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Research Article

The Effect of Aerobic Training and Octopamine Supplement on Gene Expression Levels of VEGF and PDGFR in the Heart Tissue of Rats Exposed to Deep Fried Oil

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Abstract

Background: Deep-fried oils (DFO) produce toxins that endanger people's health. Using herbal supplements along with exercise training can help improve health. The purpose of this study was to investigate the effect of aerobic training (T) and octopamine (O) supplementation on VEGF and PDGFR gene expression levels in the heart tissue of rats poisoned with DFO.

Materials and Methods: In this experimental study, 25 male Wistar rats were purchased and placed in 5 groups of 5 animals, including: 1) control, 2) DFO, 3) DFO+T, 4) DFO+O and 5) DFO+T+O.

Over a course of 4 weeks, rats in groups 2 to 5 received DFO by gavage, and rats in groups 3 and 5 ran on the treadmill 5 times a week with moderate intensity; also, rats in groups 4 and 5 received 81 µmol/kg octopamine supplement intraperitoneally 5 days a week. The Kolmogorov-Smirnov statistical test, independent samples t-test and two-way analysis of variance were used to analyze the research findings ($p<0.05$).

Results: DFO had a significant effect on increasing the gene expression levels of VEGF ($p=0.001$) and PDGFR ($p=0.002$); training had a significant effect on the reduction of VEGF ($p=0.001$) and PDGFR ($p=0.012$); also, octopamine consumption had a significant effect on the reduction of VEGF ($p=0.001$) and PDGFR ($p=0.045$).

Conclusion: It seems that aerobic training and octopamine consumption alone have anti-inflammatory effects in the heart tissue of DFO-poisoned rats, however they do not have significant anti-inflammatory interactive effects.

Keywords:
Deep Fried Oils, Aerobic Training, Octopamine, VEGF, PDGFR.

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Journal of Sports Physiology and Athletic Conditioning

1. Introduction

Cardiovascular disease (CVD) is the most common cause of death in most countries of the world, including Iran, and is the most important cause of disability as well. In most cases, premature coronary artery disease is directly related to the number and severity of atherosclerosis risk factors. Heart failure is one of the most common heart diseases, which is considered as a clinical syndrome in which an abnormality in the structure or function of the heart causes its inability to empty or fill with blood at a rate to meet the metabolic needs of the body (1).

Today, deep frying is a general cooking method in which fat is used as a heat transfer medium and during which foods with unique characteristics in terms of taste, texture and appearance are produced (2). In this method, during the frying process, simultaneously with the dislocation and transfer of temperature, the transfer of oil into the product and the exit of water from it can be envisaged (3). One of the compounds formed during high heating of foods, especially foods with high fat, is acrolein. Propenal-1 or acrolein is an unsaturated aldehyde electrophile belonging to the group of α and β aldehydes (4). The toxins produced during the fraying process cause the production of free radicals. The production of free radicals causes the initiation of inflammatory pathways related to cell death in vessels and the reduction of angiogenesis (5).

A set of factors such as increased blood flow and hemodynamic forces, increased shear stress, muscle contraction, hypoxia and some cytokines are angiogenic stimulants that cause the release of angiogenic factors and as a result angiogenesis (5).

Several angiogenic factors have been identified so far, but much attention has been paid to vascular endothelial growth factor (VEGF) and plate-derived growth factor (PDGF) (6). VEGF, as the most important angiogenic factor, causes proliferation of endothelial cells, migration and cell differentiation (6). Also, platelet-derived growth factor (PDGF) plays a role in collagen synthesis, production of extracellular matrix components, and contraction, and is also an angiogenesis regulatory factor (7). Recently, to reduce the effects of apoptosis, the role of exercise training in interaction with supplementation has been investigated. Today, herbal supplements are considered as one of the most effective supplements available.

Among these, we can refer to the antioxidant effects of octopamine supplement (8). Citrus fruit extracts, including sour orange, are traditionally used as weight loss and appetite suppressant products and sometimes as a food ingredient, but mostly as a medicinal or dietary supplement (9). One of the components of these extracts is octopamine, which mimics the sympathetic function and is considered an adrenergic substance. The effects of octopamine include antioxidant and anti-inflammatory effects as well as weight loss and fat burning effects (10). The difference in adrenergic receptor is one of the factors that causes the difference in the pharmacological effects when comparing octopamine with other biogenic amines such as norepinephrine and ephedrine (11).

The active ingredients in octopamine include various alkaloids with adrenergic activity, including synephrine. Based on the evidence, this substance affects the adrenaline system of the body, increases the rate of basic metabolism and rises the metabolism of the body to a great extent. Having the ability to generate heat is another characteristic of octopamine (12).

Several studies have shown that increasing physical activity as well as using antioxidant supplements is one of the ways to prevent cardiovascular diseases caused by nutritional errors (13).

It has been reported that regular aerobic physical activity with low to moderate intensity improves the physiological function of skeletal and cardiac muscles, reduces the incidence of a wide range of diseases, including cardiovascular diseases, and improves inflammatory factors (14). On the other hand, performing exercise training individually can offset the negative effects of fatty tissue, and also make brown fat tissue more efficient (15).

Recent evidence shows that exercise leads to major adaptations in adipose tissue, especially turning white fat into brown and making brown adipose tissue more efficient, which plays an important role in the metabolic effects of exercise on health (15).

Due to the importance and criticality of damage to the vital organs of the human body induced by fast foods and deep fried oils, as well as the anti-inflammatory effects of exercise training and octopamine supplementation, the present study was conducted to investigate the effect of aerobic training and octopamine supplementation on VEGF and PDGFR gene expression levels in the heart tissue of rats poisoned with deep-fried oils.

2. Materials and Methods

Animals selection

In this experimental study, 25 male Wistar rats, approximately 20 weeks old and with a weight range of 300 to 350 grams, were purchased from the Histogen Research Center and transferred to the laboratory and were kept under standard conditions for one week in order to adapt to the laboratory environment.

Then, on the eighth day, according to body weight, they were placed in 5 groups of 5 animals, including: 1) control, 2) DFO, 3) DFO+ T, 4) DFO + O and 5) DFO + T + O. Over a course of 4 weeks, rats in groups 2 to 5 received DFO by gavage; also, rats in groups 3 and 5 ran on a treadmill with moderate intensity 5 times a week, and rats in groups 4 and 5 received 81µmol/kg O supplement intraperitoneally for 5 days a week.

Exercise protocol

The aerobic training protocol was performed with moderate intensity so that the rats ran at a speed of 16 m/min (50% Vo_{2max}) in the first week, and the running speed reached 26 m/min (65% Vo_{2max}) in the last week. The duration of running in the whole research period was 20 minutes.

It is worth noting that in order to acclimate the rats to running on the treadmill, before starting the main training program, the rats ran at a speed of 9 m/min for 20 minutes for one week. Also, before starting each training session, they warmed up for 5 minutes at a speed of 7 m/min and cooled down for 5 minutes at a speed of 5 m/min after the end of the main training (16).

Supplement preparation and consumption

In the present study, octopamine supplement was prepared from Sigma Aldrich Co. Rats in groups 4 and 5 received 81 µmol/kg octopamine supplement peritoneally 5 days a week.

It is worth mentioning that in order to prepare octopamine for injection, it was dissolved in 9% normal saline solution (17). Based on previous studies, 8 liters of sunflower oil were used to prepare DFO. The oil was heated for 4 consecutive days, 8 hours a day at a temperature of 190 to 200° C, and depending on the available sources, food items (chicken nuggets, potatoes, chicken and protein products such as sausages and salami) were immersed in the frying oil every 30 minutes, and finally, on the fourth day, the oil was fed to rats by gavage (18).

Blood sampling and laboratory analysis

48 hours after the last training session and O supplement injection, rats were anesthetized by chloroform inhalation (15). In order to measure VEGF and PDGFR gene expression levels, the heart tissue of rats was extracted and placed inside special microtubes.

The microtubes were transferred into a nitrogen tank and kept in a -80° C freezer until cell analysis. The desired factors were measured by real-time PCR. The sequence of primers is presented in Table 1.

Table 1: Sequence of primers used in the present study

Gene	Forward (5'-3')	Reverse (5'-3')
PDGFR	*ACAGCACAGGGGTAGAAGAGTTG*	*GAGGATGGTTTTGGAGTGAGGAGG*
VEGF	*TGT GTG TGT GAG TGG CTT*	*ACC GAG AAT ACT GAA AAA AAC CC*
Gap	*AAG TTC AAC GGC ACA GTC AAG G*	*CAT ACT CAG CAC CAG CAT CAC C*

Statistical analysis

The Kolmogorov-Smirnov statistical test, independent samples t-test and two-way analysis of variance were used to analyze the research findings (P<0.05).

3. Results

VEGF and PDGFR gene expression levels in the five research groups are presented in Figures 1 and 2. The results of independent samples t-test showed that the gene and protein expression levels of VEGF (p=0.005) and PDGFR (p=0.001) in the DFO group were significantly higher than the control group.

The results of two-way analysis of variance showed that the interaction of training and octopamine supplementation had no significant effect on VEGF gene expression levels, but training had a significant effect on VEGF gene expression levels (F=32.28, P=0.001, η=0.617). Octopamine supplementation also had a significant effect on VEGF gene expression levels (F=12.61, P=0.002, η=0.387). Besides, training (F=16.56, P=0.001, η =0.453) and octopamine supplementation (F=4.57, P=0.045, η =0.186) had a significant effect on the reduction of PDGFR. However, the interaction of training and octopamine supplementation had no significant effect on VEGF gene expression levels (F=0.236, P=0.632, η=0.012).

The results of two-way analysis of variance test showed that training (F=6.83, P=0.01, μ=0.29) and octopamine supplementation (F=17.18, P=0.001, μ=0.51) had a significant effect on the reduction of NFkB, however the effect of training and octopamine supplementation was not significant in the reduction of NFkB (F=0.33, P=0.57, μ=0.02). Also, training (F=6.68, P=0.007, μ=0.37) and octopamine supplementation (F=20.08, P=0.001, μ=0.55) had a significant effect on the reduction of TNF-α, however the effect of training and octopamine supplementation on the reduction of TNF-α α was not significant (F=0.41, P=0.20, μ=0.02).

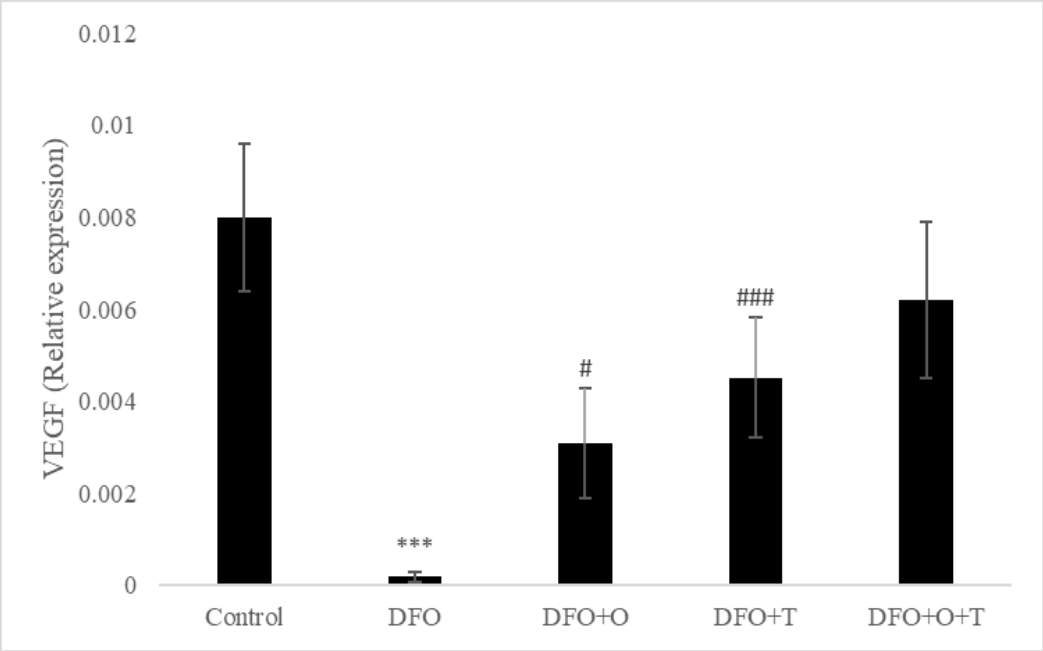

Figure 1: VEGF gene expression levels in the five research groups

*Significant reduction compared to the control group

#and ### Significant effect on the reduction of VEGF compared to the DFO group

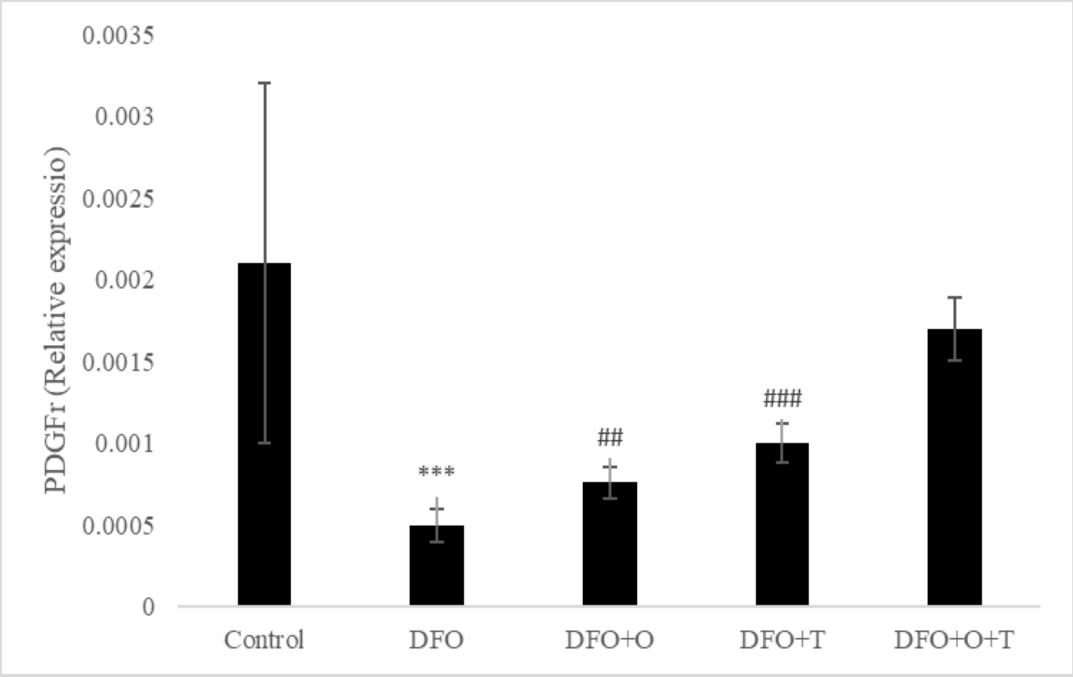

Figure 2: PDGFR gene expression levels in the five research groups

*Significant reduction compared to the control group

#and ### Significant effect on the reduction of PDGFR compared to the DFO group

4. Discussion

The results of the current research indicated that there was a significant difference in VEGF and PDGFr gene expression levels between the healthy control group and those poisoned with deep-fried oil, and as a result of poisoning with deep-heated oil, VEGF and PDGFr gene expression levels significantly decreased.

