighing 230 grams and 6 weeks old, which were transferred to the laboratory after being prepared from the Pasteur Institute of Iran, and after two weeks of familiarization and adaptation to the environment, they were divided into 3 groups. Each group consisted of 8 rats: healthy control (HC), diabetic control (DC) and resistance training (RT) and then type 2 diabetes was induced into two groups of rats. The control group did not receive any intervention. The RT and the DC groups became diabetic through a high-fat diet and STZ injection. The RT group performed resistance training according to the designed protocol. During the whole period of the research, the rats were kept and controlled at a temperature of 22±2°C, humidity 45-55% and sleep-wake cycle 12:12, with the availability of food and water.

Induction of type 2 diabetes: in rats Induction of diabetes was done in 2 groups, DC and RT, using a combination method of high-fat diet and STZ injection (HFD-STZ). For this purpose, all rats were fed a diet with 59% fat, 14% protein and 27% carbohydrates for 3 weeks (9). Normal rat food contains 570 grams of carbohydrates, 20 grams of fat, and 175 grams of protein, to which 0 grams of carbohydrates, 531 grams of fat, and 125 grams of protein were added in order to reach the percentages mentioned for inducing diabetes (9). Then, at the end, 35 mg of STZ per kilogram of body weight in a citrate buffer of 0.1 mmol/L with an acidity of 4.5, intraperitoneally after 12 hours of overnight starvation at around 9 am. was injected. Seven days after the injection of STZ, a blood sample was taken from the animal's tail to measure blood glucose by a glucometer, and the samples with blood sugar more than 300 mg/dL were determined, and it was confirmed that they were diabetic.

Resistance training protocol

In the present study, resistance training was performed using a one-meter ladder along with hanging weights from the rats' tails for 5 sessions per week. Rats climbed the ladder for three repetitions without weights and without resting between repetitions to warm up. The main part of the resistance training program consisted of 3 sets with four repetitions in each set, with 30 seconds between each repetition and 3 minutes of rest between each set. Applying resistance in the form of tying weights to the rats' tails, equivalent to 30 to 100% of the rats' body weight, was performed during the eight weeks of the training period. Also, the training intensity in the training groups increased gradually every week by increasing the amount of weight in such a way that in the first week 30%, the second week 45%, the third week 60%, the fourth week 45%, the fifth week 60%, the sixth week 75%, the seventh week 90% and the eighth week 100%, Rat body weight was applied. The angle of the ladder in these exercises was 85 degrees. After the end of the training protocol, in order to evaluate the maximum oxygen consumption, samples from a rat treadmill and a 10-step test according to the evaluation method of Leandro et al. (10) were evaluated and recorded in different groups. 48 hours after the last training session, the samples were anesthetized by a combination of ketamine (30-50 mg/kg) and xylazine (5-3 mg/kg) injections in fasting state, and the soleus muscle tissue was taken for evaluation of the expression of some genes was removed. The blood sample was also taken from the right ventricle of the heart and used to evaluate the desired variables.

Statistical Methods

In the statistical analysis section, Pearson's correlation coefficient was used to determine the relationship between variables. Also, one-way analysis of variance was used to measure the difference between groups. If there was a difference between the groups, Tuky post hoc test was used. A significance level of 5% and SPSS version 21 software was used for data analysis.

3. Results

The measured variables of the current study, including the weight of the samples, aerobic capacity, plasma concentration of EPO and FBS are listed in Table 1.

Table 1: Average and standard deviation of weight, aerobic capacity, erythropoietin concentration and fasting glucose in different research groups (M±SD).

Groups / Variables	Control	Diabetic control	Resistance training
weight (g)	312.6250±2.26385	317.5000±5.92814	310.3750±3.92565
Maximum oxygen consumption (ml/kg/min)	77.8750±2.03101	55.0000±1.30931	60.2500±2.37547
Erythropoietin concentration (ng/ml)	1.0871±.10920	.5216±.06572	1.1213±.13789
Fasting blood glucose concentration (mmol/l)	4.6597±.13092	16.5347±.29538	9.7639±.27176

Determining the relationship between EPO and FBS concentration in different research groups is shown in Table 2. The results show that there is no significant relationship between these variables in any of the groups.

Table 2: The results of Pearson's correlation test in determining the relationship between EPO and FBS concentration in different research groups

Groups	Pearson's correlation coefficient	significance
Control	-.263	.530
Diabetic control	.034	.937
Resistance training	.045	.915

Analysis of research data using one-way analysis of variance (Table 3) showed that the concentration of erythropoietin and fasting glucose in different groups have significant differences. The results of Tukey's post hoc test also showed that the concentration of erythropoietin in the diabetic group was lower than the other two groups (Table 4).

Table 3: The results of the one-way analysis of variance test regarding the concentration of erythropoietin and fasting glucose in the research groups.

	sum of squares	df	mean square	F	p
Erythropoietin (EPO)	567.766	2	283.883	4778.084	.000
Fasting blood glucose concentration (FBS)	1.815	2	.907	77.210	.000

Table 4: Tukey's post hoc test results comparing EPO and FBS concentrations in different research groups (The difference of the averages and the level of significance are specified in each case)

Variables	Groups	Diabetic control	Resistance exercise
Erythropoietin (EPO)	Control	.56550* p=.000	-.03412 p=.806
	Diabetic control	---	-.59962* p=.000
Fasting blood glucose concentration (FBS)	Control	-11.87500* p=.000	-5.10417* p=.000
	Diabetic control	---	6.77083* p=.000

4. Discussion

The first finding of the present study was that there was no significant relationship between the levels of erythropoietin and blood glucose in fasting conditions of healthy and diabetic rats. Also, resistance training could not change the amount of this relationship. Since the induction of diabetes increased the fasting glucose concentration in rats up to 3 times the normal condition, this indicates that blood glucose concentration alone cannot be a stimulus for EPO secretion. It is likely that other changes are caused by the induction of type 2 diabetes, which alters the EPO response to diabetes. Previous reports have documented the effect of EPO on lowering blood glucose levels (11,12,13). Most of these studies have been conducted on patients with diabetes or insulin resistance. In the study conducted by Katz et al. (2010), injection of large amounts of EPO led to a decrease in blood glucose in dialysis rats (5). Previous studies have reported an association between EPO levels and hypoglycemia, which indicates a potential protective effect of EPO in the treatment of diabetes (14).

It was effective (4). The mentioned study showed that in terms of ultrastructure, EPO prevents the dysfunction of pancreatic β cells, improves fragmentation of mitochondria, and increases the number of secretory granules. Administration of EPO increased the activity of antioxidant enzymes such as SOD and GSH-PX and decreased the level of MDA. Furthermore, EPO increased blood selenium in diabetic rats and produced a hematopoietic effect. The decrease in blood glucose associated with exposure to high levels of EPO may be due to an increase in the number of erythrocytes and thus their glucose uptake (15). At least two adaptive mechanisms allow mice injected with large amounts of EPO to cope with this excessive erythrocytosis. The first mechanism is the very high expression of nitric oxide (NO) and the second is the reduction of the life span of red blood cells. The second mechanism keeps red blood cells young and flexible and thus prevents excessive blood viscosity (16). Therefore, it seems that the relationship between EPO concentration and blood glucose is a cause and effect relationship during which EPO leads to the reduction of blood glucose with the mentioned mechanisms.

Another finding of the research showed that type 2 diabetes led to a significant decrease in the concentration of erythropoietin in the samples of the diabetic group. The exact mechanism of erythropoietin suppression caused by the development of type 2 diabetes is not yet known, but it seems that the increase in insulin concentration due to the development of insulin resistance in samples of the diabetic group is the most important reason for this issue (Mayes, 2015). On the other hand, resistance training was able to restore the concentration of erythropoietin to the initial levels.

The short-term and long-term effects of resistance exercise on increasing basal EPO levels have been well demonstrated (17). It has also been shown that a session of resistance activity can acutely increase many angiogenic factors such as Endothelial progenitor cells (EPCs), vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1-alpha (HIF-1α) and this response was dependent on the intensity of resistance activity (17). Therefore, it seems that resistance training, like endurance and aerobic exercises, can be effective on indicators related to hematopoiesis and angiogenesis, which was also confirmed in the present study.

Another result of the present study was changes in fasting blood glucose concentration after a resistance training program, which shows a significant difference with the control group. The results showed that while the induction of type 2 diabetes increased the FBS index up to 16 mmol/L, but the 8-week resistance training program caused a significant decrease in it and its value decreased to 11 mmol/L. The effects of endurance training and high intensity interval training on glucose metabolism in diabetic patients have been well documented (18,19,20), however, these effects have been less investigated in the case of resistance training. In a comparison conducted by Strasser et al. (2013), resistance training can improve glycemic control and insulin sensitivity possibly even more than aerobic endurance training (21). It is possible that increased lean body mass after RT is an important mediator of improved glycemic control. The increase in the number of GLUT4 transporters caused by resistance training has also been specifically discussed in various studies, because GLUT4 transporter protein expression in the plasma membrane is related to fiber volume in human skeletal muscle fibers (22).

Improvement in blood sugar control depends not only on the change in muscle mass, but also on the consequences of internal changes in the muscle. Holten et al reported improved insulin action with increased protein content of GLUT4, insulin receptor, protein kinase B-α/β, and glycogen synthase after six weeks of single-leg RT, while the untrained leg remained unchanged (23). Therefore, improving blood sugar control reduces the amount of insulin needed to clear a given amount of glucose. Resistance training can improve glucose transport in normal and insulin-resistant skeletal muscle by increasing the activation of the insulin signaling cascade (21). These exercise-induced changes improve the metabolic profile of skeletal muscle and can occur independently of significant increases in skeletal muscle mass (24).