In line with the present study, many studies have been conducted regarding the effect of deep-fried oils on the vascular endothelial growth factors, which have reported similar results. For example, Zhao et al. conducted a study on the effect of four weeks of aerobic training and octepamine on the levels of malondialdehyde and caspase-3 in the brown fat tissue of male rats fed with deep-fried oils. The findings of their study showed that the concentration of malondialdehyde increased significantly as a result of poisoning with deep fried oil.

Also, the results of the above study showed that training and octopamine supplementation had a compensatory effect on the rats that were induced by poisoning, also these interventions had a significant effect on increasing VEGF and PDGFr levels in the rats poisoned with deep-fried oils; thus, it confirms the hypothesis that training and octopamine supplementation can reduce the negative effect of fried oil on the heart tissue of poisoned rats (19).

In a study, Zarezadeh Mehrizi et al investigated the effect of eight weeks of aerobic training on the gene expression of hypoxia-inducible factor-1 (HIF-1α), vascular endothelial growth factor (VEGF) and angiostatin in the hippocampus of male Wistar rats. The result of their study showed that aerobic training caused a significant increase in the levels of HIF-1α and VEGF.

Given the changes made in the levels of HIF-1α and VEGF, aerobic training had beneficial effects on the function of the hippocampus region of the brain. The results of this study are in line with the present study. This consistency may be due to the implementation of similar feeding, maintenance and aerobic training conditions in the two above-mentioned studies (20).

In another study, Wang investigated the effect of exercise training on age-related changes in improving cerebral blood flow and capillary vessels through the re-regulation of VEGF and eNOS. The results of this study indicated that exercise can improve vascular changes caused by aging and decreased perfusion, which is associated with the re-regulation of VEGF and eNOS.

The findings of this research showed that the effective mechanisms of exercise training on the changes in cerebrovascular vessels caused by aging included the re-regulation of VEGF and eNOS gene expression in connection with the change in the oxidant and antioxidant balance, which is in line with the present study (21).

The present study showed that octopamine supplementation had a significant effect on VEGF and PDGFr gene expression levels. In line with this research, Shokri et al. investigated the effect of aerobic exercise and octopamine supplementation on the angiogenesis of visceral fat tissue of rats fed with fried oil and concluded that consumption of DFO significantly increased visceral fat, while octopamine supplementation significantly increased VEGF. This agreement can be due to similar dosage of supplementation and implementation of similar procedures in keeping and feeding the rats (22).

Another result of the present study was that aerobic training and octopamine supplementation alone had a significant effect on VEGF and PDGFr gene expression, but the interaction of aerobic training and octopamine supplementation had no significant effect on the heart tissue of rats poisoned with deep-fried oil.

Also, the results of this study showed that exercise training and supplementation had a compensatory effect on the rats induced by poisoning, and these interventions had a significant effect on increasing VEGF and PDGFr levels in rats poisoned with deep-fried oils; it confirms the hypothesis that exercise training and octopamine supplementation can reduce the negative effect of fried oil on the heart tissue of poisoned rats (23).

Many studies have been carried out in line with the present study regarding the effect of aerobic exercise and octopamine supplementation on VEGF and PDGFr gene expression levels. For example, Shokri et al. in a study investigated the effect of aerobic exercise and octopamine supplementation on the angiogenesis of rat visceral adipose tissue with fried oil and concluded that DFO intake significantly increased visceral fat, while it significantly decreased VEGF. Aerobic exercise significantly increased HIF-1 and VEGF levels, while octopamine decreased HIF-1 and VEGF levels. On the other hand, octopamine along with aerobic exercise had no significant interactive effect on HIF-1 and VEGF levels. Octopamine and aerobic exercise appear to improve the process of visceral adipose tissue angiogenesis, which is impaired by DFO, and hence reduce the damage caused by DFO feeding (22).

Conclusion

The results of the present study showed that deep-fried oils lead to an increase in VEGF and PDGFR gene expression levels in the heart tissue of rats poisoned with deep-fried oil, however training and octopamine supplementation alone lead to a decrease in VEGF and PDGFR gene expression levels in the heart tissue of rats poisoned with deep-fried oil.

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Funding

This study did not have any funds.

Compliance with ethical standards

Conflict of interest None declared.

Ethical approval The present study followed the ethical principles of working with laboratory animals in accordance with international laws and according to the rules of the Ethical Committee of Laboratory Animals of Islamic Azad University.

Informed consent Informed consent was obtained from all participants.

Author contributions

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

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Research Article

The effect of aerobic training and curcumin supplementation on the expression of IGF-1 gene in muscle rat

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Abstract

Background: The purpose of this research was to investigate the effect of four weeks of moderate aerobic training and nanocurcumin supplementation on IGF-١ gene expression and its receptor in the soleus muscle of rats.

Materials and Methods: 32 male Wistar rats, approximately eight weeks old, with a weight range of 200±10 grams, were prepared and kept for one week under standard conditions of light, temperature, and humidity. Rats were randomly divided into 4 groups of 8 series: healthy control group, aerobic training group, nanocurcumin group and aerobic training + nanocurcumin group. The training protocol was for 4 weeks, 5 days a week, in the first week, 20 minutes of activity with a speed of 18 meters per minute, and finally in the 4th week, it reached 35 minutes of activity with a speed of 20 meters per minute. Nanocurcumin supplement was also used for each animal at 80 mg / kilogram of body weight. One-way analysis of variance and Bonferroni's post hoc test was used at the significance level ($P<0.05$).

Results: The results showed an increase in IGF-1 and IGF-1R gene expression levels in the soleus muscle in all groups compared to the control group.

Conclusion: The combination of training and nanocurcumin supplementation probably increases the antioxidant capacity of the body and causes a greater increase in the expression of the IGF-1 gene and IGF-1R in skeletal muscles, which indicates more anabolic effects.

Keywords:
IGF-1R, IGF-1, curcumin

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Journal of Sports Physiology and Athletic Conditioning

1. Introduction

Inactivity due to industrial life or injury can be associated with an increase in the loss of muscle mass and increase the level of disability in people. Two important factors in improving body composition are daily sports activity and nutritional interventions. The best and biggest changes in the body happen when proper nutrition programs are implemented at the same time as regular sports activities (1). Improving body composition, which means increasing muscle mass and reducing unnecessary fat mass, is one of the most important elements of physical fitness, which improves sports performance and prevents people from suffering from diseases related to chronic inflammation caused by obesity and overweight (2). The production of free radicals and oxidants is an inevitable necessity of running sports programs, and many sports adaptations are the result of the release of these destructive molecules that make the body stronger against them (3). All kinds of sports activities increase the secretion of insulin-like growth factor (IGF-1) and increase the size of muscles as well as the strength and power of skeletal muscles. Insulin-like growth factor-1 is one of the best growth factors and has many beneficial results of physical activity. One of the most important functions of IGF-1 is regulating protein synthesis in skeletal muscles and promoting body growth (4). IGF-1 after binding to the IGF-1R receptor, phosphorylates an insulin receptor substrate intracellular adapter protein (IRS-1), which phosphorylates Phosphoinositide 3-kinase (PI3K), followed by Akt phosphorylation (5). Mammalian target of rapamycin (mTOR) is a target Downstream of Akt, mTOR activity is closely related to anabolic/catabolic balance. The IGF-1/Akt/mTOR pathway is essential in promoting muscle hypertrophy (6).

Skeletal muscle cell size is determined by the balance between the synthesis of new proteins and the degradation of old proteins. Under physiological conditions, the rate of protein synthesis and degradation is balanced and myofiber size is maintained. Under physiological conditions, the rate of protein synthesis and degradation is balanced and myofiber size is maintained. In the condition of weight loss, on the contrary, myofiber protein degradation is accelerated and the rate of protein synthesis is suppressed, resulting in muscle weakness and fatigue (7). IGF-1 can regulate protein synthesis and degradation pathways (6). In a study conducted on young healthy subjects, high levels of circulating IGF-1 were negatively associated with body fat, body mass index, and total cholesterol, and positively associated with aerobic fitness and muscular endurance, In contrast, lower levels of IGF-1 were associated with various pathological conditions including chronic diseases, inflammation and malnutrition (3).

Recently, the complementary role of curcumin in the anti-atrophy effects of skeletal muscles has been shown. Taking this supplement by increasing the antioxidant capacity and reducing the oxidants leads to the development of muscle hypertrophy processes. This polyphenol has anti-inflammatory, antioxidant and anti-tumor effects and inhibits aging processes in skeletal muscles by directly removing ROS. Curcumin interacts with various molecular targets including cytokines, growth factors, proteins, enzymes and receptors (8). The results of a study showed that curcumin improved hypoglycemia in rats by increasing IGF-1 gene regulation and improving oxidative stress caused by diabetes (9).

Although curcumin has been investigated in various clinical conditions, studies to evaluate its effect on muscle changes caused by exercise training are rare.

Considering that so far, there is no study on the simultaneous role of curcumin and aerobic training with moderate intensity and has not investigated the changes in the expression of the IGF-1 gene and its receptor in skeletal muscle, and considering the existence of contradictions in the results of previous researches, in this study, the effect of moderate aerobic training and nanocurcumin supplementation on the expression of the IGF-1 gene and its receptor IGF- 1R was investigated in soleus muscle of rats.

2. Materials and Methods

In this experimental and fundamental research, 32 Wistar male rats, approximately eight weeks old and weighing 200±10 grams, were obtained from the Pasteur Laboratory Animal Breeding and Reproduction Center (Tehran, Iran) and then transferred to the Animal Sports Physiology Laboratory. Animals had free access to standard laboratory food and water. Rats were kept for one week under standard conditions in terms of proper ventilation and 12-hour dark and light period, temperature of 22 ± 3 degrees Celsius and humidity of 50 ± 3%. Then the rats were randomly divided into 4 groups of 8 series. Healthy control group, aerobic training group, nanocurcumin group, aerobic training + nanocurcumin group.

Estimation of maximum running speed

For the test to determine the maximum speed, the animals performed the warm-up program at a speed of 5 meters per minute for 5 minutes. Then they started the test with a speed of 9 meters per minute (5 minutes for each of the speeds). Next, the speed of the conveyor belt was increased by 2 meters per minute until the animal reached a stop. The criterion of the animal's disability was the inability to return to running on the treadmill within 10 seconds (10,11,12).

Exercise protocol

First, for one week, the rats were prepared to perform aerobic activities and familiarize themselves with a special treadmill. The training protocol for 4 weeks, 5 days a week, in the first week was 20 minutes of activity at a speed of 18 meters per minute and finally in the 4th week, 35 minutes of activity at a speed of 20 meters per minute (Table 1) (13).

Table 1: Aerobic training protocol

type of training	week	intensity	duration	Frequency
	1	18 m/min	20 min/day	5 day/week
	2	18 m/min	25 min/day	5 day/week
	3	20 m/min	30 min/day	5 day/week
	4	20 m/min	35 min/day	5 day/week

Nanocurcumin supplementation

Chitosan (500 mg) was dissolved in 2% acetic acid solution (50 mL) and mixed with curcumin in ethanol (1 mg/mL). 15 ml of 1% TPP solution was added drop by drop under constant magnetic stirring. Then the solution was stirred for 1 hour and centrifuged at 10,000 rpm for 30 minutes.The obtained pellet was resuspended in water and further lyophilized to obtain chitosan nanoparticles encapsulated in curcumin. The size and morphology of formed nanoparticles were analyzed using scanning electron microscope (SEM), particle size with Zeta sizer device and product stability with DLS device. Commercially manufactured nano curcumin by Exir Nano Sina Company (Tehran, Iran) was used as a comparative sample of product quality. After preparing the solution, 80 mg per kilogram of body weight was gavage for each animal (14).

Gene expression evaluation method

All stages of keeping and slaughtering rats were done according to the criteria of Pasteur Laboratory Animal Ethics Committee (Tehran, Iran). 48 hours after the end of the research and supplementation protocol, all rats were anesthetized by intraperitoneal injection of a combination of ketamine (70 mg/kg) and xylazine (3 mg/kg) and the soleus muscle was removed with a surgical kit by a specialist. It was taken out and after washing, it was frozen in a saline solution and stored in a refrigerator at -80. The expression of genes was measured by designing the primers of the studied genes whose sequence is in Table 2.

Table 2: Sequence of genes

	Gene	Sequence
1	IGF1	Forward: GGTAGGGTAGGTTGGAAATG
		Reverse: GCGAAGGTCTTGGTCACATC
2	IGF-1R	Forward: AAGTSGGTAATGGCATGAGA
		Reverse: GTTTGGTTTCCCACGGCTTC

Statistical analysis

In order to analyze the data, descriptive and inferential statistics were used. At the level of descriptive statistics, indicators such as mean, standard deviation, and in the inferential statistics section were used to analyze the data, one-way Anova and Bonferroni's post hoc test were used at the significance level ($P<0.05$).

3. Results

According to the results of the Kolmogorov-Smirnov test, the distribution of the data related to the research variables is normal. Moderate aerobic training and nano curcumin supplementation had a significant effect on IGF1 gene expression in rat muscle cells. The results of Bonferroni's post hoc test showed that there was a significant difference between the control group and the experimental groups and the training group and the nanocurcumin group with nanocurcumin + training group ($p=0.000$). The figure1 shows the comparison of IGF1 values.

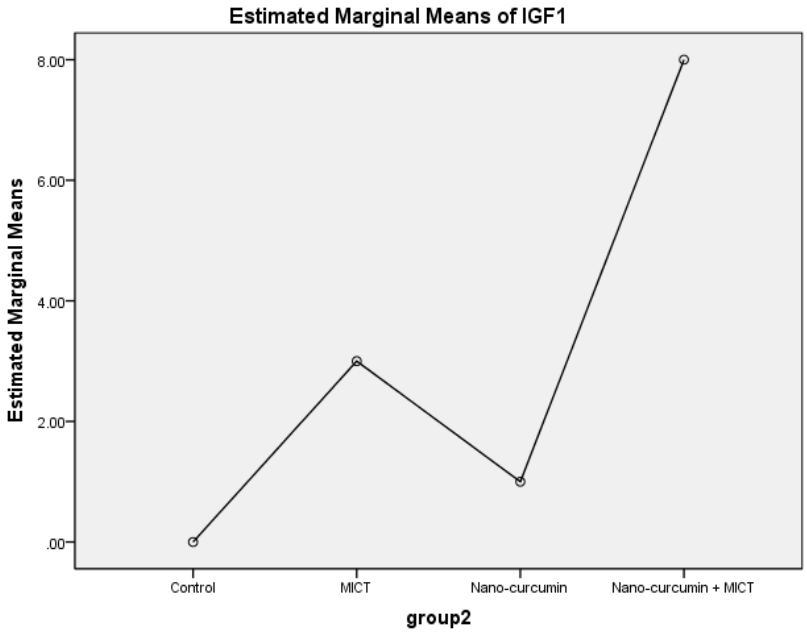

Figure 1: Comparison of IGF1 expression in four groups: control, training, nanocurcummin, nanocurcumin + training

The result of moderate aerobic traning and nano curcumin supplementation has a significant effect on the expression of the IGF-1R receptor gene in rat muscle cells.

The results of Bonferroni's post hoc test showed that there is a significant difference between the control group and all the groups. The figure2 shows the comparison of IGF-1R values.

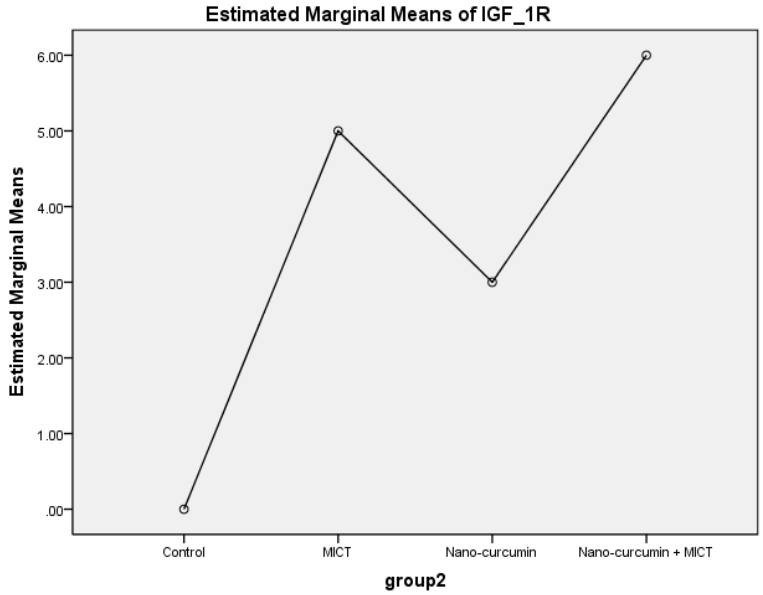

Figure 2: Comparison of IGF-1R expression in four groups: control, training, nanocurcumin, nanocurcumin + training

4. Discussion

In the present research, the IGF-1 gene expression values of soleus muscle increased in all groups compared to the control group

Sports training and nanocurcumin supplement alone are a suitable and sufficient intervention to increase muscle IGF-1 gene expression. In the case of lack of training, the level of IGF-1 gene expression in the soleus muscle will decrease, which can be prevented to a significant extent only by taking nanocurcumin supplements, and as a result, muscle wasting can be prevented (15). There is evidence that curcumin increases the serum activities of antioxidants such as superoxide dismutase (SOD). Curcumin can destroy various forms of free radicals such as reactive oxygen and nitrogen species (ROS and RNS, respectively) and regulate the activity of GSH, catalase and SOD enzymes in neutralizing free radicals. Also, it can inhibit ROS-producing enzymes such as lipoxygenase/cyclooxygenase and xanthine hydrogenase/oxidase (15). In this research, it seems that nanocurcumin supplementation alone has prevented the reduction of IGF-۱ by developing the antioxidant capacity of the body and especially skeletal muscles، caused by injuries or skeletal problems, it has useful and significant effects that should be further investigated in other researches. When nanocurcumin supplementation was combined with moderate aerobic training, IGF-1 gene expression reached the highest level and showed an increase of 8.17 times compared to the control group.

Although all kinds of sports activities can increase the expression levels of the growth hormone gene as well as IGF-1, studies have shown that strength training increases the levels of IGF-1 more than other sports training (16).

It seems that due to the high production of oxidants, aerobic training moderates IGF-1 gene expression to some extent compared to resistance training. For this reason, resistance training creates a greater increase in muscle mass. Maybe combining an effective substance in reducing oxides and free radicals along with sports training with the development of antioxidant defense will reduce the modulatory effects of IGF-1, which seems to have played such a role in the present research. In the present study, the increase in IGF-1R gene expression in the soleus muscle of Wistar rats was significantly increased in all three intervention groups compared to the control group. All of the effects of IGF-1 are induced by binding to its specific cell surface receptor, the IGF-1R. If the IGF-1 receptor is not expressed or upregulated, none of the anti-apoptotic or anti-proteolysis pathways will be established and the breakdown of cellular proteins will begin (17). The amount of proteins in the body is the result of a homeostasis between the analytical and anabolic regulatory genes, and one of the most important and key pathways is IGF-1, which without its receptor will not have any effect on the cell and subsequent processes. . So binding of IGF-1 to its receptor is one of the most necessary and important cellular pathways to maintain muscle mass (17).

In chronic diseases such as metabolic syndrome or diabetes mellitus, the IGF-1 receptor expression levels decrease on the cell surface, which in the long run leads to the loss of a large amount of muscle tissue in sick people. Sports activity of any kind has the ability to increase the expression of IGF-1R on the cell surface. In fact, the increase of cellular receptors is one of the most important effects of training, especially aerobic training (18).

Conclusion

The obtained results indicate that aerobic training and nanocurcumin consumption alone increased the expression of IGF-1R and IGF-1 genes in muscle, but the combination of training and nanocurcumin supplementation probably caused a greater increase in IGF-1R and IGF-1 genes expression by increasing the body's antioxidant capacity, which indicates more anabolic effects. According to the positive effect of aerobic training and the consumption of nano curcumin in the subjects of this research and based on the similar results of past human research, it is suggested to implement the aerobic training method used in this research to increase the anabolic power and for synergistic effects, nano curcumin supplement be consumed in the amounts used in this research.

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

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Research Article

The Effect of Tapering Period with and without Creatine Supplementation on Hormonal Responses of male football Players

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Abstract

Background: The aim of this study was to examine the effects of a tapering period with creatine supplementation on hormonal responses of men football players.

Materials and Methods: The study sample included 18 male soccer players of the first division league (mean age: 18.77 ± 1.26 years, height: 174.5± 5.77 cm, and weight: 64.07± 6.99 kg) that were in the specific conditioning period who were randomly divided into two equal groups: Taper alone (n=9) and Taper with creatine supplementation (n=9). The levels of blood hormonal parameters including testosterone, cortisol and testosterone to cortisol ratio (T/C) were measured before and after the tapering period (10 days). Paired t-test and independent T-test was used to examine the differences within and between groups, respectively.

Results: The results showed that the cortisol levels in the post-test compared to the pre-test decreased significantly in both groups ($p \leq 0.05$) ; While, there was no significant difference in testosterone and T/C ($p \geq 0.05$). Also, no significant difference was observed in the levels of testosterone, cortisol and T/C between the two groups.

Conclusion: The taper period can reduce the cortisol levels of male soccer players, but creatine consumption has no significant effect on the levels of cortisol, testosterone and T/C ratio in the taper period.

Keywords:
Taper, Anabolic hormones, Katabolic hormones, Elite soccer players.

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1. Introduction

The efforts of athletes, coaches, and sports science experts have always been to identify the facilitating and limiting factors of sports performances (1). Football players cover a distance of about 10 kilometers with 70 to 80% of maximum oxygen consumption. Long-term activity with this intensity is dependent on glycogen and metabolic substrates. In a soccer match, the reliance on each energy system is constantly changing. Getting the desired training balance is complex and depends on special knowledge about the metabolism and physiological changes of the athletes of that sport (2,3).

The levels of testosterone and cortisol in the plasma indicates the anabolic and catabolic reactions of the tissues. Also, the ratio of testosterone to cortisol is used as an indicator of training load (4). The increase of some hormonal indicators during special football activities is one of the training adaptations; Also, overreaching or overtraining also shows similar compensatory adaptations (5). 10-30% of professional players suffer from overtraining syndrome at the end of the competition season, which affecting the hypothalamus-pituitary-adrenal (HPA) axis is one of the most important effects of this syndrome (6). On the other hand, a rest period at the end of the competition season is common in all sports fields, in order to prevent proteolysis and a decrease in the performance of physiological indicators (hormones, immune system, etc.) caused by overtraining (7). Appropriate and correct adjustment of sports exercises requires deep sciences related to sports performance, including physiology, psychology, nutrition and technical aspects (8).

One of the key elements of the athletes' physical preparation is the period of gradually reducing the training load in the weeks before the start of the competition, which is commonly known as Tapering. The taper period prevents accumulated fatigue caused by long periods of training without reducing training adaptations and helps to maintain the athlete's fitness and performance. Decreasing training load requires a careful plan, which is often used synonymously with maximizing performance. In general, a taper period is defined as a period of several days to several weeks in which the volume of training is gradually reduced while the intensity of training is maintained relatively. However, the combination of volume and intensity applied during the taper period depends on the type of sport and the desired adaptations for a successful sports competition (9).

Most of the conducted studies have investigated the effect of the taper period in individual sports (mainly endurance sports) such as running, swimming, cycling, rowing and triathlon (10,11). While there is little information about the effect of reducing the training load in team sports including football.

However, professional soccer players who participate in constant club competition and international tournaments seem to have little time for tipper, which is likely to impair their performance - it depends on a combination of physical, physiological, psychological, tactical, and technical factors - in important competitions (12).

A large number of athletes use energizing nutritional supplements to improve the quality and quantity of training and actually maintain their performance in competitive conditions. It is possible that additional nutrients may be necessary for athletes during intense training (13). In fact, under certain circumstances, energizing supplements can have positive effects on athletic performance, body composition, and strength (14). Due to the many demands of football, including shuttle, fast and explosive movements and jumps, which are often performed with short recovery periods during 90 minutes of competition (15), football players can take energizing nutritional supplements including creatine to benefit. Creatine supplementation increases the amount of muscle phosphocreatine at rest, which can be effective as an immediate phosphate transporter for ATP regeneration during activity. Increasing creatine in athletes allows access to a higher training load, reduces training fatigue, and improves performance (16). It has been shown that a short period of creatine consumption (5-7 days) increases total creatine of muscle by 20-50% .Also, creatine can increase the synthesis or decrease the breakdown of proteins (16). The coordinated metabolic function between dietary supplements such as creatine and physiological factors can play an important role in strengthening hormonal reactions and improving sports performance (17). However, few studies have been conducted on the effect of creatine supplementation on the changes in the reactions of anabolic hormones, and their results are not consistent with each other.

Considering the physical demands of football and considering the common beneficial effects of reducing the training load and short-term creatine supplementation on hormonal adaptations to improve sports performance, it is worth investigating whether the use of both of these strategies in football players, does it have an effect on the performance of the anabolic-catabolic hormone index and the positive balance of these hormones or not?