Conclusion

In Conclusion, the results of the present study showed that resistance training helps to reduce FBS in diabetic rats with HFD-STZ method. It seems that the stimulation of EPO and the renewal of muscle tissue, as well as the increase of energy consumption in muscle tissue, is one of the possible mechanisms of this issue. The general conclusion about the effect of the mentioned variables on muscle tissue in humans and the generalization of the results of this research to humans should be done with caution, but what is certain is that resistance training can be suggested as a solution to improve disease-related indicators. Type 2 diabetes in humans noted. Future studies will clarify more facts in this regard.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: F.S., Y.K., A.B., S.A.; Methodology: F.S., Y.K., A.B., S.A.; Software: Y.K., A.B., S.A.; Validation: Y.K., A.B., S.A.; Formal analysis: F.S., Y.K., S.A.; Investigation: F.S., Y.K., S.A.; Resources: F.S., Y.K., A.B., S.A.; Data curation: F.S., Y.K., A.B., S.A.; Writing - original draft: F.S., A.B., S.A.; Writing - review & editing: F.S., Y.K., A.B., S.A.; Visualization: F.S., Y.K., A.B., S.A.; Supervision: F.S., Y.K., A.B., S.A.; Project administration: F.S., Y.K.; Funding acquisition: F.S., Y.K., A.B.

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Research Article

The effect of eight weeks of aerobic training on some apoptotic factors of elderly men

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Abstract

Background: Considering the significant increase in apoptosis in the elderly, which is related to cardiovascular diseases, cancer, etc. The purpose of this research was to investigate the effect of eight weeks of aerobic exercise on some factors of apoptosis in elderly men was investigated.

Materials and Methods: This research was conducted using a semi-experimental method with 30 healthy elderly men aged 60 to 75 years. who voluntarily participated in this research project and were randomly divided into two experimental (15 people) and control (15 people) groups. The experimental group participated in aerobic exercises for 8 weeks (three sessions of 1 hour per week) based on the special recommendations of the American College of Sports Medicine (ACSM) for the elderly. The control group did not have any physical activity during this period. Serum caspase-8, P53 and IGF-1 were measured by ELISA method. Dependent t-test and independent t-test were used to check the intra-group and inter-group differences of variables. Data analysis was done using SPSS version 20 software and the significance level was considered $p \leq 0.05$ in all steps.

Results: The results showed that eight weeks of aerobic exercise increased the serum levels of caspase 8(P=0.0001) and decreased the serum levels of P53(P=0.0001) and IGF-1(insulin-like growth factor) (P=0.0001).

Conclusion: According to the findings of the present research, it seems that eight weeks of aerobic exercise significantly increases the serum levels of caspase 8 and decreases the serum levels of P53 and IGF-1 in elderly men. According to these results, more research is needed in this field.

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1. Introduction

Aging is a natural process that includes all living beings, including humans, and aging is accompanied by physiological and psychological changes (1). Demographers consider the age of 60 to 65 as the beginning of old age (2). In the year 2019, for the first time in human history, the number of elderly people in the world exceeded children (under five years old). It is also expected that by 2050, the number of elderly people aged 60 and over will double and the number of people aged 80 and over will increase three times (3). In Iran, although currently less than %10 of the population is made up of elderly people aged 60 and over(4), but this ratio is expected to increase rapidly from 2030 and reach about %30 of Iran's population in 2050(5). Scientists have tried for a long time to express the basics of the evolution of aging specifically, one of the main hypotheses that was later proven by microscopic studies in tissue sections is the acceleration of the aging process with an increase in programmed cell death or apoptosis(6). Apoptosis is a regular program of cell death that is very important in terms of physiology and occurs from two pathways inside (mitochondria) and outside the cell (death receptor) with morphological changes such as cell wrinkling, chromatin condensation and DNA fragmentation (7). In the extracellular pathway, the binding of important ligands such as IL-1β (Interleukin 1 beta), TNFα (Tumor necrosis factor alpha) and Fas to death-inducing membrane receptors causes the activation of caspase-8 (Cysteine-dependent aspartate-directed protease 8). Activated caspase 8 can directly activate executive caspases, and executive caspases such as caspase 3 are activated in the next steps by initiator caspases and start the caspase cascade, while in the intracellular pathway (to title of the most important path in causing apoptosis) mitochondria and endoplasmic reticulum are at the center of the process and under the influence of stress factors (glucocorticoids, cytokines, nitric oxide and oxygen reactive species) they induce apoptosis by activating caspases (8). Caspases are part of the family of cysteine proteases and play a central role in the initiation and execution phase of apoptosis. Therefore, the evaluation of caspase activity as a biochemical marker of apoptosis is relevant. Other important factors are also involved in the control of apoptosis, which either prevent its creation, such as IGF-1 (insulin-like growth factor), Bcl-2 (B-cell leukemia 2), or accelerate the process of apoptosis, such as Bax and p53 (9). IGF-1 is a peptide hormone of 70 amino acids that is secreted by the liver. This hormone plays a major role in preventing apoptosis and controlling aging. According to research results, IGF-1 can reduce apoptosis in several ways. Sports activity increases IGF-1 hormone in the body, IGF-1 also leads to inhibition of apoptosis in two ways. The first pathway is the activation of PI-3K and then Akt. The second path is the activation of HSPs (8, 10). On the other hand, P53 is also the most reliable serum marker for inducing cell apoptosis (11). This protein is considered a cell protection factor in a low or physiological state, but if the damage has passed the threshold, it will be a pre-apoptotic factor and, in that case, it will cause changes and start the transcription of apoptosis-helping factors.

Also, p53 induces apoptosis through the non-caspase pathway by increasing Bax protein and releasing cytochrome c from mitochondria and extends apoptosis by decreasing Bcl-2 (12). As it has been mentioned, the amount of apoptosis increases during old age, so it is expected that the process of apoptosis will be strongly affected by the change in mitochondrial integrity caused by increasing age (13). Also, studies have shown that exercise as a method non-medicinal treatment can change cell apoptosis by changing and modulating different factors. But some researchers stated that high-intensity exercise can accelerate the process of apoptosis(14, 15), while unlike intense sports activity, performing moderate and continuous exercises probably reduces apoptosis.(16-18) However, in conflicting studies, intense activity has been associated with a decrease in apoptosis and moderate activity has been associated with an increase in it. For example, Jokar et al (2022) showed that 4 weeks of high-intensity interval training leads to a significant decrease in P53 protein in diabetic rats(19). In Kazemi et al.'s study (2018), a period of aerobic exercise was associated with a significant increase in caspase 3 (20), in another study, eight weeks of aerobic exercise did not have a significant effect on the level of caspase 3 (21). Due to the conflicting results of sports training in young and sick samples, there has been less comprehensive study on the effect of aerobic training on the amount of apoptosis indicators in elderly samples. Therefore, the purpose of this research is to investigate the effect of eight weeks of aerobic training on some apoptotic factors in elderly men.

2. Materials and Methods

The current research was semi-experimental and of an applied type, which was carried out in the form of a pre-test, post-test design with a control group on the elderly of Borujerd city who met the conditions to enter the study. The criteria for entering the samples into the study were as follows. Age of people should be from 60 to 75 years, Have the ability to exercise, Do not have any physical disease or history of any special disease, Be interested in participating in the sports program, Do not participate in sports programs inside or outside the retirement center.Then, through the questionnaire of sports medical records and practical test, subjects who had a history of taking any supplements or drugs in the past month or had a specific disease or disorder were excluded from the research. The demographic characteristics of the subjects were measured and recorded, and after the examination by the doctor and the explanation of the research objectives, written consent was obtained from the participants. The purpose of obtaining this consent was to confirm their willingness to participate in the study and to inform them that they can withdraw at any time if they do not wish to participate in the study. The research samples were randomly divided into two groups of 15 people, control and experimental.

Selected aerobic exercise protocol

The subjects performed the selected exercise program 3 times a week for 8 weeks. It is worth noting that this exercise program was implemented based on the specific recommendations of the American College of Sports Medicine (ACSM) for the elderly and based on the principles of exercise science adapted from the recommendation of Dekker et al (2014) (22). A treadmill was used to implement the walking program.

The duration of each training session was 15 to 30 minutes. The subjects' heart rate while working on the treadmill was continuously adjusted using the heart rate monitor of the control device and to maintain the intensity of the exercise within the designated range. At the beginning of the activity, due to the possibility of low physical fitness, the duration and intensity of the activity was gradually increased, so that at the beginning of the activity, the duration of the training session was considered 15 minutes, and after two weeks of the activity, the subjects did 20 minutes, and in the following weeks with They worked for 30 minutes. During this interval, the intensity of the activity also increased gradually from 60% of the maximum heart rate at the beginning and up to 75% of the maximum heart rate (23).

Blood sampling and biochemical evaluation

Blood was taken from the subjects fasting in two stages 24 hours before the start of the training protocol and then 24 hours after the end of the last session of the training protocol from the antecubital vein while sitting. To determine the serum levels of caspase-8 (with a sensitivity of 0.051 ng/ml) and protein p53 (with a sensitivity of 5.59 ng/l) by the ELISA method and based on the instructions of the manufacturer of the kits of Technology Bioassay Laboratory (intra-test coefficient of variation less than 8 and 10 percent) were done. Also, IGF-1 values were measured by ELISA method with the kit of Mediagnost, Germany (with a sensitivity of 0.09 ng/ml with intra-assay coefficient of variation less than 6.8 and 6.7%).

Statistical analysis

The homogeneity of the variables in the research groups was determined using Levin's test, and the normality of the data was determined using the Kolmograph Smirnov test. Dependent t-test was used to examine intra-group differences in variable values, and independent t-test was used to examine inter-group differences. Data analysis was done using SPSS version 20 software and the significance level was considered as $p \leq 0.05$ in all steps.

3. Results

The characteristics of the research subjects are presented in Table 1.

Table 1: Mean and standard deviation related to the age, height and weight of the subjects

Group / Variable	Experimental	Control
Age	63±6.8	60.4±2.7
Weight(kg)	81.8±7.8	79.7±6.02
Height(cm)	164.8±7.8	169.5±4.6

Figure.1- shows the serum content of caspase-8 in the study groups before and after the intervention. The value of this index increased in the experimental group after a period of aerobic exercise, so that in the experimental group, the average of caspase-8 before The intervention was 8.82, which reached 10.58 after eight weeks of aerobic exercise. Also, in the control group, the average value of caspase-8 was 8.55, which reached 8.56 after eight weeks. The level of significance obtained from the paired t-test indicates that the intra-group changes of caspase-8 are statistically significant only in the experimental group. (P=0.0001) In contrast, no significant changes were observed in the control group (P=0.67). Also, the examination of inter-group changes in caspase-8 values using independent t-test shows that the changes between control and experimental groups are significant (P=0.0001).