On the other hand, it is possible that the use of supplements and nutritional considerations can have a positive effect on the balance of anabolic-catabolic hormones during periods of reduced training load, which requires more extensive research (18). Therefore, considering the importance and position of football, as well as the role of the tapering period and nutritional considerations on the physiological and functional indicators of athletes, such as the effects of anabolic-catabolic hormones, especially testosterone and cortisol, on the mental-functional changes of athletes, the aim of this study was to compare the effect of a tapering period with and without creatine supplementation on the hormonal responses of football players.

2. Materials and Methods

The participants of this study were 18 football players of the Premier League of Tehran province province (average age: 18.77 ± 26.1 years, weight: 64.07 ± 99.6 kg, and height: 174.50 ± 77.5 cm), who were selected through purposeful sampling and randomly divided into two experimental groups (G1: taper with creatine supplement, G2: taper alone). First, 18 football players working in the Tehran Premier League, who were in a special training season and had the conditions to enter the study, were randomly divided into two equal groups. At first, all research steps and possible benefits and consequences were explained to the participants, and those interested in participating in the research completed a written consent form.

The load reduction period was performed after the completion of a 4-week conditioning period for these players. In this course, the researcher, in coordination with the team's coaches, considered the amount and intensity of the players' physical, technical, and tactical exercises almost identically. However, due to the limitations in controlling the volume and intensity of team athletes, there was a possibility of a slight increase in the intensity or volume of some players. Twenty-four hours after the end of the last session of the special conditioning period, and 24 hours after the end of the tapering period, in order to measure dependent variables, venous blood samples were taken from the participants after 8-12 hours of fasting. At each stage, 5 cc of blood were taken from the internal cubital vein of the participants and immediately transferred to a specialized laboratory for analysis. Testosterone and cortisol levels were measured before and after the tapering period by enzyme-linked immunosorbent assay (ELISA).

Intervention

Nutritional supplementation

Subjects were randomly divided into two experimental groups, include: 1) G1: Tapering period with creatine supplementation(N=9), G2: tapering alone(N=9). The participants of group one received 10 grams of creatine supplement twice a day during the tapering period for 10 days. The second group only did the tapering program without creatine supplementation. The participants of both groups maintained their diet and physical activity routine during the study. And they were asked not to have any physical activity and exercise beyond the designed protocol.

Conditioning and Tapering period

The tipper period, which was applied at the end of the special preparation period and before the start of the competition, included reducing the frequency of training (from 5 sessions to 4 sessions per week), reducing the volume of training (from 90 minutes to 60 minutes per session while maintaining the intensity of the training). The amount of training load changes was considered based on the recommended index of reducing training load in team athletes (1). The conditioning training period (befor tapering period) included the following parts: 1) Fifteen minutes of warm-up, 2) Ten minutes of individual technical movements with the ball, 3) Twenty minutes of high-intensity group tactical training, 4) Fifteen minutes of speed training and plyometrics and 5) Playing football for twenty minutes and finally 6) Cooling down for ten minutes. The exercises of the tapering period included: 1) Ten minutes of warm-up, 2) Ten minutes of group tactical exercises with high intensity, 3) Ten minutes of speed and plyometric exercises, 4) Twenty minutes of playing football and 5) Ten minutes of cooling down.

Statistical analysis

The Kolmogorov Smirnov test was used to evaluate the normality of the data distribution, and the Levine's test of homogeneity of variance were used to establish the homogeneity of variance between groups. Dependent t-test was used to examine within-group differences before and after the test, and independent t-test was used to examine between-group differences. Data were analyzed using SPSS software version 26. Significance level was considered to be equal or less than 0.05.

3. Results

The participant demographic are presented in Table 1. Therefore, within-group changes of testosterone and cortisol serum levels and T/C ratio are presented in Table 2. All groups reported homogeneous variance (P > .05). Also, at the beginning of the study no significant differences were observed between the groups in levels of cortisol, testosterone and T/C ratio (P<0.05).

The results of this study showed a significant decrease in the level of cortisol hormone after the post-test compared to the pre-test in both groups (Taper group: P=0.02; and Taper with creatine group: P=0.04); While there were no significant changes in the levels of testosterone and the testosterone to cortisol ratio (T/C) (Taper group: P=0.3, P=0.07, Taper with creatine group: P=0.09, P=0.06). Also, no significant difference was observed in the levels of testosterone, cortisol and T/C between the two groups(P≥0.05).

4. Discussion

The aim of this study was to investigate the effect of creatine supplementation during the tapering period on anabolic and catabolic hormones. The results showed that reducing the training load during the tapering period with and without creatine supplementation leads to a decrease in serum cortisol levels and has no effect on testosterone level and T/C ratio (Figures 1,2,3). Therefore, reducing the training load during the tipping period leads to improved performance due to the reduction of training stress and improvement of recovery before the competition.

Tapering is a way to relieve the fatigue caused by long training periods without reducing training adaptations. Reducing the volume of training while maintaining the intensity of training in a period of several days to several weeks is called tapering. The volume and intensity of the exercises in the tapering period depends on the type of sports (19). Studies have shown that a 7-21 day tapering period improves performance. Among the factors that play a role in this case, hematological, biochemical, and hormonal factors, immune system, and mental-psychological factors are mentioned (2,3,7,8). The levels of testosterone and cortisol in the plasma indicates the anabolic and catabolic reactions of tissues. The results showed the testosterone levels increased in both groups, but it was not statistically significant (Figure 1). Several studies have reported increased testosterone levels (6,20); While Consistent with the results of this study, several studies did not report significant changes (14,17).

The results of this study showed that the level of fasting blood cortisol in the post-test compared to the pre-test was significantly reduced in both groups.

But statistically, no significant difference was observed between the two groups (Figure 2); Consistent with the results of this study, several studies have reported decreased in cortisol levels (4,5,6); In contrast the studies of Mojica et al. did not find significant changes (19).

Casteel et al. (1991) observed a decrease in resting cortisol by 23-30% and an increase in testosterone concentration by about 22% during tapering period in swimming competitions, at the same time as a 3.2% improvement in performance (21), also Driesenderfer and Colleagues (2002) reported a 5.3% increase in serum testosterone and a 4.6% decrease in urinary cortisol in cyclists during 10 days of tapering period along with a 1.2% improvement in performance (22). The mechanisms of testosterone increase after a period of load reduction can be through the relationship of the pituitary gland and the response of the temporal processes of exercise intensity, the positive stimulating effects of androgenic-anabolic activity during load reduction sequences, which are characterized by the reduction of physiological stress levels. (6).

Hortbagy et al. (1993) reported a significant increase in testosterone and the T/C ratio along with a decrease in cortisol during 14 days of reduce load and suggested that a short-term tapering period could indicate increased tissue stimulation, and this hormonal change is a reflection of the state of the muscle tissue or functional output responses before blood sampling, which is dependent on the stimulation of the intensity and volume of exercises (23).

On the other hand, Mujica et al. did not observe significant changes in the amount of testosterone, cortisol, T/C ratio and performance during 800 meters running during a 6-day tapering period; While in another study on the same group, authors reported an increase in testosterone levels in parallel with improved performance (24). Based on this, it has been suggested that long-term training and low-intensity training prevent the stimulation of anabolic processes by testosterone; While in intermittent intense training, this stimulation of anabolic processes is facilitated (24).

Creatine supplementation increases the amount of muscle phosphocreatine at rest, which can be effective as an immediate phosphate transporter for ATP regeneration during activity. Also, increasing free creatine in muscles at rest can increase regeneration during and after exercise and also facilitate energy transfer from mitochondria to parts where ATP is consumed. On the other hand, increasing the role of tampons (buffering) for hydrogen ions that prevent acidosis of muscle cells is also important for football players. Increasing the concentration of creatine in athletes allows training with higher training loads, reduces fatigue, and increases muscle hypertrophy, which can improve performance (7,14,16,17). Creatine can increase the synthesis or decrease the breakdown of proteins. Based on this, it is possible that creatine consumption can help to increase the effectiveness of the tapering period after the competition season or the training period with high intensity or volume of training (18).

The coordinated metabolic function between nutritional supplements such as creatine and physiological factors can play an important role in strengthening hormonal reactions and improving sports performance (18). Few studies have been conducted on the effect of creatine supplementation on the changes in the responses of anabolic hormones, and their results are inconsistent. However, in this study, the short-term use of creatine supplements could not have significant changes in the 10 days of tapering period on the levels of these hormones (figure 1-3). In general, it can be concluded that the training model (intensity, volume, and training pattern) and the time of tapering period can have a more influential role than food supplements. However, this issue needs more and more detailed investigations. Researchers have used the T/C ratio as an important indicator of exercise stress (18,21). The results of this study showed that the T/C ratio increased in the tapering and tapering groups with creatine supplementation in the post-test compared to the pre-test; but it was not statistically significant (Figure 3). Also, there was no significant difference in T/C ratio between two groups.

Most of the studies conducted in the field of muscle damage and anabolic-catabolic balance have used the T/C ratio (19.24). Martinez et al. (2010) reported a favorable anabolic-catabolic balance during the study of cortisol, testosterone levels and T/C ratio of elite basketball players during the competition season. And they found it useful to use the ratio of cortisol, testosterone and T/C changes in preventing stress and controlling periods of returning to the initial state during the competition season (25).

Table 1: Demographic characteristics of the participants

Variable	Group	
	G1 (N=9)	G2 (N=9)
	Mean ± SD	Mean ± SD
Age (year)	18.11 ± 0.33	19.44 ± 1.50
Height (cm)	175.66 ± 6.41	173.33 ± 5.15
Body Mass (KG)	64.41 ± 6.51	63.70 ± 8.73

G1: Tapering period + creatine supplement; G2: Tapering period alone; N: Participant number; SD: Standard Deviation

Table 2: Within-group changes of testosterone and cortisol serum levels and T/C ratio

Variables	Group	N	Pre-test	Post-test	P value
			Mean ± SD	Mean ± SD	
Testosterone	G1	9	8.1 ± 3.15	8.88 ± 2.74	0.70
	G2	9	6.28 ± 2.78	6.83 ± 3.57	0.30
Cortisol	G1	9	155.60 ± 25.74	140.60 ± 26.66	**0.04 ***
	G2	9	164.7 ± 44.66	129.6 ± 35.74	**0.02 ***
T/C	G1	9	0.051 ± 0.01	0.064 ± 0.02	0.06
	G2	9	0.038 ± 0.01	0.055 ± 0.03	0.09

G1: Tapering period + creatine supplement; G2: Tapering period alone; N: Participant number; SD: Standard Deviation, T/C: testosterone to cortisol ratio

*** Significant at level P<0.05**

Conclusion

Therefore, in general, the results of this study showed that reducing the training load during the tapering period in team athletes such as football who have a relatively short and intense conditioning period can reduce the levels of catabolic hormones such as cortisol and improve the catabolic-anabolic index.

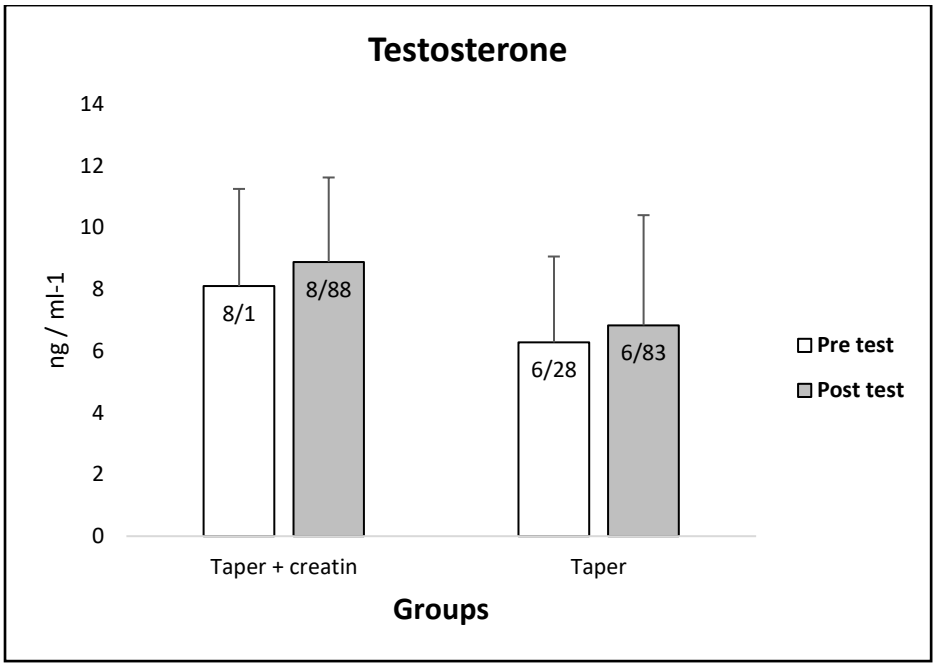

Figure 1: Intragroup changes in testosterone level pre-test and post-test.

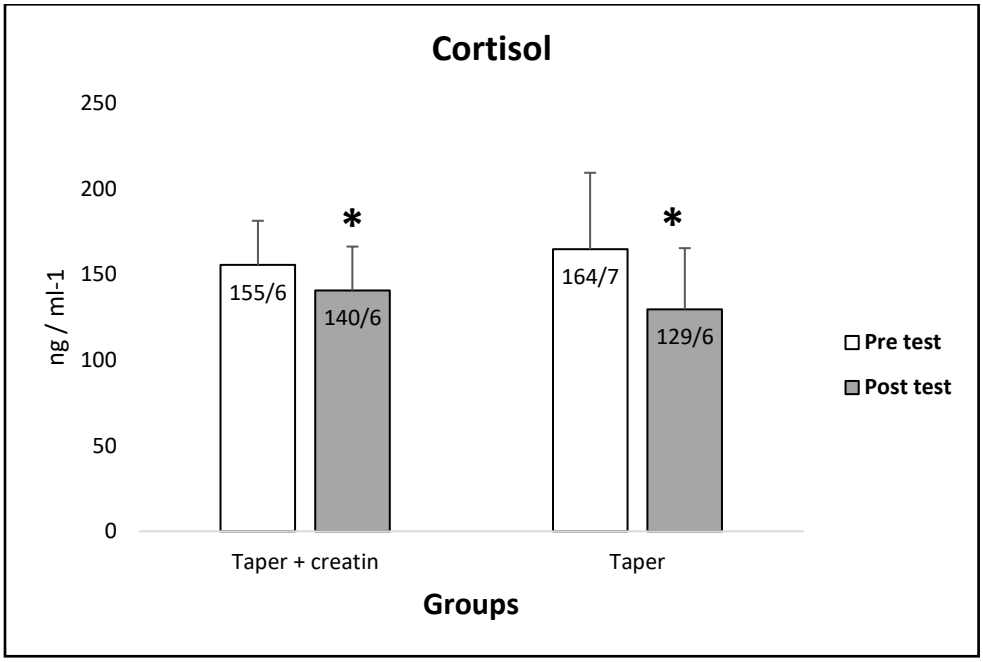

Figure 2: Intragroup changes in cortisol level pre-test and post-test.

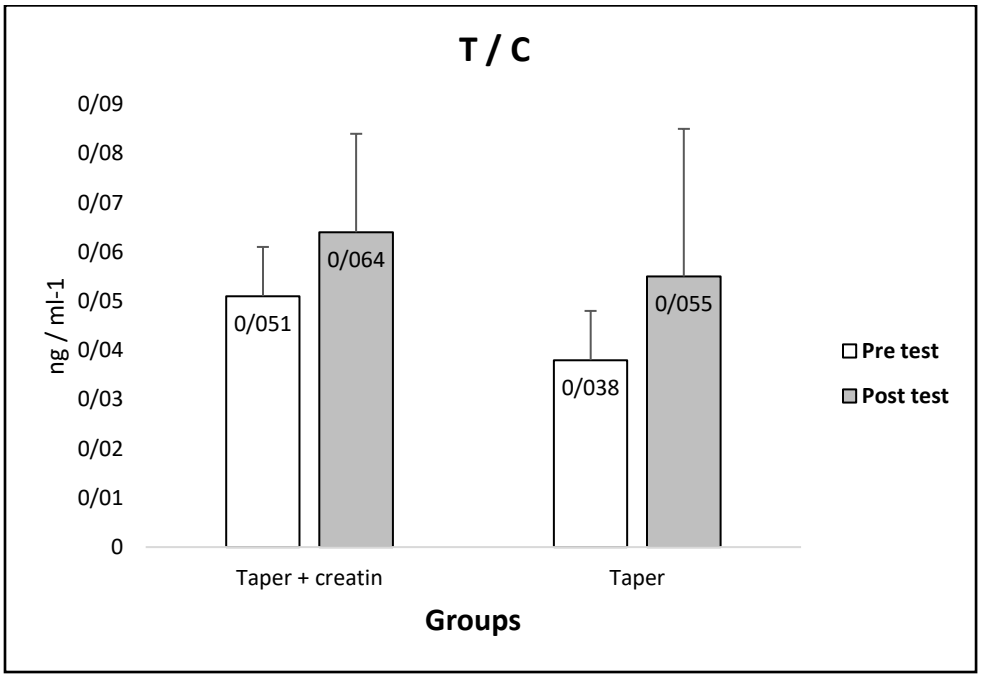

Figure 3: Intragroup changes in testosterone to cortisol ratio level pre-test and post-test.

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

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Research Article

Two methods of aerobic and combined training on biomechanics of Vessels in patients after bilateral femoral artery coronary grafting

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Abstract

Background: Cardiovascular disease is one of the most common causes of death in the world and its prevalence increases with age. For the purpose of cardiac rehabilitation after heart disease, performing exercise training causes functional and structural adaptations in patient's cardiovascular system and consequently reduces mortality from related diseases. Therefore, the aim of this study was to investigate the effect of two methods of aerobic and combined exercise training biomechanics of blood in middle-aged patients after bilateral femoral artery coronary bypass grafting surgery.

Materials and Methods: In this semi-experimental study with a pre-post test design, 68 middle-aged men (mean age 56.19± 1.26 years) were studied after bilateral femoral artery coronary bypass grafting surgery. Subjects were randomly and availably divided into 3 groups: aerobic (n =20) and combined (aerobic + resistance) (n =20) exercise training, and control groups (n =28). Subjects in the intervention groups performed 8 weeks of training/3 sessions per week. Each training session in aerobic and combined groups was considered for 40 minutes with the intensity of 70-85% heart rate reserved, and 60 minutes with the intensity of 40-80% one repetition maximum for each patient, respectively. In order to analyze the data, Leven, MANOVA and Bonferroni statistical tests were used at the significance level of P≤0.05.

Results: The results of one-way MANOVA test showed that the levels of functional capacity, ejection fraction and maximal oxygen consumption were increased significantly after aerobic and combined exercise training compared to control group (p <0.05). However, Bonferroni post hoc test showed no significant differences between functional capacity, ejection fraction and maximal oxygen consumption post-test levels in aerobic and combined exercise training groups (p> 0.05).

Conclusion: the findings of this study show that both aerobic and combined exercise training can improve the heart functional variables in middle-aged patients after bilateral femoral artery coronary bypass grafting surgery, and this improvement levels appears to be independent of the types of training.

Keywords:
biomechanics of vessels, Aerobic and Combined Exercise Training, Arterial Stiffness, Coronary Artery

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1. Introduction

Today, cardiovascular disease (CVD) is the main cause of death worldwide (1, 2). According to the World Health Organization, CVD was the main cause of death in the world (22%) and Iran (35%) in 2002 (3) and it is likely to be the cause of 33% of all deaths worldwide in 2030. (4). On the other hand, coronary artery bypass surgery (CABG) or the rehabilitation of blocked arteries in CVD patients causes some adverse effects such as irregularity and variability in heart rate and disturbance in the tone of the vagus nerve, which indicates a malfunction in the ventricles. It is the left side of the heart (5). The inability of these diseases, clinical treatments, and high treatment costs at different ages (6) has caused much research to be carried out to develop effective strategies to prevent and improve the disease. Therefore, it seems necessary to address the clinical problems caused by aging, especially in patients with CVD, and preventing secondary events after CABG and the progression of the Atherosclerosis process in them is of great importance (7). One of the most important causes of CVD is arteriosclerosis (8), so atherosclerosis of the aorta, coronary, carotid, and peripheral arteries, including the brachial and femoral arteries, is at the top of CVD diseases in people over 40 years old or Middle-aged, with an age range of 40-65 years (9). Considering that atherosclerosis or the accumulation of lipid deposits begins in childhood and increases in older ages and with narrowing of the arteries (10, 11) and subsequently, disruption of blood supply to the heart, brain, and other peripheral organs lead to heart attack, stroke and Lower limb ischemia occurs (12, 13). This can justify the increase in peripheral vascular occlusive diseases and age (14). In other words, the pathogenic changes of atherosclerosis progress with increasing age (15) and finally lead to clinical problems and death (16).

Therefore, it seems that increasing age and gender (more men than women) are uncontrollable risk factors in the development of CVD (17).

In addition, aging is associated with an increasing decrease in the levels of maximum oxygen consumption (VO2max) (18), functional capacity (FC) (19), and a decrease in ejection fraction (EF) (20) in the heart. VO2max is an indicator of maximum cardiorespiratory performance, aerobic fitness, and how the heart system works, which decreases with age, and its decrease is a known risk factor in CVD mortality. Therefore, the reduction of VO2max along with increasing age is effective in increasing the risk of mortality in middle-aged and elderly people (18). Another main cause of heart failure is a decrease in EF, an indicator of the function of the left ventricular of the heart (20). When the muscle strength of the heart decreases so much that the decrease in EF reaches less than 40%, we will witness heart failure in a person (21). FC is also the maximum ability of a person to perform a sport or physical activity beyond the level at rest. A decrease in FC has been observed after the onset of coronary artery disease followed by CABG (7).

Also, increasing age (2, 22, 23) combined with an unhealthy lifestyle (24) such as reducing the amount of sports activity (23) and inactivity is associated with an increasing increase in CVD risk factors (22). Inactivity is a modifiable risk factor in CVD (25), while sports activity is known as the most effective intervention in improving age-related performance (20) and because of its preventive and protective effects against CVD (26) and prevention of deaths caused by it (27) Many experts recommend regular physical activity, which among the adaptations caused by sports activity can increase the shear stress caused by blood flow.

it pointed out on the arterial walls and finally the improvement of endothelial function during sports activities. Also, endurance exercises have potential anti-ischemic effects and increase coronary blood flow by strengthening capillary density (26). In general, a sport or physical activity beyond the amount of rest will improve FC or the peak ability of a person in patients with CVD (7). In addition, volume overload on the heart caused by endurance and aerobic exercise leads to an increase in the volume of cavities and eccentric hypertrophy of the left ventricle of the heart (28), so it is logical that this type of exercise can increase VO2max levels. The increase in VO2max after sports training is related to the increase in the function of the left ventricle of the heart and subsequently to the increase in the maximum output of the heart (central adaptation) (29). Other adaptations caused by endurance sports activity, such as a decrease in vascular resistance, an increase in blood volume, an increase in EF, and an increase in the oxidative capacity of skeletal muscles can also increase VO2max levels (30). Khorramdel et al. (2015) investigated the effect of 8 weeks (three sessions per week) of Pilates exercises and balanced movements on VO2max levels in middle-aged women and showed that a period of exercise improved VO2max in middle-aged subjects (31). Bahramian et al. (2018) studied 10-week-old rats suffering from myocardial infarction and showed that 6 weeks (5 sessions per week) of intermittent aerobic activity in 3 different intensities could increase EF levels and they stated that exercise training, regardless of Due to its intensity, it can improve the structure and function of the left ventricle of the heart, however, increasing the intensity causes better effects (32). In this regard, the findings indicate that moderate-intensity sports activity can reduce CVD in elderly people, however, it seems that middle-aged men should exercise more intensely in order to achieve its protective benefits. pay (27, 33).

Therefore, although the endurance and aerobic exercises by improving cardiovascular fitness bring many health benefits to the elderly (18), its quantitative and qualitative indicators in the development of VO2max in the middle-aged population are still unknown. However, it seems that the development of CVD can be prevented by changing lifestyles and controlling modifiable risk factors (12).

Moderate intensity continues training (7) are considered cardiac rehabilitation programs. Therefore, by using cardiac rehabilitation programs after CABG, functional capacity (FC) and quality of life can be improved in middle-aged patients (7), and compared to only drug therapy, it can further reduce the death rate caused by CVD. 34). By creating structural adaptations in the left ventricle, rehabilitation exercises help the contractility of the heart and adjust the vagal tone, which is associated with an increase in EF (35). However, the findings indicate that the cardiac rehabilitation program in the form of submaximal aerobic exercises (36) and moderate intensity (34) is an effective treatment and rehabilitation program after CABG (36).) and is considered one of the most common types of cardiac rehabilitation programs (34), but since in ischemic heart patients (7) and after CABG, there is a decrease in muscle mass and strength, followed by a decrease in VO2peak and subsequent reduction of FC and quality of life (37), it is believed that by increasing muscle strength we will achieve performance optimization in this segment (7). Therefore, in order to increase muscle strength and aerobic capacity, resistance exercises can be used in addition to aerobic exercises (7). Therefore, strength training is recommended as part of the rehabilitation program in cardiac patients (38) and it is assumed that combined rehabilitation protocols (resistance-aerobic) can bring a greater improvement in FC values after CABG in adults.

Existing studies have indicated the effect of different methods of cardiac rehabilitation programs (combined (7) and aerobic (7, 36)) on FC (7) of cardiovascular patients, however, few studies have investigated the effect of combined exercises and Aerobic exercise has been performed in middle-aged men after CABG surgery, and the best type of rehabilitation program that can achieve more favorable effects on the biomechanical behavior of blood and vascular structure of these patients has not yet been determined. Therefore, assuming that exercise training is effective, the purpose of this study was to investigate the effect of two methods of aerobic and combined exercise on factors affecting heart function, including FC, EF, and VO2max in middle-aged male patients after bilateral femoral artery CABG surgery.

2. Materials and Methods

In this semi-experimental study, with a pre-and post-test research design, a causal-comparative model, and an applied type, from within the statistical population of 2648 middle-aged cardiac patients 40 to 65 years old (W.H.O) underwent coronary artery bypass grafting (968) had been performed in Tehran Heart Center Hospital, and among 382 male coronary artery transplants, 68 middle-aged people who were two to three weeks after their operation was introduced to the rehabilitation center of Tehran Heart Center Hospital, the subjects of the present study They gave. Subjects were randomly selected and placed in three groups: 1) aerobic exercise training (20 people), combined exercise training (20 people), and control group (28 people). The type and severity of the disease were diagnosed by the doctor present in the clinic.

By filling in the questionnaire of personal information and physiological health, complete explanations were given to the subjects regarding the purpose of the research, the method of conducting it, and the confidentiality of the information, and a consent letter was obtained to declare the consent of the subjects to participate in the research. The article is based on letter number 101/1000-2 dated 4/31/2018 from the University/Research Institute of Movement Sciences and has ethics approval.