Figure.2- shows the serum content of P53 in the studied groups before and after the intervention. The value of this index decreased in the experimental group after a period of aerobic exercise, so that in the experimental group, the average P53 before the intervention was equal to 573.5, which reached 537.4 after eight weeks of aerobic training. Also, in the control group, the average value of P53 was 552.3, which reached 551.7 after eight weeks. The significance level obtained from the paired t-test indicates that the intra-group changes of P53 are statistically significant only in the experimental group. (P=0.0001) In contrast, no significant changes were observed in the control group (P=0.45). Also, the examination of inter-group changes in P53 values using independent t-test shows that the changes between control and experimental groups are significant (P=0.0001).

Figure.3- It shows the serum content of IGF-1 in the studied groups before and after the intervention. The value of this index decreased in the exercise group after a period of aerobic exercise, so that in the experimental group, the average of IGF-1 before the intervention It was equal to 66.55, which reached 63.4 after eight weeks of aerobic training. Also, in the control group, the average value of IGF-1 was 65.85, which reached 65.75 after eight weeks. The level of significance obtained from the paired t-test indicates that the intra-group changes of IGF-1 are statistically significant only in the experimental group. (P=0.0001) In contrast, no significant changes were observed in the control group (P=0.45). Also, examining the inter-group changes in IGF-1 values using the independent t test shows that the changes between the control and experimental groups are significant (P=0.0001).

4. Discussion

the present study was conducted to investigate the effect of eight weeks of aerobic exercise on some apoptosis factors in elderly men. The findings of the present study showed that eight weeks of aerobic training causes a significant increase in the serum levels of caspase 8. In this regard, Khan Souz et al. (2020) showed an increase in caspase-8 after eight weeks of endurance training in infarcted rats.(24) In another study, eight weeks of resistance training increased caspase-8 in diabetic rats (25). Contrary to the findings of the present study, it was shown that exercise training is associated with a decrease in caspase-8 activity levels in the heart tissue of obese rats.(26) In another non-equivalent study by Kim and colleagues (2010), they showed that eight weeks of training decreases the amount of caspase-8 (27). The reason for the discrepancy is probably the age difference of the subjects. Considering that the study subjects of Kim et al were young, but the samples of the present study were elderly, the increase of caspase-8 could be due to the rapid development of apoptotic factors in old age (28). Another finding of the present study was a significant decrease in P53 after eight weeks of aerobic exercise. In line with the findings of the present study, Sharifi et al. (2012) reported a decrease in p53 protein levels in trained men(29), in the same vein, a significant decrease in p53 levels in rats after eight weeks of resistance training (climbing the fence with carrying closed load to the tail of rats) was reported.(30) in another parallel study, Bahman Baglo and colleagues (2021) also showed that endurance training decreases p53 protein levels in diabetic rats(31) and also Hooshmand Moghadam et al (2021). They investigated the effect of twelve weeks of resistance training on some markers of apoptosis in elderly men and reported a significant decrease in p53 levels.(32)

Contrary to the present finding, it was shown that short-term compound training is accompanied by a significant increase in p53 protein.(33) In this regard, Ghorban Alizadeh and his colleagues (2020) investigated the effect of 12 weeks of aerobic training on the expression of the P53 gene. The results showed that 12 weeks of training Aerobic exercise is associated with an increase in the expression of the P53 gene.(34) One of the important factors in the conflict is the difference in the age of the research subjects. In the current research, elderly rats have been used, and considering that it has been proven that the amount of AMPK protein in elderly rats decreases in comparison with young rats(35) and on the other hand, AMPK protein will lead to the activation of p53 protein. Therefore, the reduction of AMPK protein in elderly rats is associated with the reduction of P53 protein.(36) In general, in relation to key proteins such as P53, exercise training can show contradictory results; Because factors such as duration, intensity, recovery time and other factors are very important in the results on this protein (31) Recent studies show an important function of p53 protein in regulating the IGF-1/AKT/mTOR pathway to regulate energy metabolism. IGF-1 was another variable investigated in this study. The presented results show that eight weeks of aerobic training causes a significant decrease in the amount of this protein. In line with the present research, Kordi et al (2019) showed a significant decrease in IGF-1 levels after eight weeks of aerobic and combined exercise in elderly men (37).

Bagheri et al (2015) investigated the effect of eight weeks of combined exercise on GH and IGF-1 in the serum of elderly women and showed that eight weeks of combined training is associated with a decrease in IGF-1, which is consistent with the findings of the current research (38), but Shabani et al (2017) in a non-aligned study showed that resistance training increased IGF-1 The elderly are associated (39) In addition, Tisai et al (2015) showed that 12 months of resistance training in elderly men increases the level of IGF-1 (40). The contradiction of the existing reports is probably due to the difference in the type of training, the training used In Shabani et al.'s research, it was resistance, but in this research, aerobic exercises were used. Considering that the source of energy in aerobic exercises is mainly provided by the breakdown of fat tissue, and then free fatty acid (FFA) and glycerol increase, and the increase in free fatty acid by affecting the hypothalamus causes an increase in somatostatin and further inhibition of GH (41) and because growth hormone stimulates the synthesis of IGF-1 in the liver, inhibition of GH is associated with a decrease in IGF-1 synthesis(42, 43) Among the limitations of the present study, we can mention the lifestyle, individual differences and hereditary factors of the subjects.

Conclusion

According to the findings of the present research, it seems that eight weeks of aerobic exercise significantly increases the serum levels of caspase 8 and decreases the serum levels of P53 and insulin-like growth factor (IGF-1). However, due to the increase of caspase-8, more investigation is needed in this field.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: S.H.M., B.A., M.KH.; Methodology: S.H.M., B.A., M.KH.; Software: S.H.M., B.A., M.KH.; Validation: S.H.M., B.A., M.KH.; Formal analysis: S.H.M., B.A., M.KH.; Investigation: S.H.M., B.A., M.KH.; Resources: S.H.M., B.A., M.KH.; Data curation: S.H.M., B.A., M.KH.; Writing - original draft: S.H.M., B.A., M.KH.; Writing - review & editing: S.H.M., B.A., M.KH.; Visualization: S.H.M., B.A., M.KH.; Supervision: S.H.M., B.A., M.KH.; Project administration: S.H.M., B.A., M.KH.; Funding acquisition: S.H.M., B.A., M.KH.

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Research Article

Effects of acute intensive exercise on hormone response in children, adolescents, and youth athletes

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Abstract

Background: The transition from childhood to adulthood is associated with many physiological changes that result from hormonal changes. Despite this, it has been reported that hormonal response to exercise can be age-dependent.the purpose of this study was to investigate growth hormone, insulin, testosterone, and cortisol response to acute intensive exercise in children, adolescents, and youth male athletes.

Materials and Methods: Twenty-nine eligible football players volunteered to participate in the study. Participants assigned to three groups: children (age = 10.88 ± 0.92, n=9), adolescents (age = 14.40 ± 1.17, n=10), and youth (age = 17.70 ± 0.82, n=10). The Bruce Protocol Stress Test was performed as an acute intensive exercise on treadmill. Plasma hormones were measured before and after the exercise.

Results: The acute intensive exercise leads to a significant increase in circulating levels of testosterone (p = 0.02) and Cortisol in children (p = 0.001). In the adolescent group, only a significant increase in GH (p = 0.001) was observed. In the youth group a significant increase in GH (p=0.05) and testosterone (p=0.001) was observed. However, insulin levels did not change significantly after intensive exercise in all groups. Results showed that there were no significant differences between hormonal changes within the three groups.

Conclusion: The results showed that the basal levels of some hormones and their changes after exercise were different. However, the pattern of hormonal changes after acute intensive exercise was similar in children, adolescents, and young athlete boys.

Keywords:
Acute exercise, Hormone response, Different ages, Athletes.

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Journal of Sports Physiology and Athletic Conditioning

1. Introduction

The participation of children, adolescents, and youth in exercise programs improved their health and sports performance. However, the physiological response to exercise are not the same at different ages. Accordingly, today one of the areas that have recently been of interest to researchers is the studying of hormonal responses to exercise (1). Some researchers have reported that the hormonal response to exercise differs in childhood and adulthood. In adults, plasma insulin levels decrease during moderate intensity prolonged exercise (75% VO_{2max}) (2). It is well known that puberty is associated with a period of insulin resistance and pubertal adolescents also demonstrate insulin resistance during exercises (3).

Furthermore, in adults, GH levels increase during exercise (4), but the function and physiological effects of this hormone, especially in children and adolescents, are not fully understood. Growing individuals may exhibit different or excessive GH response patterns during exercise (5), (6). Surprisingly, few studies have studied plasma GH response in children and adolescents during intensive exercise. Marin et al. (7) reported that the GH response to exercise significantly increased with increasing puberty stage. Pomerantset al (8) reported the rise of serum GH concentration was highest in the group with Tanner stage III during 30 min aerobic exercise.

Additionally, testosterone and cortisol are known to play a significant role in metabolism and puberty (9). Testosterone increases dramatically in adolescent boys during puberty. With exercise, there is also a small but significant increase in circulating testosterone levels in adolescent boys (10), (11), (12).

The effect of intensive exercise or exercise training on hormonal response, especially testosterone, has been studied mainly in adults and in resistance training. In addition, systematic characterization of the hormone response to exercise in children and adolescents has not been done nearly as wisely as it has been studied in adults. One of the first to measure hormones during exercise in children was Fahey in 1979 (13). No differences in hormonal responses to exercise were found among 27 boys at different stages of puberty, perhaps because only one type of exercise was examined and because only a few hormones were measured (testosterone, GH, and insulin). Since then, only a handful of studies have reported circulating levels of select hormones in children and adolescents, and often the responses are measured only at moderate-intensity exercise.

Sellami et al illustrated (1) total testosterone and cortisol increased with acute exercise (Wingate Anaerobic-Test). Adebero et al (14) greater increase in serum cortisol in men compared with boys (men:242%; boys:64%) and reduced serum Testosterone (men:-14.7%; boys:-33.9%) in response to intensive multitask exercise reported. Among hormones, cortisol is the main catabolic hormone with different responses to various exercises. cortisol catabolic hormone secreted by the adrenal gland, which plays a great role in metabolism. Studies show that cortisol concentration has a linear relation to the exercise intensity (15). Mazdarani et al (16) increases of salivary cortisol concentration in pre pubertal girls of 10- 11 years old after official basketball competition are reported.