After the patients were referred to the cardiologist and the doctor's approval to participate in the research, the subjects were introduced to the imaging center to perform the pre-examination tests one day before the start of the training programs. Before starting the test, the patients were explained about the purpose of the research and then the consent forms for the research were completed by the patients. Then the patients began aerobic and combined exercise programs under the supervision of a nurse familiar with monitoring and a researcher at the rehabilitation center of Tehran Heart Center Hospital. The subjects of the aerobic exercise group performed eight weeks of submaximal aerobic exercise protocol/three sessions per week and each session lasted 40 minutes with treadmills, arm ergometers, and exercise bikes. In each session, after warming up, the patients first run on a treadmill for 10 to 20 minutes with an intensity of 70% of the reserve heart rate reserve, which was calculated according to Karvonen's formula, and with a maximum speed of five kilometers per hour at the beginning of the session and In the continuation of the training sessions, they increased to 85% of the reserve heart rate and increased to a maximum speed of nine and a half kilometers per hour. Then, they continued to exercise with an arm ergometer and a stationary bike for 8 to 10 minutes, respectively, with an intensity of 50 watts, which increased to 80 watts during the sessions.

The subjects of the combined exercise group (70% aerobic and 30% resistance) first exercised for 40 minutes according to the aerobic exercise protocol and then did resistance exercise twice a week for 20 minutes with four hip adductor machines. They did seat chest presses, leg extensor, and abdominal. The intensity of these exercises was initially 40 to 50% of one maximum repetition (RM1) and then to 60 to 70% of RM1 with 8 to 12 repetitions in 2 to 3 sets. It should be mentioned that during the training period, the relevant officials constantly checked the heart rate and the training pressure in order to prevent excessive pressure and not harm the patient in case of possible training pressure on the patient. In order to investigate the effect of sports training on the desired parameters, after eight weeks of aerobic and combined training, a post-test was taken from the subjects. Also, to evaluate the values of the emptying fraction, the echocardiograph model VIVD3 made by General Electric of America was used, and to determine the levels of functional capacity and maximum oxygen consumption, an exercise test was used on the Kansas USA model treadmill. Functional capacity is expressed based on MetS, and each MetS is equivalent to 3.5 liters of oxygen per kilogram of body weight per minute.

The subjects of the control group were selected from the patients who did not visit the rehabilitation center. In addition to nutritional recommendations, all three groups of patients were advised to walk (three days a week). It should be noted that a number of coronary artery graft patients either refused to continue this research due to personal reasons, or due to death, repeated MI and hospitalization, or absenteeism for more than two sessions, the researcher excluded them from continuing the research.

Statistical analysis

To describe the data in descriptive statistics, mean and standard deviation were used. In addition, based on the size of the samples in the research groups, firstly, the normality of the distribution of the studied variables was checked using the Kolmogorov-Smirnov (K-S) test, and after confirming the normal distribution of the data, to determine the homogeneity of the error variances of the dependent variables. In all groups, Levin's test was used, and to investigate the effectiveness of aerobic and combined exercise methods on selected variables of heart function, the one-way MANOVA test was used, and to determine the location of differences and comparison between groups in the groups, Bonferoni post hoc test was used at the level P≤۰/۰۵significance was used. Also, SPSS version 24 statistical software was used to analyze the raw data.

3. Results

Table 1 shows the basic characteristics of the subjects such as age, height, weight, resting, and maximum heart rate in all three groups separately.

Table 1: Descriptive statistics indicators are related to subjects' background variables (mean ± standard deviation) in the research groups.

Background variables	Groups		
	Aerobic training	Combined training	Control
Age (Year)	671/55±6	031/76±7،54	109/11±5،58
Weight (Kg)	807/44±6،79	578/44±7،76	417/50±8،75
height (Cm)	822/32±5،174	052/40±4،171	389/72±5،171
resting heart rate (thud . minutes)	398/20±13،80	517/32±11،83	285/56±10،78
Maximum heart rate (thud . minutes)	431/60±14،131	962/88±12،122	733/33±16،128

To investigate the effectiveness of aerobic and combined exercise methods on selected blood variables, the one-way MANOVA test was used and the results of this test were reported in Table 4-9. According to the results of the one-way MANOVA, the group effect was not significant for the pre-test values in any of the variables under study, so there was no significant difference between the pre-test values of the groups in the selected blood biomechanical variables. Also, the results of the MANOVA test related to the post-test values showed that in the variables of blood flow velocity in the systolic phase, the intensity of blood flow in the systolic phase of the group effect was not significant, in the sense that there is no significant difference between the post-test values of the groups in these variables, but for The post-test values of blood flow velocity in the diastolic phase and blood flow intensity in the diastolic phase of the group, the effect was significant in the sense that there is a significant difference between at least one pair of groups.

In the following, Bonferroni's post hoc test was used to investigate the post hoc comparisons in blood flow speed and intensity variables in the diastolic phase, the results of which are reported in Table 2.

Table 2 : Summary of one-way MANOVA test results related to the selected values of vascular biomechanics

The dependent variable	Time	Aerobic exercise group			Combined training group			control group		
		Number	Average	standard deviation	Number	Average	standard deviation	Number	Average	standard deviation
Diastolic resting lumen diameter Dd	Pre-test	25	579،0	110،0	25	514،0	092،0	18	555،0	156،0
	post-test	25	589،0	103،0	25	603،0	119،0	18	540،0	153،0
Resting systolic lumen diameter Ds	Pre-test	25	695،0	137،0	25	672،0	105،0	18	665،0	106،0
	post-test	25	712،0	117،0	25	759،0	157،0	18	672،0	163،0
Intimal thickness ratio lmt	Pre-test	25	132،0	272،0	25	124،0	032،0	18	146،0	069،0
	post-test	25	116،0	024،0	25	112،0	020،0	18	147،0	052،0
Media in diameter lumens Lmt.dm	Pre-test	25	127،0	032،0	25	117،0	025.	18	127،0	046،0
	post-test	25	136،0	036،0	25	121،0	029،0	18	13،0	040،0

Table 4-12 : Summary of post-test results for paired comparisons of blood flow speed and intensity in systolic and diastolic phases.

Variable	Time	Group		Mean Difference	Amount P
Intimal thickness ratio **LMT**	Pre-test	Aerobic training	Combined training	0.008	1.000
			Control	-0.014	0.924
		Combined training	Control	-022،0	307،0
	post-test	Aerobic training	Combined training	004،0	000،1
			Control	-031،0	009،0
		Combined training	Control	-035،0	003،0
Blood flow intensity in the diastolic phase **PDMM**	Pre-test	Aerobic training	Combined training	002،0	000،1
			Control	-039،0	919،0
		Combined training	Control	002،0	000،1
	post-test	Aerobic training	Combined training	008،0	000،1
			Control	074،0	033،0
		Combined training	Control	-066،0	071،0

According to the results of Table 10-4, the post-test related to blood flow speed in the diastolic phase showed that there was no significant difference between the groups under study in the pre-test. In the post-test, there is only a significant difference between the aerobic exercise group and the control group, but there is no significant difference between the other groups.

Also, the results of the post hoc Bonferroni test related to blood flow intensity in the systolic phase also showed that there was no significant difference between any of the pairs of groups in the pre-test, and in the post-test, there was only a significant difference between the aerobic exercise group and the control group.

4. Discussion

Sports training has strong and significant effects on the morphology of blood vessels. Structural changes appear following functional changes in the vessels and lead to improved blood flow. Exercise causes angiogenesis (and Arteriogenesis) in the formation of vessels with high transmissibility, in fact, angiogenesis is the result of the balance between positive (negative) angiogenic and angiostatic regulators of blood vessels. Another important aspect of the effect of exercise on capillaries is the initiation and continuation of arteriogenesis. Considering that arteriogenesis leads to the formation of vessels with high transfer capability, which is able to compensate for the loss of function of blocked vessels, the establishment of arteriogenesis causes vascular adaptation. Angiogenesis and arteriogenesis are very sensitive to local mechanical conditions. An increase in shear stress levels caused by exercise leads to a decrease in vascular resistance and an increase in tissue perfusion. An increase in wall shear stress causes an increase in the production of nitric oxide, which results in a decrease in the degree of contractility of vascular smooth muscle and strong vasodilation. Vascular adaptations resulting from regular and continuous aerobic exercise activities include lower arterial stiffness in people with higher aerobic capacity, protection against systemic oxidative and inflammatory stress, increased endothelium-dependent vasodilation capacity, as well as increased coronary blood circulation due to increased Nitric oxide production. Animal studies and clinical observations have provided evidence that shows that there is a significant correlation between regular physical exercise and an increase in the diameter of the lumen in the coronary artery.

Exercise can also exert beneficial effects against atherosclerosis by increasing the flow of endostatin, which prevents the expansion of atherosclerotic plaque by blocking angiogenesis in the plaque tissue. Endurance activities also improve angiogenesis by reducing endostatin plasma levels. The results of the present study in the variables of blood vessels also showed that, except for the ratio of intima thickness, there was no significant difference between the groups in the post-test values. Although there was no significant difference between the groups in the diameter of the resting lumen in the systolic and diastolic phases, the lumen diameter increased in both the diastolic and systolic phases from the pre-test to the post-test in both groups of aerobic exercise and combined exercise. The results of the follow-up test related to the ratio of intima thickness showed that there are significant differences between the aerobic and combined exercise groups with the control group, and it is significantly less for the aerobic and combined exercise group than the control group. These results were in line with the studies of Maiorana et al. (2011), Tiejsen et al. (2011), Tiejsen et al. (2012), and Okamoto et al. (2007). In this way, it can be stated that aerobic exercise improves the structure of the internal damaged surfaces of the vessels and leads to an increase in the blood flow rate resulting from the shear stress force of the inner layer of the vessel. On the other hand, increasing the intensity of strength training causes a sharp increase in blood pressure compared to aerobic training. The exact cause of these changes has not been determined, but strength training is known as a strong stimulator of the parasympathetic nervous system and causes an increase in vascular contraction. Long-term aerobic exercise regimens improve cardiovascular function, reduce total environmental resistance, and increase the conduction of the circulatory system.

This fact can be observed in healthy subjects without any basic risk factors, in elderly people as well as those with cardiovascular risk factors. Following exercise training in cardiovascular patients, the peak aerobic capacity is increased, and the increase in maximum cardiac output is consistent with the stability of the average aortic pressure, as a result of which peripheral resistance is also reduced in patients with hypertension, type 2 diabetes, metabolic syndrome, Stable cardiovascular disease, myocardial infarction, and heart failure all benefited from the exercise program compared to those who did not participate in any exercise program. In fact, people with cardiovascular risk factors will benefit more. In healthy individuals, a more intense and prolonged exercise protocol is required to produce measurable changes in cardiovascular parameters, whereas older, healthier subjects may benefit from less intense exercise regimens. In addition, it has been proven that only the veins that have experienced high shear forces during sports training have improved their performance. Also, research has shown that exercise training in coronary artery bypass graft candidates significantly increased the endothelium-dependent vasodilation capacity and, when compared with matched sedentary volunteers, increased the average peak blood flow velocity of the left internal artery. . It has been reported that the risk of a heart attack in relatively sedentary people is 0 to 6 times higher during exercise than at rest compared to people who do regular exercise. However, the risk of sudden cardiac death or myocardial infarction increases transiently during high-intensity exercise.

Conclusion

In general, the results obtained from the present study showed that performing eight weeks of aerobic and combined exercise programs at the same rate can increase the intima-media thickness ratio and blood flow intensity in middle-aged men after bilateral femoral artery coronary artery bypass grafting. Therefore, to improve the health status after coronary artery bypass surgery and prevent the progression of atherosclerosis in middle-aged men, it is recommended to include aerobic and combined exercise programs in their treatment process.

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Author contributions

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

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Research Article

Effect of 8-week metabolic resistance training and Chlorogenic acid supplement on the expression of UCP1: A randomized clinical trial

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Abstract

Background: Obesity and overweight is one of the major public health challenges all around the world. The aim of this study was to evaluate the simultaneous effect of eight weeks of metabolic resistance training (MRT) and Chlorogenic Acid (CGA) supplementation on weight loss, expression level of UCP1in overweight women.

Materials and Methods: The present study was a randomized clinical trial performed in 2022 on a sample of overweight women in Iran. Participants in the study were randomly divided into four groups including combined 8-week course of MRT training and CGA supplementation, 8-week course of MRT, CGA supplement, and the control group. Intervention included three training sessions per week and the duration of each session was 45 minutes. The supplementation arms were also received 400 mg / day CGA extracted from green coffee beans. Expression level of Uncoupling Protein 1 (UCP1) was the main interested outcome that assessed pre and post intervention.

Results: In the MRT exercise group, UCP1 expression increased by 4.3 units on average over the 8-week intervention. The highest increase was observed in participants who received both CGA supplement and MRT exercise where UCP1 increased from 22.5 (1.2) to 28.0 (3.5) over the study period ($P<0.05$). No significant increase was observed in CGA supplementation group.

Conclusion: MRT exercises with and without CGA supplementation stimulated expression level of UCP1 mRNA.

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1. Introduction

Obesity is one of the leading public health challenges worldwide (1). It is estimated that the prevalence of obesity has increased triple times since 1975(2). The number of overweight adults exceeded 1.9 billion in 2016 and 650 million of them were obese(2). Obesity is an independent risk factor for a couple of cancers, diabetes mellitus, cardiovascular diseases, and hypertension and through this pathway has a major contribution to the burden of disease (1, 3).

Several different approaches like exercise training have already been introduced to combat obesity and overweight. According to previous studies, both aerobic and resistance training could lead to favorable outcomes in obese people (4, 5). However, higher efficacy has been shown for metabolic resistance training which is the combination of both aerobic and resistance training (6). The distinctive feature of these exercises is their very small volume, however, it has been shown that the implementation of such exercises leads to an increase in the activity of aerobic and anaerobic enzymes and an increasing amount of both oxidative and glycogenic enzymes, as well (7, 8).

Dietary supplements are another approach to weight reduction and it is illustrated that combined approaches including both dietary supplements and exercise training may bring more benefits (9). There are pieces of evidence regarding the efficacy of Chlorogenic Acid (CGA) in weight reduction in overweight people(10). According to the available evidence, CGA could affect glucose metabolism and fat formation which consequently leads to accelerated weight loss (10, 11).

The possible mechanisms of these interventions have remained almost unclear. It is already shown that practical approaches modulate gene expressions and through this pave increase fat-burning and energy consumption in the cell and the whole body. Uncoupling Protein 1 (UCP1) is the type of protein that contributes to the heating and fat-burning process(12, 13). Therefore, any increase in the expression level of UCP1 can increase the amount of energy consumed by the cell and it is associated with burning reserved fat and consequently weight loss.

In the current, study we aimed to investigate the effect of MRT and CGA supplements on the expression level of UCP1. We also assessed the combined effect of these two approaches in this regard.

2. Materials and Methods

Study design and study participants

We performed an open-label randomized clinical trial on 40 overweight women. The study was carried out on a convenient sample of overweight women referring to a fitness gym in Tehran-Iran in 2022. Inclusion criteria were Body Mass Index (BMI) between 25 and 28 and physical activity in the past six months to maintain health. Not using dietary supplements, medications, underlying diseases related to the investigated variables, and not having any pulmonary diseases were the other inclusion criteria. We excluded cases with no willingness to participate and study participants were allowed to leave the study at any phase.

Intervention

The study participants were randomly assigned into four groups including control groups (no intervention), CGA supplement, MRT exercise, and dual supplement and exercise group who received both CGA supplement and MRT exercise interventions concurrently. The number of training sessions was three a week and the duration of each session was 45 minutes including 10 minutes of warm-up, 30 minutes of metabolic resistance training, and 5 minutes cool down. Details of the exercise intervention are provided in supplementary Table 1. The intensity of the exercise was 60-70 percent of the maximum heart rate of the participants (7). 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(14).

Supplementary Table 1: The MRT program for 8-weeks Micro cycle

Complete 1-6 in circuit fashion	Weeks 1-2, 60 seconds rest	Weeks 3-4 45 seconds rest	Weeks 5-8 30 seconds rest
1. Skipping 10 yards down and back	Reps:2 Rounds: 2	Reps:3 Rounds: 2 (3), 3(4)	Reps: 4 Rounds: 3(5-6), 4(7-8)
2. Agility ladder Half ladder, In In Out Out	Reps:2 Rounds: 2	Reps:3 Rounds: 2 (3), 3(4)	Reps: 4 Rounds: 3(5-6), 4(7-8)
3. Modified barbell Hang Clean and Press 15Kg	Reps:5 Rounds: 2	Reps:8 Rounds: 2 (3), 3(4)	Reps: 10 Rounds: 3(5-6), 4(7-8)
4. Medicine Ball, Move to mountain 10 yards, 8Ib med bar or DB	Reps:1 Rounds: 2 5 med balls/DBs	Reps:1 Rounds: 2 (3), 3(4) 6 med balls/DBs	Reps:1 Rounds: 3 (5-6), 4(7-8) 8 med balls/DBs
5. TRX Bodyweight Row	Reps: 5 Rounds:2	Reps:8 Rounds: 2 (3), 3(4)	Reps: 10 Rounds: 3(5-6), 4(7-8)
6. Med Ball Overhead Slams 8-Ib med ball	Reps: 5 Rounds:2	Reps:8 Rounds: 2 (3), 3(4)	Reps: 10 Rounds: 3(5-6), 4(7-8)
7. Finisher: Bike Sprints	Reps: 4(1), 5(2) Workload: 1.0 kg Work/Rest 10 seconds/ 30 seconds	Reps: 5(3), 6(4) Workload: 1.5 kg Work/Rest 10seconds/20seconds	Reps: 5-6(5), 7-8(6) Workload: 2.0 kg Work/Rest 10 seconds/ 15 seconds

Data collection and outcome assessment

Demographic and biometric data including age, weight, height, BMI, medical history, and volume of physical activity was collected for each participant. We also collected data on lipid profile and gene expression using blood samples taken at the baseline and after eight weeks of intervention. Study participants were asked not to have vigorous exercise two in the last two days leading to blood sampling. Blood sampling was performed from the participant's right-hand vein at 8 AM and all study participants were fasting in the last 12 hours. We collected data on HDL, LDL, and triglyceride using a photometric approach. UCP1 was assessed using genomic DNA extraction. We used real-time PCR to determine the UCP1 expression level. All procedures were repeated at the end of the study after an 8-weeks intervention.

Randomization and concealment

We used a stratified balanced block randomization approach for the random allocation of the study participants into four investigated groups. We generated six blocks of 4 using A and B letters (for example, AABB) and numbered them 1 to 6. Then we throw a dice to determine the training intervention status for each participant. We determined the training intervention status for 4 participants in each attempt according to the dice number and its associated AB block. Overall, ten dice throws were performed for all participants. In this stage, we had two groups (20 participants in each group) that were allocated to either training intervention (Group A) or non-intervention (Group B). Then, we repeated the same procedure as the previous step in each group separately to determine whether or not participants would receive nutrition intervention.

The letter A represented nutrition intervention, and the letter B was the marker of no intervention. Finally, we had a two-letter combination of A and B (repeat was allowed) for each study participant to determine the type of intervention that they were supposed to receive. All combinations were numbered 1 to 40 and then received an 8-digits code containing the numbers and letters. We wrote the letter combinations on paper and put them into a sealed envelope. All pockets were given to the research group. After the enrolment of each participant, we announced the envelope code to be opened and repeated the same process until the last participants. The research group was unaware of the following code over the case enrollment period.

Sample size

We calculated the sample size using the comparing two means equation for sample size calculation. In this equation, α was considered as 0.05 and the power study was 80%. We also assumed the effect of the intervention (MRT training) on UCP1 expression level was 2.5 times higher than the control group (15). The sample size of the study was estimated to be 10 people for each arm of the study, and due to the presence of four groups in the study, the final sample size was 40 people.

Statistical analysis

We described continuous variables as mean and standard deviation. Baseline characteristics were compared using One-way ANOVA. We also assessed within-group variability pre and post-study using paired t-test. We also compared post-intervention UCP1 between group variability using One-way ANOVA. We also investigated the effect of each intervention on UCP1 expression level using a multiple linear regression model. Age and other baseline characteristics including BMI, HDL, LDL, TG, and baseline level of UCP1 expression were entered into the model as possible confounders. Then we used a backward approach to generate the best-fitted model. We also drew a change in UCP1 expression versus a change in body weight. The association between these two variables was investigated using simple linear regression. All statistical analysis was performed using Stata software (Ver 17.0, College Station, Texas, USA). P-values <0.05 were considered significant.

3. Results

Overall, the average age of the study participants was 40.4 (5.5) years and there was no statistically significant difference between the study arms (P-value= 0.948). We also found no significant difference in other baseline characteristics including weight, BMI, HDL, LDL, and TG among the compared groups (P-value>0.05) (Table 1).

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.

The average pre-intervention expression level of UCP1 was 22.3 (4.9) in the control group and remained almost constant over the study period (post-study= 22.1±4.2), while in supplement group UCP1 slightly increased from 22.9 (3.4) to 23.6 (5.1), although it was not statistically significant (P-value>0.05). In the MRT exercise group, UCP1 expression increased by 4.3 units on average over the 8-week intervention. The highest increase was observed in participants who received both CGA supplement and MRT exercise where UCP1 increased from 22.5 (1.2) to 28.0 (3.5) over the study period (P-value<0.05) (Table 2).

Table 2: Pre and post-intervention expression of UCP1 by each arms of the study

Group	Pre-intervention	Post intervention	Difference	P-value
Control	22.3 ±4.9	22.1 ±4.2	0.1 ±1.9	0.549
Supplement	22.9 ±3.4	23.6 ±5.1	0.6 ±3.5	0.552
Exercise	22.7 ±4.4	27.0 ±2.6	4.3 ±2.2	<0.001
Ex/Supp	22.5 ±1.2	28.0 ±3.5	5.4 ±3.1	<0.001
P-value	0.982	0.006	0.003	----

We compared the effect of each intervention versus the control group using multiple linear regression and observed that the effect of exercise was 5.0 times higher than the control group (95% CI= 2.5, 7.4). This effect reached 5.5 in the exercise-supplement group and dual intervention led to 5.5 units (95% CI= 3.1, 7.9) average increase in UCP1 expression level in comparison to the control group. The observed association was statistically significant (P-value<0.001). Using the CGA supplement solely has led to a 1.2 unit increase in the average expression level of UCP1. However, the observed change was not statistically significant (P-value= 0.306) (Table 3).

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

Group	Regression coefficient	95% CI	P-value
Control	Reference		
Supplement	1.2	-1.1, 3.6	0.306
Exercise	5.0	2.5, 7.4	<0.001
Supp/Exercise	5.5	3.1, 7.9	<0.001

Model is adjusted for age, baseline BMI, HDL, LDL, and UCP1. CI= Confidence Interval

We also investigated the association between change in UCP1 expression level and change in body weight and observed that each unit increase in UCP1 could lead to 1.3 Kg weight loss (95% CI= -1.9, -0.8) that was statistically significant (P-value<0.001) (Table 4). Figure 1 illustrated the association between weight and UCP1 expression in each arm of the study (Figure 1).

Table 4: The association between change in UCP1 expression and weight change in overall and stratified by each arm of the study

Group	Regression coefficient	95% CI	P-value
Control	0.08	-1.2, 1.2	0.987
Supplement	-0.4	-4.4, 3.6	0.808
Exercise	-0.9	-1.6, -0.3	0.010
Supp/Exercise	-1.8	-2.6, -0.9	<0.001
Overall	-1.3	-1.9, -0.8	<0.001

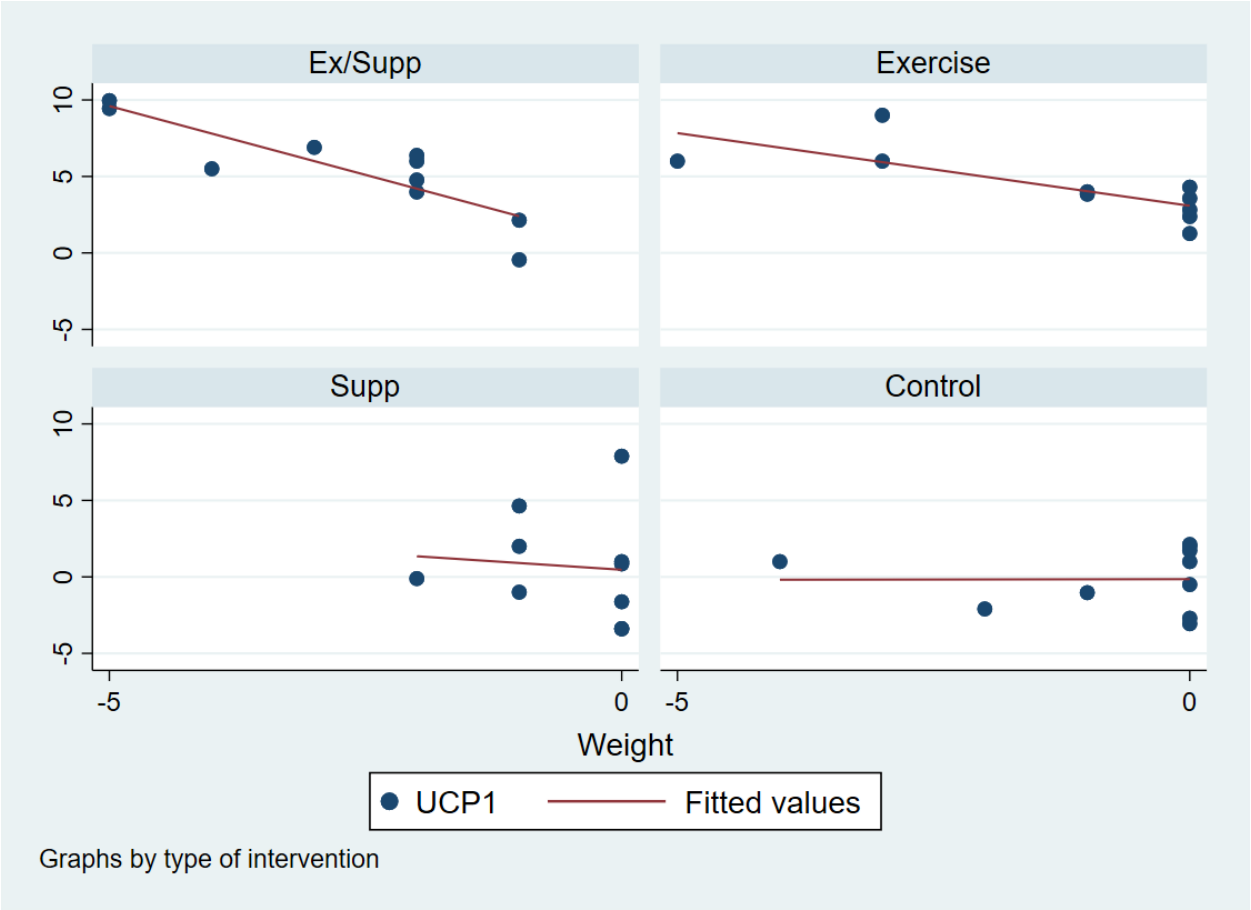

Figure 1: The association between change in UCP1 expression level and weight lost by type of intervention

4. Discussion

The current study aimed to investigate the effect of 8-weeks MRT training and CGA supplement on expression level of UCP1. According to our findings, participation in 8-week MRT training was associated with increased expression level of UCP1. However, such was not observed in CGA supplement group. Moreover, we observed no interaction effect when both interventions were given to the study participants concurrently and change in UCP1 expression was virtually as same as the exercise group. We also found statistically significant inverse association between change in UCP1 level and weight lost. In other word, increase in UCP1 expression was associated with decrease in body weight over the study period.