The available data on hormone responses to exercise in children and adolescents are sometimes conflicting and limited by the age, gender, and training status of the individuals. In addition, understanding the hormonal response to exercise may help to form better training recommendations for youth of all ages. The purpose of this investigation is to evaluate the effects of acute intensive exercise on hormone response in children, adolescents, and youth soccer players.

2. Materials and Methods

Subjects

Seventy male soccer players of were recruited to participate in this study (Table1). Twenty-nine players with at least 3 years of sports experience, volunteered to participate in the study. Individuals with history of any chronic medical condition or use of any medication were excluded from participation. By football federation classification, participants were divided into three groups of children (n=9, 10–12 years old), adolescents (n=10, 13–16 years old), and youth (n=10, 17–19 years old). The university research council approved the study. Written informed consent was obtained from participants and parents. Subjects were requested to observe normal sleep patterns (with at least 8 hours of sleep), normal daily activity patterns, and dietary patterns before the test, and avoid any physical activity, excessive consumption of food, supplements, medication, coffee, tobacco, and cocoa up to 48 hours before taking a blood sample.

Measurements

Anthropometry. Body height to the nearest 0.1 cm and body weight to the nearest 100 g were determined by standard methods, using a SV-Seca 710 stadiometer and beam scale weight (Seca Precision for Health, Hamburg, Germany). Body mass index (BMI) was calculated as weight/height^2(kg/m^2).

Exercise protocol

Each subject performed the Bruce test on a treadmill as an acute intensive exercise. It was performed until exhaustion. Subjects were vigorously encouraged during the high-intensity phases of the exercise protocol. The Bruce protocol is a standard exercise test that is comprised of seven stages of three minutes each. Stage 1 of the Bruce protocol is performed at 1.7 miles per hour and a 10% gradient. At three-minute intervals the incline of the treadmill increases by 2% and treadmill speed in the first stage to the seventh stage was 2.7, 4, 5.5, 6.8, 8, 8.8 and 9.8 km/h respectively. The subjects performed the test on three separate days. To reduce the effect of circadian rhythm, all blood samples were collected at the same time per day from 7:30 to 9:00 AM.

Blood samples. Blood samples were taken before and after acute intensive exercise then transported to the lab and immediately centrifuged at 3000 rpm, at 4°C for 20 min. GH serum were determined by ELISA with the use of the Biochem Diagnostics kit (Diagnostic System Laboratories. Canada). Intra-assay coefficient of variation (CV) was 3.3–4.5%, inter-assay CV was 5.5–12.9%, and the sensitivity was 0.03 ng/ml. Serum insulin levels were determined by ELISA with the use of the Mercodia kit (Sweden). Intra-assay CV was 1.3–2.6%, inter-assay CV was 5.2–6.2%, and the sensitivity was 0.26IU/ml. Serum cortisol levels were determined by ELISA with the use of the Biochem Diagnostics kit (Diagnostic System Laboratories, Canada). The intra- and inter-assay CV for this assay were 3.2 and 6.8%, respectively. Testosterone serum concentrations were determined by ELISA with the use of the Biochem Diagnostics kit (Diagnostic System Laboratories. Canada). Intra-assay coefficient of variation (CV) was 5.6%, inter-assay CV was 3.2–4.7%, and the sensitivity was 0.04ng/ml.

Statistical Analysis

SPSS software (version 24) was used for data analysis. The changes in variables were analyzed by ANOVA between 3 groups with LSD post hoc test and paired t-tests in each group. Statistical significance was set at P≤0.05. All data are reported as Means±Standard deviation (M±SD).

3. Results

Anthropometric characteristics of participants are presented in table1.

Table 1: Anthropometric characteristics of participants (M±SD)

	Children(n=9)	**Adolescent**(n=10)	**youth**(n=10)
Age(year)	10.88±0.92	14.40±1.17	17.70±0.82
Mass(kg)	27.06±1.27	56.33±3.35	71.00±1.64
Height(cm)	131±3.14	165±2.11	174±4.29
BMI(kg/m^2)	15.69±0.40	20.51± 0.83	23.07±0.24

The results of the paired t-test for comparing pretest and posttest in groups are shown in Table2.

Table 2: paired t–test comparison

	Groups	**Pretest** (M±SD)	**Posttest** (M±SD)	**t**	**sig**
GH(ng/ml)	Children	1.20±0.59	1.42±0.47	-1.25	0.24
	Adolescent	2.36±0.46	2.71±0.57	-6.61	0.001*
	Youth	2.92±0.94	4.03±1.17	-2.20	0.05*
Insulin(ul/ml)	Children	6.95±2.64	6.09±3.36	-0.14	0.89
	Adolescent	5.57±2.53	5.31±2.93	-0.52	0.69
	Youth	8.61±2.80	8.03±4.08	1.22	0.25
Testosterone(ng/ml)	Children	1.99±2.16	2.88±2.36	-2.84	0.02*
	Adolescent	5.68±1.89	6.23±2.37	-1.81	0.1
	Youth	7.81±0.94	8.15±0.83	-3.92	0.001*
Cortisol(mg/dl)	Children	9.27±2.13	10.03±2.41	-1.86	0.001*
	Adolescent	10.92±2.98	12.18±3.30	-1.53	0.15
	Youth	15.96±5.87	15.23±2.54	0.39	0.7

*Within-group changes from pretest-posttest (P<0.05).

GH. At baseline, there was significant difference between groups (F=14.61, P=0.00). Youth GH was significantly higher than children (p=0.00) as well as adolescents GH was significantly higher than children (p=0.00). After intensive exercise, GH levels increased significantly in adolescents (p=0.001) and youth (p=0.05). But no significant changes were noted in children (p=0.24). There was no significant difference between hormonal changes in the three groups (Fig1).

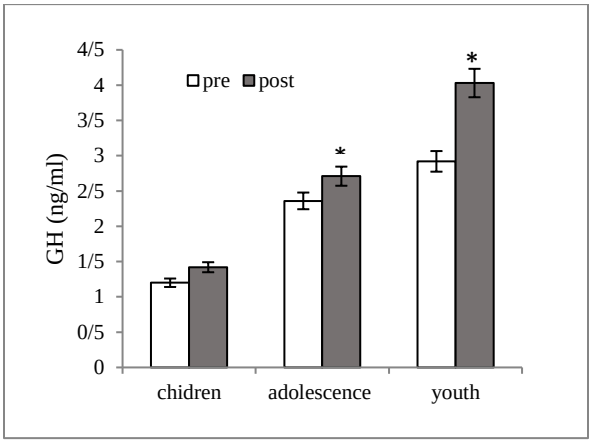

Figure 1: GH changes in groups following the acute intensive exercise

Insulin. At baseline, there was no significant difference between groups (F=3.27, P=0.54). After intensive exercise, insulin levels did not change significantly in any of the groups. (Fig2)

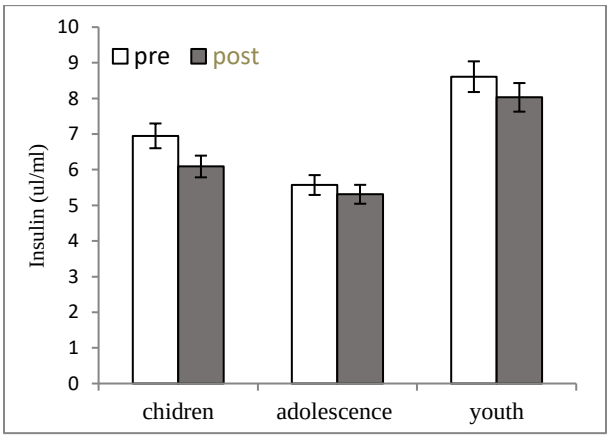

Figure 2: Insulin changes in groups following the acute intensive exercise

Testosterone. At baseline, there were significant differences between groups (F=27.04, P=0.00). Youth testosterone was significantly higher than adolescents (p=0.02) and children (p=0.00). Also, adolescents' testosterone was significantly higher than children's (p=0.00). After intensive exercise testosterone levels increased significantly in children (p=0.02) and youth (p=0.05). But no significant changes were noted in the adolescent (p= 0.1). There was no significant difference between hormonal changes in the three groups. (Fig3)

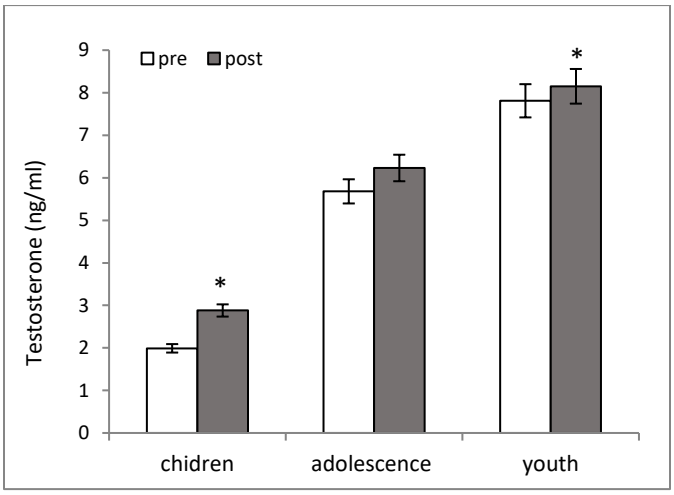

Figure 3: Testosterone changes in groups following the acute intensive exercise

Cortisol. At baseline, there was significant difference between groups (F=7.14, P=0.003).

Youth Cortisol was significantly higher than adolescents (p=0.02) and children (p=0.00). There were no significant difference between adolescents and children (p=0.65). After intensive exercise Cortisol levels increased significantly in Children (p=0.001). There was no significant difference between hormonal changes in three groups. (Fig4)

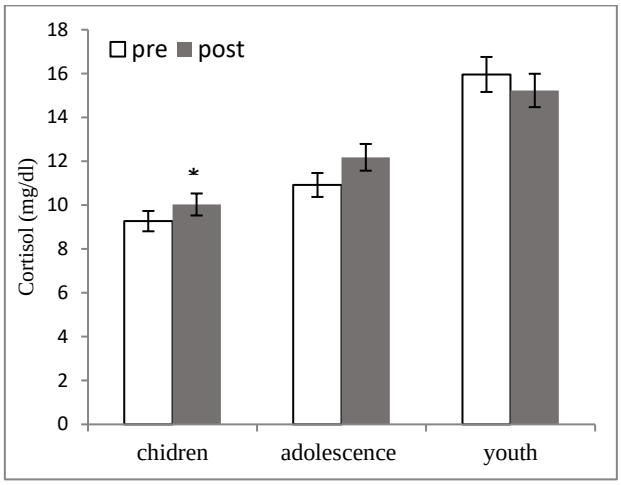

Figure 4: Cortisol changes in groups following the acute intensive exercise

4. Discussion

In the present study, we examined the effects of acute intensive exercise on hormones response in children, adolescent and youth male football players. It is noted that exercise stimulates GH secretion(17). The GH response to exercise depends on the duration and intensity of the exercise, the fitness level of the individual, the timing of blood sampling, and age(18).

GH may play a central role in the regulation of the utilization and storage of energy (9). Previous studies that examined the effects of endurance and anaerobic-type exercise on GH suggested that the exercise should be appropriate to cause a significant metabolic effect (above the lactic anaerobic threshold) to stimulate GH secretion (19). We found that a graded intensive exercise led to a significant increase in GH levels in adolescent and youth.

Socratis et al (20) and Yamaner et al (21) reported that GH concentration significantly increases after intensive exercise in boys. The results of a study showed that GH concentration did not increase after an acute Incremental exercise until 70% maximal oxygen consumption in 10-year-old children (5). In the current study, GH concentrations increased following exercise in all groups, but a significant increase was observed only in adolescent and youth subjects, indicating a more eager exercise-stimulated GH secretion in puberty, approving that exercise-induced GH increase parallels puberty development(22). The combination of rapid growth, high levels of exercise performance, and spontaneous increase in anabolic hormones during puberty suggests the possibility of integrated mechanisms linking exercise to the highest levels of GH secretion in adolescents and young adults. This indicates that an increase in growth hormone is associated with an increase in body mass and athletic capacity during growth (23).

Testosterone is considered the major developer of muscle growth and subsequent increase in muscle strength in response to resistance training(24). In this study, testosterone level increased after intensive exercise, but only in children and youth it was significant. The effects of exercise on hormones were examined in numerous papers and there were some supporting results as well as opposing ones. The majority of studies confirmed an increase in testosterone levels after endurance and resistance exercise. Wegner et al (25) demonstrated acute increases in testosterone concentration immediately after the maximal time trial in adult and adolescent. The increase in testosterone depends largely on the exercise workload and intensity, muscle mass involved, and the athlete fitness level.

In the current study, testosterone levels of the adolescent increased but not significantly. Activation of the hypothalamic-pituitary-gonadal axis causes the progressive secretion of testicular sex hormones, mainly testosterone, during puberty, which are important for biological and behavioral changes. Perhaps these increased testosterone levels due to puberty limit its greater secretion capacity after intensive exercise (21), it seemed reasonable to assume that the alteration in this hormone concentration might affect explosive-type performance as observed in soccer.

Our findings are similar with results from 9 to 10-year-old children, who were exercising with 180–190 bpm for 12 min but they are contradicted from 15-year-old adolescents, who were exercising with 65–75% HRmax for 15 min (25).

In adult, intensive bouts of exercise are known to affect an increase in the circulating concentrations of testosterone and cortisol (25), (26). The training intensity, training duration, adaptation levels of the athletes to training, load/rest ratio, and nutritional differences may affect the results of the studies. Researchers have suggested multiple mechanisms such as stimulation of testosterone secretion by promoting dilatation of vessels and increased blood flow in muscle tissue, increased LH production, and an increase in lactate accumulation following exercise(27). It has also been suggested that there was an increase in sympathetic function due to training, which may lead to a more rapid testosterone response (28).

During exercise, muscle activity increases glucose uptake independently of insulin. (29). Indeed physical activity induces greater insulin sensitivity by increasing glucose transporters in the cell membrane.In adults, plasma insulin levels decrease during prolonged moderate exercise (75%VO_{2max}) (2). In light of decreased insulin sensitivity in prepubertal period and enhanced glucose-induced insulin secretion during puberty (3), specific responses would not be surprising in children and adolescents during physical activity.

Although in this study, decrease insulin after acute exercise was not significant in any of the groups ($p>0.05$). One of the possible reasons is that the subjects are athletes who have less hormonal changes due to the increase of cell membrane receptors. Despite this observation, lack of data makes the characterization of age-related changes in plasma insulin levels after exercise impossible.

Cortisol has been shown to be an indicator of physical and mental stress (28). Despite some inconsistent findings most studies have shown increases in concentrations of cortisol in response to exercise. In the present study, acute intensive exercise did not result in significant change in adolescent and youth groups' cortisol concentration. Only in children did cortisol concentration increase significantly. These data are not in agreement with observations made in 15 to 16-year-old males (12). But Budde et al(30) Showed that Saliva cortisol significantly increases after 12 min high-intensity exercise in 9–10 years old primary school students.

The higher cortisol concentration seen in soccer players was likely an adaptation to exercise induced stress. With regard to our results, it is important to point out that in a single sample analysis the interpretations based on the concentration of hormones in the blood needs to be done with great caution. Soccer has been categorized as high-intensity intermittent exercise and repeated bouts of maximal effort sprints are observed in this sport. It has been previously reported that an increase in physical performances may be related and explained by an increase in plasma testosterone levels coupled with a decrease or maintenance of plasma cortisol levels (31). Probably because of the higher level of physical fitness in adolescent and youth athletics than in children, the intensity of exercise was not sufficient to significantly increase cortisol.

Conclusion

This study investigated the hormone responses to acute intensive exercise in children, adolescents, and youth male athletes. The results showed that the basal levels of some hormones and their changes after the exercise were different. An acute intensive exercise resulted in a significant increase in circulating testosterone in children and youth, whereas the growth hormone was significantly increased after the exercise in the youth and adolescents. and cortisol increased significantly only in children. This hormonal response may affect not only the muscular system, but also other target organs (immune, cardiopulmonary, etc.) Therefore, performing intense exercises by children and its possible effects on other tissues need more research. Although the pattern of hormonal changes was similar in the three groups, it is important to note that the physiologic outcomes of these hormonal changes are also determined by the intensity of exercise as well as age category.

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This study did not have any funds.

Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants and parents.

Author contributions

Conceptualization: M.F., R.S.; Methodology: M.F., M.A. R.S.; Software: M.F., R.S.; Validation: M.F., R.S.; Formal analysis: M.F., R.S.; Investigation: M.F., R.S.; Resources: M.F., R.S.; Data curation: M.F., R.S.; Writing - original draft: M.A., M.F., R.S.; Writing - review & editing: M.F., R.S.; Visualization: M.F., MA., R.S.; Supervision: M.F., R.S.; Project administration: M.F., R.S.; Funding acquisition: M.F., MA., R.S.

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Research Article

Effect of 8-week simultaneous metabolic resistance training and Chlorogenic acid supplement on the expression level of BMP2, BMP4, BMP6, and BMP7: A randomized open label clinical trial

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Abstract

Background: BMP has critical role in development, growth, and differentiation of cell. There is enough evidence regarding the role of BMPs in lipid accumulation and homeostasis The current study aimed to evaluate the simultaneous effect of eight weeks of metabolic resistance training (MRT) and Chlorogenic Acid (CGA) supplementation on expression level of BMP2, BMP4, BMP6, and BMP7 in overweight women.

Materials and Methods: We carried-out a randomized clinical trial performed on 40 overweight women in Iran 2020. We randomly assigned the study participants into four groups including combined 8-week course of metabolic resistance training (MRT) training and 400 mg chlorogenic acid (CGA) supplementation, 8-week course of MRT, CGA supplement, and the control group. Intervention included three MRT training sessions per week and the duration of each session was 45 minutes. The training exercise intervention was 10 minutes of warm-up, 30 minutes of metabolic resistance training, and 5 minutes of cool-down. The supplementation arms were also received 400 mg / day CGA extracted from green coffee beans. Expression level of BMP2,4,6, and 7 was the main interested outcome that assessed pre and post intervention.

Results: We observed significant decrease in BMP2 level in combined intervention group in compared with the control group (Regression coefficient= -2.7, 95% CI=-5.0, -0.4). Moreover, we observed that combined intervention has decreased BMP4 level and the observed difference was statistically significant (Regression coefficient= -6.2, -1.7, -10.6). No significant effect for MRT and CGA group was reported regarding BMP2, and BMP4. Neither combined nor separate form of CGA and MRT had no significant effect on BMP6 and BMP7 (P-value>0.05).

Conclusion: Simultaneous MRT exercises and CGA supplementation prohibited expression levels of BMP2, and BMP4. However, they had no significant effect separately. There was no association between the interventions and expression level of BMP6, and BMP7.

Keywords:
metabolic resistance training, green coffee, obese and overweight women, Bone/body morphogenic proteins

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1. Introduction

Obesity is a global health problem with an increasing trend over the past decade across the world (1). It increases risk of many chronic diseases and also is associated with higher risk of all-cause mortality (2). Lack of physical activity, sedentary lifestyle, and high calories fast-food and diet are possibly the main risk factors of obesity (3).

Exposure to exercise and dietary factors could regulate neurogenesis in adults that involves in different phases of cell cycle including cell proliferation, differentiation, survival and integration into functional circuits(4, 5). Bone/body morphogenic proteins (BMPs) are type of growth factors that are affected by such exposures. Although inducing bone and cartilage formation are the original ability of the BMPs, there are enough evidence regarding the role of BMPs in lipid accumulation and homeostasis (6, 7). BMP family has at least 20 members with different roles in development, growth, and differentiation. For instance, BMP8 is known as a driver of thermogenic response in brown adipose tissues and BMP7 could lead fat browning through regulation of insulin signaling (8, 9). On the other hand, BMP2 and BMP4 have already been shown as regulator of insulin sensitivity (10, 11). According to previous studies BMPs could regulate insulin resistance and they are correlated with metabolic syndrome, as well (10, 12, 13).

As it already mentioned exposure to exercise and some specific dietary supplement could regulate level of BMPs. Previous studies reported that exercise training has various effects on different member of BMP family. It could induce expression of BMP7 (14), while it had inhibitory effects on BMP2 and BMP4 (10).

Despite from lack of evidence, the results of previous studies regarding the effect of exercise on BMPs are controversial. Moreover, dietary supplement like chlorogenic acid (CGA) was almost ignored and simultaneous effects of these two factors have never been investigated. In the current study, we aimed to investigate the effect of 8-weeks metabolic resistance training (MRT) and CGA supplement on level of different BMPs including BMP2, BMP4, BMP6, and BMP7. We also determined the effect of simultaneous MRT and CGA on level of the investigated BMPs.

2. Materials and Methods

The current study was an open-label randomized clinical trial that was performed on 40 overweight women. The study participants were taken using a convenient sampling scheme among women with a body mass index between 25 and 28 who participated in a fitness gym in Tehran-Iran. Having physical activity in the past six months, Not using dietary supplements, or medications, having underlying diseases related to the investigated variables, and not having any pulmonary diseases were the other inclusion criteria. Women with no willingness to participate were excluded from the study. This study was reviewed and approved by XXX ethics committee and all study participants completed a written informed consent prior to the study. The study participants were allowed to leave the study at any phase.

We investigated the effect of CGA supplement, MRT exercise, and simultaneous supplement and exercise versus a control group and the study participants were randomly assigned to one of these groups using a stratified balanced block randomization approach. The training exercise intervention was 10 minutes of warm-up, 30 minutes of metabolic resistance training, and 5 minutes of cool-down. The intensity of the exercise was 60-70 percent of the maximum heart rate of the participants (15). The overall duration of each training session was 45 minutes and it was repeated three times a week. We also used Chlorogenic Acid (CGA) extracted from green coffee as the dietary supplement. Each participant in the supplement group received 400 mg of the green coffee extract on daily basis (16). More detail about sampling, randomization, and interventions are described elsewhere.

We collected data on Demographic and biometric data including age, weight, height, BMI, medical history, and volume of physical activity for each participant. Moreover, data on lipid profiles were also collected as baseline characteristics. Lipid profile was determined using a blood sample taken at baseline and repeated after an 8-weeks study period. All study participants were fasting over the last 12 hours before sampling and sampling was done from their right-hand vein at 8 AM. BMPs expression levels including BMP2, BMP4, BMP6, and BMP7 were the main investigated outcomes that were assessed using DNA extraction. Real-time RT-PCR was used to determine the expression level of BMPs. All procedures were repeated at the end of the study after an 8-weeks intervention.

Statistical analysis

We checked the normality assumption and provided the mean and standard deviation (SD) if this assumption was met. Otherwise, we presented the continuous variables as the median and interquartile range (IQR). For dichotomous variables frequency proportion was reported. The baseline characteristics were compared over the four investigated groups to assure that randomization generated comparable groups. We used one-way ANOVA or its non-parametric equivalence for continuous variables and the between-group difference of dichotomous variables was compared using the Chi-square test. Pre and post-study within-group variability was investigated using the U-Mann Whitney test. We also used multiple linear regression to investigate the effect of each intervention on the expression level of BMPs after adjustment for potential confounders. Age, BMI, baseline BMP, HDL, LDL, and TG were entered into the model and then we sued backward approach to generate the best fitted model. We created separate regression model for each investigated BMP. All statistical analysis was performed using Stata software (Ver 17.0, College Station, Texas, USA). P-values <0.05 were considered significant.

3. Results

We enrolled 40 overweight women into the four groups including control, CGA supplement, MRT training, and CGA/MRT combined group. Table 1 compared the baseline characteristics including age, weight, BMI, HDL, and LDL. And TG among the compared groups and found no statistically significant difference (P-value>0.05) (Table 1).

The average BMP2 expression level at baseline was 26.1 (5.8) in the control group. It was 25.4 (5.1) in the CGA group, 23.9 (5.4) in the MRT group, and 24.9 (3.4) and the combined MRT/CGA group. After 8 weeks, the BMP2 expression level in the control group, CGA, MRT, and combined groups reached 25.7 (6.3), 25.5 (4.7), 22.4 (6.5), and 21.9 (4.9), respectively (Figure 1). The median of the effect size in the combined CGA/MRT group was 2.9 which was statistically significant (P-value= 0.013). No statistical pre and the post-intervention difference was observed in other groups (P-value>0.05) (Table 2).

The average expression level of BMP4 was 22.7 (4.4) at baseline and 25.1 (7.1) after an 8-weeks study period. The average level of BMP4 in the CGA supplement group was 23.4 (4.6) and 23.8 (5.2) at baseline and after the 8-week intervention. We observed 3.1 units decrease in the BMP4 expression level in the exercise group where the average BMP4 was 23.9 (4.6) at baseline and 20.8 (9.1) at the end of the study. However, the observed difference was not statistically significant (P-value=0.072). Moreover, the average level of BMP4 was 24.6 (7.3) at baseline and decreased to 17.5 (15.5) after 8 weeks of CGA/MRT intervention. This decrease in the BMP4 level was statistically significant (P-value=0.021) (Table 2) (Figure 1).

We also compared the expression level of BMP6 and BMP7 before and after the study period and observed no statistically significant group within the all investigated groups (Table 2).

The effect size of each intervention was compared versus the control group adjusted for all possible confounders. According to table 1, the combined CGA/MRT has led to a 2.7 decrease in the BMP2 expression level and it was statistically significant in comparison to the control group (0.021). The post-test average expression level of BMP4 in the combined intervention arm was also 6.2 units (95% CI= 1.7, 10.6) lower than the control group and the observed difference was statistically significant (P-value=0.008). We also spotted a non-significant decrease in the expression level of BMP2 and BMP4 in the exercise group compared with the control group (P-value>0.05) (Table 3). We found no statistically significant association between the expression level of BMP6 and the applied interventions including CGA, MRT, and combined CGA/MRT. Such a pattern was observed regarding BMP7 expression level in the multiple linear regression model, as well (Table 3).

Table 1: Study participants baseline characteristics by type of intervention

Characteristics	Control	Supplement	Exercise	Exercise/Supp	P-value
Age, year	39.6 (4.7)	40.6 (7.0)	41.1 (4.9)	40.4 (5.9)	0.948
Weight, Kg	67.1 (5.2)	69.8 (3.3)	69.2 (5.1)	68.3 (3.5)	0.632
BMI, Kg/m^2	26.5 (1.4)	26.6 (1.3)	26.9 (1.2)	26.1 (1.2)	0.555
HDL, mg/dL	65.3 (6.8)	64.1 (5.4)	64.3 (8.1)	63.9 (9.3)	0.977
LDL, mg/dL	99.9 (19.9)	104.0 (20.3)	100.4 (25.6)	103.5 (22.5)	0.990
TG, md/dL	108.7 (35.8)	104.3 (22.9)	108.9 (15.9)	113.1 (36.1)	0.170

All variables presented as mean and standard deviation.

Table 2: Pre and post-intervention expression of BMP2, BMP4, BMP6, and BMP7 by each arms of the study

	BMP2		BMP4		BMP6		BMP7	
Group	Post-Pre	P-value	Post-Pre	P-value	Post-Pre	P-value	Post-Pre	P-value
Control	-0.2 (3.1)	0.714	0.5 (10.9)	0.556	3.6 (17.7)	0.375	-1.3 (12.4)	0.769
Supplement	0.04 (2.1)	0.752	0.4 (8.0)	1.00	-3.6 (16.9)	0.161	-3.5 (8.7)	0.130
Exercise	-2.0 (0.7)	0.075	-3.1 (6.8)	0.130	1.8 (16.9)	0.878	-6.1 (9.6)	0.322
Ex/Supp	-2.9 (3.6)	0.013	-7.1 (7.5)	0.019	0.8 (6.1)	0.798	0.02 (20.2)	0.734

The effect sizes are provided be median and interquartile ranges.

Table 3: Multiple linear regression to investigate the effect of each intervention on expression level of UCP1 adjusted for possible confounders

	BMP2		BMP4		BMP6		BMP7	
Group	Reg (95% CI)	P-value	Reg (95% CI)	P-value	Reg (95% CI)	P-value	Reg (95% CI)	P-value
Control	Reference		Reference		Reference		Reference	
Supplement	0.4 (-1.8, 2.6)	0.716	-2.5 (-8.7, 3.5)	0.557	-5.6 (-13.2, 1.8)	0.135	-0.01 (-7.7, 7.7)	0.996
Exercise	-1.2 (-3.4, 0.1)	0.261	-2.3 (-6.3, 2.2)	0.442	-2.4 (-9.8, 4.8)	0.495	-3.8 (-11.5, 3.9)	0.323
Supp/Exercise	-2.7 (-5.0, -0.4)	0.021	-6.2 (-1.7, -10.6)	0.008	-3.1 (-10.6, 4.3)	0.402	-3.9 (-12.1, 4.3)	0.339

Model is adjusted for age, baseline BMI, and baseline BMP level. CI= Confidence Interval

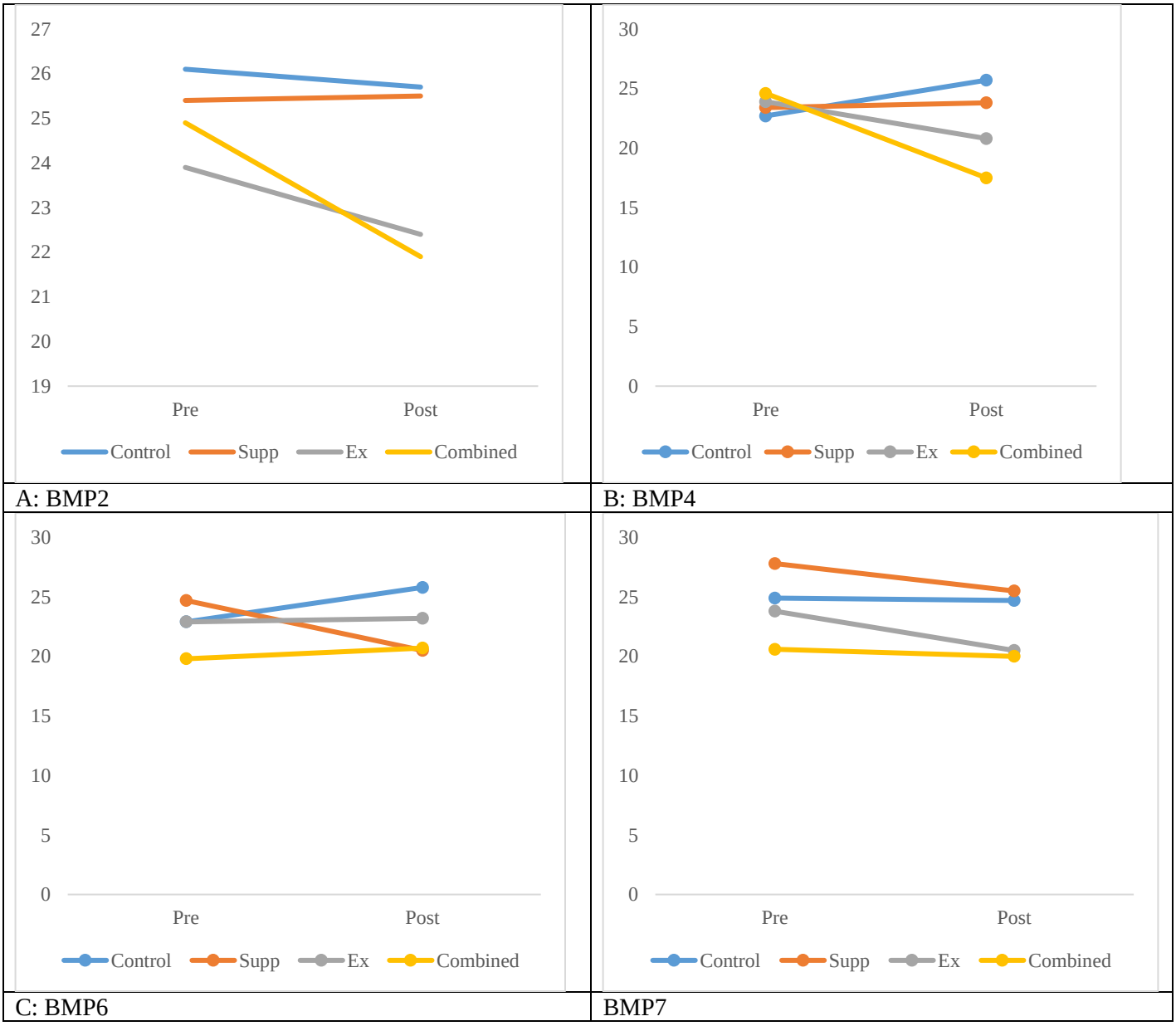

Figure 1: Average expression level of BMP2, BMP4, BMP6, and BMP7 before and after 8-weeks study period by type of intervention

4. Discussion

We performed the current study to investigate the effect of CGA supplementation and MRT exercises on the expression level of the BMP family including BMP2, BMP4, BMP6, and BMP7. We also aimed to assess the effect of 8-week simultaneous CGA supplementation and MRT exercises on the BMPs expression levels. According to our data, we found simultaneous CGA supplementation and MRT exercises could significantly inhibit the expression of BMP2 and BMP4. The such decreasing pattern was also observed for the MRT group. However, the observed association was not statistically significant indicating that the CGA supplement might work as a booster. Moreover, we found no significant effect for CGA and MRT intervention either solely or in the combined form regarding the expression level of BMP6 and BMP7.

We found that the combined CGA and MRT intervention could inhibit BMP2 and BMP4 expression levels. According to our findings average expression level of BMP2 and BMP4 in the CGA/MRT group was 2.9 and 7.8 units lower than the control group. However, such effects were not observed in the exercise and supplement group when they were given to the participants separately. There is limited evidence regarding the effect of exercise and CGA supplements on the BMP family. However, according to previous studies, BMP has a critical role in the regulation of hemostasis (17). BMP2 and BMP4 as the closest relatives have already been shown as factors promoting white adipogenesis through the induction of peroxisome proliferator-activated receptor (PPAR) (18, 19). According to the Leipzig Cohort, there was a positive correlation between BMP2 and BMI and diabetic status, especially in visceral adipose tissues(20).

Such findings were consistent with ours since we showed reducing BMP2 has led to weight and BMI reduction. Sadeghi et al, reported that different intensities of resistant training had no effect on the expression level of BMP4 (10). They also observed a downward non-significant trend between the intensity of resistance training and the post-intervention level of BMP4 (10). Their findings were in favor of the current study as we found no association between resistant training and BMP4. The data regarding the effects of CGA supplements on BMP2 and BMP4 was limited. In one study by Fujita et al. CGA had no effect on the expression of BMP4 that was similar to our findings (21). However, as we added the CGA supplement our data in CGA/MRT group was unique and we found a significant reduction in BMP4 level in this group. In another study by Majerczak et al no association between BMP4 level in the heart muscle of mics and exercise was observed that confirmed our findings (22). They also illustrated that exercise training could significantly reduce the level of BMP4 in the tibia indicating that the effect of exercise on BMP4 level might depend on the type of tissue(22). It has been argued that BMP4 tended to be accumulated in obese and overweight people since it could inhibit insulin secretion from beta cells (10). There are also evidences regarding the association between BMP4 level and Insulin resistance (23). Activation of IRS-1 inhibitors and insulin signaling was the proposed contribution of BMP4 in the upregulation of insulin resistance (17). Increasing the BMP4 level could reduce lipolysis and consequently increase the weighting of adipose tissues through Smad signaling pathway (17).

We also investigated the effect of resistance training and CGA supplement on the expression level of BMP7 and BMP6 and observed no statistically significant association in this regard. Rodrigues et al. against our findings have shown that training exercises could upregulate the level of BMP7(14). They also showed BMP7 was inversely correlated with body weight2(14). It seems that BMP7 leads to full activation of brown adipogenesis and affects processes such as the induction of primary regulators of brown fat such as PRDM16 and PGC-1. Also, the BMP7 protein increases the number of specific markers of brown fat (UCP1), adipogenic transcription factor PPARy, CCAAT binding proteins, and induction of p38 mitogen-activated mitochondrial kinase biogenesis and PGC-1α-dependent pathways (14). However, the studies conducted on the effect of exercise training on the expression level of BMPs were limited and there are serious differences in the method of conducting these studies. Also, the conducted studies were mostly performed on animals and the generalization of their findings to humans has serious limitations. These reasons can be considered the main reasons for the differences observed in the findings of the present study with previous studies.

The current study is one of the first attempts to investigate the simultaneous effect of MRT training and CGA supplementation on BMPs expression. In this sense, the findings of this research were unique as we had a comprehensive approach regarding BMPs and investigated the expression level of BMP2. BMP4, BMP6, and BMP7. Conducting the study on a human sample with no lost-to follow-up cases in all intervention and control groups was the main strength of the current study. However, the interpretation of our findings must be done in light of our limitations.

The impossibility of blinding was the main limitation of the current study, which could bias the findings of the present study. However, in this study, we tried to avoid contact between the intervention and control groups, and the people of these groups participated in their exercise programs separately. Moreover, the small sample size was the other limitation that reduced our power.

Conclusion

Simultaneous MRT exercises and CGA supplementation prohibited expression levels of BMP2, and BMP4. However, they had no significant effect separately. We also found no association between MRT exercise/CGA supplement with BMP6 and BMP7 either in combined or separate form. More human studies are required.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: M.T., F.GH., SH.S., KH.E.; Methodology: M.T., F.GH., KH.E.; Software: M.T., F.GH., SH.S., KH.E.; Validation: M.T., F.GH., SH.S.; Formal analysis: F.GH., SH.S., KH.E.; Investigation: F.GH., SH.S., KH.E.; Resources: M.T., F.GH., SH.S.; Data curation: M.T., F.GH., KH.E.; Writing - original draft: M.T., F.GH., SH.S., KH.E.; Writing - review & editing: M.T., SH.S., KH.E.; Visualization: M.T., F.GH., SH.S., KH.E.; Supervision: M.T., SH.S., KH.E.; Project administration: M.T., SH.S.; Funding acquisition: M.T., SH.S., KH.E.

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Research Article

Evaluation of the consumption of authorized and illegal supplements among ski athletes in Tehran

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Authorized supplement, unauthorized supplement, athlete, skiing.

Abstract

Background: In line with the general policy of quantitative and qualitative development of sports by relying on scientific and research achievements and in order to keep sports clean from the harmful and destructive phenomenon of inappropriate use of drugs and supplements, an exorbitant amount is paid annually to track drugs and control doping. The purpose of this research is to investigate the consumption of permitted and unauthorized supplements among skiing athletes in Tehran.

Materials and Methods: According to the nature of the subject and research objectives, the present research uses the analytical descriptive research method along with the survey method, which was conducted in the field by presenting a standard questionnaire. The statistical population in this research is 103 people from the first to tenth place in national championships. In this research, after collecting data, we use factorial variance analysis, Friedman test and linear regression to perform statistical analysis.

Results: The findings of the research showed that according to the results of this research, we find that all the selected variables in this research, which include (effects on muscles, physical effects, doping and motivation), have a significant effect on the consumption of sports supplements by They have athletes. Also, considering that the selected sample is from the first to tenth champions of the country's skiing championships, most of these people have an acceptable quality of sleep without any particular problem.

Conclusion: It seems that the variables of this research include effects on muscle, physical effects, doping and motivation. The test results showed that the importance and ranking of these criteria are different among athletes. The comparison of the average ranks shows that the most important variable influencing why sports supplements are used among ski athletes was the positive effects on the muscles and improving their performance. The third and fourth are ranked for doping and motivation, respectively.

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1. Introduction

Fasting exercise is an important part of the life of many people. Some do it for fun and some do it professionally. he does the increasing attention of people to this phenomenon makes sports an international base industry all over the world. In spite of all the benefits and benefits derived from sports, one of the problems and problems associated with it, especially in the last few decades, is the excessive attention given to officials, coaches and athletes to the results of sports competitions and marginal issues such as improving the appearance of the body. A means of increasing the volume and mass. It may have caused nutritional disorders caused by the habit of consuming energizing substances among young people (1-3) In line with the general policy of quantitative and qualitative development of sports by relying on scientific and research achievements and in order to keep sports clean from the phenomenon Harmful and destructive misuse of drugs and supplements, an exorbitant fee is paid annually to track drugs and control doping. Unfortunately, despite the efforts of sports federations to preserve the sports dignity of the champions, with the growing growth of sports clubs, drug abuse has taken on a special and dangerous form and exposed the young people of the countries to terrible complications, even death, and on the other hand, the culture of sports and respect, It has been invaded (4). With the advancement of the sciences of sports physiology, metabolism and nutrition, it has been proven that diet and nutritional intake have an effect on the performance of athletes (5). But the benefits of nutritional supplements have not been fully proven as the athletes themselves believe.

On the other hand, smuggled supplements (which are manufactured and supplied illegally and with low-quality raw materials) or prohibited (which contain energizing substances) as well as some food supplements that are not indicated on their labels, but contain non-food substances such as Prohormones and stimulants and each has its own side effects (6,7) The consumption of various food supplements is also increasing dramatically all over the world, which includes daily vitamins, supplements, herbal supplements and performance enhancers. At present, a large number of supplements that are commonly bought and sold in the market, not only have not been assured about their consumption, nor have they been proven safe and harmless by reliable scientific research. but there are concerns from the athletes of different sports for their consumption (8,9) Athletes find that certain foods or herbs help their athletic performance. For example, from the leaves of a kind of tree to fight with They used fatigue from training or hard work, or they used a kind of mushroom because of its high protein percentage (10,11) Today, the use of these substances to increase sports performance is very widespread, and a large group of athletes in different disciplines have a history of using these substances or are currently using them (12). Sports supplements are classified into two categories: permitted and non-permitted, non-permitted supplements refer to those substances that cause abnormal changes in the level of improvement in a person's sports performance by influencing factors such as stimulation of the nervous system, blood pressure, and vasodilation. Abnormal increase of growth hormone and testosterone and sometimes blood volume and other factors.

Among the illegal supplements are stimulants such as amphetamine, ephedrine, cocaine, asteroids, asteroid compounds, etc. (13,14). Researches that have been carried out abroad in order to investigate the consumption of drugs, energy supplements, the awareness and attitude of athletes and non-athletes regarding doping and its harmful effects, show that athletes are more exposed to the consumption of such substances and the consumption patterns of various substances in Different sports are different (15). Since the interest of young people in the sport of skiing is increasing all over the world and also in Iran, which will naturally lead to the tendency to abuse these substances, and considering that so far a study in This has not been done in Tehran, this study has been designed and carried out in order to investigate the use of permitted and unauthorized supplements in ski athletes in Tehran, to identify the existing weaknesses and strengths based on the results and to propose basic solutions to In order to improve and solve the existing obstacles and problems, it is a small step to prevent injury to the country's athletes. The main purpose of this research is to investigate the consumption of permitted and unauthorized supplements among skiing athletes in Tehran.

2. Materials and Methods

According to the nature of the subject and research objectives, the present research uses the analytical descriptive research method along with the survey method, which was carried out in the field. Also, due to the length of the research, it was cross-sectional. The library method has been used to collect information in the field of literature and research background. In this way, the required information has been collected by reading books and articles and researches of other researchers. Also, the field method has been used to collect information to investigate the research questions. In this research, in order to achieve the set goals and collect the necessary information from a standard questionnaire according to reliable sources. The questionnaire to investigate the consumption of permitted and unauthorized supplements among ski athletes is designed in a practical way and in three parts, the first part is to obtain the necessary information and individual characteristics of ski athletes, the second part is for the time and duration of the use of supplements during sports and the third part of this questionnaire was compiled using the opinions of ski athletes in this field and foreign article. The questionnaire to investigate the consumption of permitted and unauthorized supplements among ski athletes is designed in a practical way and in three parts, the first part is to obtain the necessary information and individual characteristics of ski athletes, the second part is for the time and duration of the use of supplements during sports and the third part of this questionnaire was compiled using the opinions of ski athletes in these field and foreign articles. The questionnaire in this research was prepared and compiled using the articles of (16,17).

3. Results

Considering that in our research, we have 1 dependent variable, i.e. (amount of consumption of sports supplements) and 4 independent variables, which include :Effects on muscle, physical effects, doping, motivation and sufficient information to perform statistical tests on them, it is first necessary to determine the normality of the variables; So that parametric or non-parametric test suitable with variables can be selected to check the data. We obtain the normality of the research variables by checking the skewness and kurtosis.

Skewness and standard error of skewness along with its skewness and standard error are reported. Now, in order to find out whether the distribution of the desired variables is normal or not, we need the Z-statistic of skewness and the Z-statistic of kurtosis. Z skewness is obtained by dividing the skewness by the standard error of skewness, and Z kurtosis is also obtained in the same way. It should be noted that the values of these two Z values should be in the range of -1.96 to +1.96 in order to determine the normality of the variables.

Table 1: Crookedness and Elongation of variables

Statistics					
		Effects.on.muscle	Physical effects	doping	motivation
number	a valid	103	103	103	103
	missing	0	0	0	0
crookedness		-.553	-.671	.500	.623
Standard error of the square		.241	.241	.241	.241
Elongation		-.586	-.891	-1.055	1.344
standard error of elongation		.478	.478	.478	.478

Considering that all the statistics of the variables are between -1.96 and +1.96;

Therefore, all the variables are normal and we use factor variance analysis test to test the variables.

Table 2: table of Friedman's statistical test

Test statistics	
Number	103
The amount of df	3
Significance level	.000
Friedman test	

Table 2: is related to statistical significance. The chi square value obtained in this test is equal to 134.241, which is at an error level of less than 0.05. Meanwhile, the significance of the test itself is equal to 0.000, which indicates that there is a relationship. The significance of Friedman's test means that the ranking of the criteria related to the research is meaningful from the point of view of the statistical community and the skiing athletes have different rankings of the variables of this research.

According to the statistical analysis, we find that all the selected variables in this research, which include (effects on muscles, physical effects, doping and motivation), have a significant effect on the consumption of sports supplements by athletes. Also, considering that the selected sample is among the first to tenth champions of the skiing field, most of these people have an acceptable quality of sleep without any particular problem. And in the section related to the general mental health questionnaire, we found out that according to the successes of these people, all of them have high general mental health.

The variables of this research include effects on muscle, physical effects, doping and motivation. According to the results of this research, the significance level of each of these variables should be less than 0.05. that this problem is observed, the variables of physical effects, doping and motivation are less than 0.05, so it should be noted that the variables of physical effects, doping and motivation also have an effect on the consumption of sports supplements.

Also, according to the Friedman test, the independent variables of this research were ranked as follows: Effects on muscle (3.21), physical effects (3.14), doping (1.93) and motivation (1.73)

4. Discussion

In this research, it should be determined that all the people of the statistical community start using supplements according to their needs and the recommendations of bodybuilding trainers and nutritionists. In this research, it was revealed that 52% of respondents use protein powders, 48% of amino acids, 57% of vitamin supplements, 61% of athletes use creatine, 77% of glutamine and 31% of testosterone during their sports activities. have used In addition, it was found that the consumption of amino acids and creatine is higher among men and protein powders among women. From a total of 103 people in the research sample, it was found that 19 of the sample athletes use sports supplements before starting training, 10 people (9%) of the respondents to the questionnaire use sports supplements during training. and 74 athletes take sports supplements after training. In addition, 20% of the respondents (20 people) less than 5 grams, 52% (53 people) between 5 and 10 grams, 21% (22 people) between 10 and 20 grams and 7% of the sample athletes also more They use 20 grams of sports supplements. Also, it is clear in this research that most of the amino acids are consumed by the athletes before starting the training and protein powders, creatine and glutamine are used by the athletes to increase the efficiency of the muscles and accelerate the recovery of the muscles after training. becomes Vitamin supplements are also used more during training than other supplements to increase the body's energy (18).

Considering that the people in the sample are national champions, they mostly consume sports supplements according to the programs provided to them by the official bodybuilding coach and nutrition and drug experts. According to the extracted information, it is determined that 49% of the athletes who responded to the questionnaire use the program provided to them by the official bodybuilding trainers, and 43% of these respondents seek the help of a nutritionist and medicine specialist. And only 7% of people use sports supplements arbitrarily or with the guidance of other people. According to the research data, it is determined that 40 people (40 percent) of the statistical sample use sports supplements due to the promotion and improvement of sports performance and 33 people (33 percent) use sports supplements due to the increase in strength and muscle mass. The total is 77% of the entire society and this shows that athletes and especially skiing champions use sports supplements for the reason of improving their sports performance and increasing their strength.

Also, according to the information provided, it is determined that 15 people, equivalent to 15% of the sample, use supplements to speed up the body's recovery and delay fatigue. Also, about 12 people (12 percent) use these supplements for the following reasons:1) meeting metabolic needs (preserving health). 2) Preventing oxidative destruction of muscles. 3) strengthening the immune system. According to these materials, it is clear that all the sample people use different sports supplements in some way and most of this use is aimed at increasing their strength and improving their sports performance in skiing.

Conclusion

It seems that the variables of this research include effects on muscle, physical effects, doping and motivation. The test results showed that the importance and ranking of these criteria are different among athletes. The comparison of the average ranks shows that the most important variable influencing why sports supplements are used among ski athletes was the positive effects on the muscles and improving their performance. The third and fourth are ranked for doping and motivation, respectively.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the present research is the result of the findings of Mosab Master's thesis of Islamic Azad University, Tehran East Branch.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: A.K., A.I.; Methodology: A.K., A.I.; Software: A.K., A.I.; Validation: A.K., A.I.; Formal analysis: A.K., A.I.; Investigation: A.K., A.I.; Resources: A.K., A.I.; Data curation: A.K., A.I.; Writing - original draft: A.K., A.I.; Writing - review & editing: A.K., A.I.; Visualization: A.K., A.I.; Supervision: A.K., A.I.; Project administration: A.K., A.I.; Funding acquisition: A.K., A.I.

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