We observed that MRT exercise either with or without the CGA supplement could increase the expression level of UCP1. Such findings have been previously reported by Daneshyar et al (15). Xu et al have also shown that resistance training could double the expression level of UCP1 in rats which was consistent with our findings(16). Several endocrine mechanisms have been suggested in previous studies for the increasing effect of exercise training on the UCP1 level. According to Daneshyar et al, exercise training increases sympathetic nervous system activity and norepinephrine secretion (17). Exercise-induced norepinephrine binds to β3 adrenergic receptors and therefore induces a signaling pathway leading to UCP1 gene expression in subcutaneous and brown fat cells(17). An increase in thyroid hormones due to contribution in exercise training leading to an increase in volume and activity of the DIO2 enzyme was the alternative mechanism suggested by Daneshyar et al (17).

It is also argued that long-term exercise training increases the secretion of Irisin from muscles and fat tissues that could be considered as a possible pathway to justify the increasing effect of exercise training on UCP1 gene expression(17). Apart from endocrine factors, some proteins like Peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α) might have an effect on exercise-induced UCP1 expression. PGC-1α is a key regulator of energy metabolism(18). According to previous studies, there is a strong correlation between PGC-1α and exercise-induced UCP1 expression (18, 19).

Although more studies have shown that training exercise could increase expression of UCP1, this is still and controversial issue. Flouris et al, in contrast with our findings illustrated that exercise training could not stimulate the expression of UCP1 mRNA(20). Differences in the study design, type and volume of the exercise, and some other confounding factors like temperature, and gender of the participants could justify the observed difference in the previous studies.

We also observed no effect for CGA regarding the stimulation of UCP1 mRNA expression. There was lack of evidence in this regard and the available studies were limited to rats. The study performed by Ye et al reported that CGA was associated with a remarkable increase in the expression of adipogenic and thermogenic genes in Brown Fat Tissues (BAT) (21). Such findings were in contrast with our data. There was also evidence regarding the effect of CGA on the expression of PGC-1α as a modulator of energy metabolism and fat oxidation (22). Huang et al, have also shown that CGA could decrease body weight and obesity through the expression of PPARα which is the opposite of our findings (23).

The study by Sudhakar et al has also shown that CGA has positive effects on the expression of UCP1 and PGC-1α and through this pathway promotes the browning of white adiposities (24). It should be noted that there is a lack of human studies regarding the effect of CGA on the expression of UCP1 and all previous researches were in-vitro or animal-based studies performed on rats. Moreover, we had a small sample size that reduced the power of the study to investigate the association between CGA and UCP1 expression.

The current study is one of the first attempts to investigate the cumulative effect of MRT training and CGA supplementation on UCP1 expression. In this sense, the findings of this research are unique. Conducting the study on a human sample as well as no lost-to follow-up cases in the all intervention and control groups were the main strengths of the current study. However, this study had some limitations that must be considered in interpreting of the results. 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 that to avoid contact between the intervention and control groups, and the people of these two groups participated in their exercise programs separately.

Conclusion

According to our findings MRT exercises with and without CGA supplementation stimulated expression level of UCP1 mRNA. There was no such effect for CGA solely. More human studies are required.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval This study was reviewed and approved by Ethics Committee and review board Sport Science Research Institute of Iran (Ethic Code: 95RI-REC-2202-1483 (RS)). The trial was registered at Iranian registry of Clinical Trial irct.ir (IRCT2020051104740).

Informed consent Informed consent was obtained from all participants.

Author contributions

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

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Research Article

The effect of a period of Pilates and the supplementation of Royal Jelly on inflammatory indexes of diabetic obese women

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Abstract

Background: The use of supplements, herbal extracts, and exercise training for the treatment of diseases and metabolic disorders has increased among people. Thus, the aim of this study was the study of The effect of a period of Pilates and the supplementation of Royal Jelly on inflammatory indexes of diabetic obese women.

Materials and Methods: In this quasi-experimental study 44 volunteer diabetic obese women participated and They were randomly divided into Four groups of 11 controls, Royal Jelly, Pilates, Pilates + Royal Jelly .Pilates exercise training was performed during 8 weeks (3 sessions per week, 60 min per session). The subjects consumed a supplement group of 1000 mg of royal Jelly daily for 8 weeks. covariance analysis test were used to examine the in-group differences and between the research groups.

Results: The results showed that eight weeks of Pilates and Royal Jelly Supplements significantly reduced the TNF-α, CRP and IL-6 diabetic women(P=0.001). In addition, serum glucose and serum insulin also decreased significantly in during eight weeks of Pilates and supplementation of the royal Jelly(P=0.001). Vo2max significantly Increase in training, training + supplement groups (P=0.001).

Conclusion: According to obtained results, it appears that Pilates training plus Royal Jelly extract consumption have better effect on serum inflammatory factors in obsess women with diabetes type 2

Keywords:
Pilates, Royal Jelly, TNF-α, CRP, IL-6, Type Two Diabetes

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1. Introduction

Type 2 diabetes is a manageable chronic disease that affects more than 285 million people in the world. According to the report of the World Health Organization, in the year 2000, almost two million Iranians had diabetes. It is predicted that the number of people with type 2 diabetes will increase to 6.4 million by 2030 (1). Environmental and genetic factors, insulin resistance and dysfunction of beta cells are involved in the development of this disease (2). Diabetes and obesity are closely related, and obesity, especially visceral obesity, is one of the important factors in type 2 diabetes (3). Inflammation is one of the most important causes of diabetes, which is associated with an increase in pro-inflammatory cytokines. The progression of type 2 diabetes with insulin resistance is influenced by anti-inflammatory mediators such as IL-6 and TNF-α (4). Tumor necrosis factor alpha (TNF-α) as a pro-inflammatory cytokine that is produced by most cells of the body's defense system, endothelial cells, smooth muscle cells and adipose tissue and as a possible pathophysiological factor in atherosclerosis. Diabetes, cardiovascular disease, etc. are considered (5). IL-6 is another protein that interacts with TNF-α and IL-1 and is secreted from some body cells, especially muscle tissue and fat. Both cytokines TNF-α and IL-6 disrupt insulin signaling, and in laboratory studies, TNF-α injection induces insulin resistance through ERK1/2, JNK, and MAPK pathways, which destroys the mediated signaling pathway. insulin receptor substrates (IRS). On the other hand, the increase in IL-6 plasma levels leads to an increase in SOCS-3 and a decrease in the expression of IRS1(6). Longitudinal studies have shown that regular exercise reduces IL-6 levels and regular activity may suppress low-grade inflammation (7).

It has also been found that in inflammatory diseases such as diabetes, it is related to the increase of serum levels of IL-6 and CRP (8). CRP is a biomarker of general inflammation that is secreted in response to the accumulation of inflammatory cytokines (9). Increased CRP is associated with increased blood pressure, increased body mass, metabolic syndrome, diabetes, dyslipidemia, infection and chronic inflammation (10). On the other hand, research has shown that regular exercise has anti-inflammatory effects and suppresses low-grade systemic inflammation, which can play a role in diabetes management (11). Royal jelly is one of the natural supplements that has been traditionally used for some medical applications for a long time. This substance is secreted from the pharyngeal and submandibular glands of young worker bees and is used to feed the young larvae (for a few days) and the queen (until the end of life). Royal jelly has many important compounds with biological activity such as free amino acids, proteins, sugars, fatty acids, salts (for example, iron and calcium) and vitamins (mainly thiamine, riboflavin and niacin) (12). It has been proven that royal jelly has activities such as relaxing blood vessels and reducing blood pressure, anti-tumor activity, blood lipid lowering effects and anti-inflammatory effects (13). In a research, Etemad and Zahali (2018) showed that royal jelly supplementation caused a significant decrease in the levels of inflammatory indicators such as TNF-α, CRP and IL-6(14). In another study, Panahi (2014) concluded that royal jelly caused a significant decrease in CRP levels in inactive women, which can be effective in preventing cardiovascular diseases (15).

Considering the conflicting results from past researches and the absence of a study that investigated the interactive effects of sports activities and royal jelly consumption on the concentration of TNF-α, CRP and IL-6, in this research the researcher sought to investigate the effect of Pilates training. And the use of royal jelly on the inflammatory indicators of obese diabetic women.

2. Materials and Methods

The method of the present study was semi-experimental with a pre-test-post-test design. The present study was conducted based on the ethical principles of the Islamic Azad University, Tehran East branch, and with the code of ethics committee. The subjects were 44 type 2 diabetic obese women with an age range of 25-35 years and a body mass index greater than or equal to 30, who were selected voluntarily. The final selection of the subjects was as follows: by the available sampling method, among 110 obese people with type 2 diabetes in the 8th district of Tehran who had applied based on a public call, after completing the questionnaire, 44 people were selected as a sample in Available and targeted were selected in this research. The criteria for entering the study were: obese diabetic women aged 25-35 years, body mass index greater than or equal to 30, fasting blood sugar 150 to 250 mg/dL and glycosylated hemoglobin 7 to 10.5, no diseases Cardio-vascular, skeletal-muscular and metabolic, non-menopausal of all subjects, not using insulin and not having any diabetic complications (neuropathy, nephropathy, retinopathy), not participating in regular sports activity more than one session a week during 6 months.

In the past, not smoking, not having a history of diabetes for more than 5 years, and not taking more than one type of oral anti-diabetic pill at night (all subjects were taking metformin in the same amount). Then these subjects were randomly divided into four control groups (11 people), Pilates training (11 people), Royal Gel (11 people), Pilates training + Royal Gel (11 people). Before the start of the study, in a briefing session of all programs, the correct way of performing exercises and possible risks were explained to the participants, and all participants filled out the consent form. In this form, it was emphasized that participating and withdrawing from the study by the applicant is completely free and optional, and all the information of the applicant is completely confidential, and the research results will be published in the form of general and group information. Also, due to the fact that one of the limitations of this study was the lack of strict control of the subjects' diet, so they were asked not to change their usual and daily diet and to avoid consuming any additional food and nutritional supplements.

The training groups did Pilates exercises for 8 weeks, three sessions per week and 60 minutes per session, but the control group and the supplement group were engaged in their daily activities during this time and did not participate in any sports activity program. Each training session included: 10 minutes of warming up, 40 minutes of Pilates exercises and 10 minutes of cooling down. Pilates exercises also included stretching exercises and strengthening exercises (activation).

Table 1: Pilates exercise protocol

movement	repetition	round	intensity	rest	Description	Type of exercise
-	-	-	9-10	-	minutes 10	General warm-up
dogfish, color canvas, roll-up, single-leg stretch, ,cobra	4-8	1	14-16	8 Inhale and exhale	Inhale and exhale one movement at a time	Stretching exercises
Pull-ups, pull-ups, twisting pull-ups, hunched, criss-cross	8 -4	1	16 -14	One) · breath (only	Inhale and exhale one movement at a time	Strengthening exercises
-			9-10	-	minutes 10	(Activation)

In order to measure the anthropometric variables, the weight was measured with minimum clothing and without shoes and using a digital balance with an accuracy of 100 grams. Height was measured using an inflexible tape measure while standing next to a wall. Body mass index was calculated with the ratio of weight (kilograms) to the square of height (meters). The maximum oxygen consumption (VO2MAX) of the subjects was evaluated using the Rockport test. The subjects in the supplement groups also consumed 1000 mg of Royal Oral Gel manufactured by S.A. Martine Nieto Pharmaceutical Company in Spain daily for 8 weeks (16). To measure biochemical variables, blood sampling was done 24 hours before and after the last training session at 7-8 in the morning and after 8-10 hours of fasting. In each step, 5 ml of blood was taken from the subjects' brachial vein. To separate the serum, the blood samples were kept in an incubator at 37 degrees for 30 minutes and centrifuged at 3000 rpm for 10 minutes and finally the serum was extracted. Then the extracted serum was frozen at -20°C to measure TNF-α, CRP, IL-6, glucose and serum insulin. The levels of IL-6 and TNF-α were measured using a human kit (manufactured by BOSTER, China) with a sensitivity of less than 3.0, respectively. Pico grams per milliliter and 1/. Pico grams per milliliter was determined using the ELISA measurement method with a Biotek ELISA reading device made in the United States. CRP was measured using a bionic laboratory kit made in Iran with a sensitivity of 1.95 mg/liter using a mindray Bs-800 device. Serum insulin level using Mercodia AB lab kit made in Sweden by ELISA method with 15/ sensitivity. micro units per milliliter and the percentage of intra-subject variation was measured as 6.48. Fasting glucose was also measured by glucose oxidase enzyme method (Pars Azmoun, Iran) with a sensitivity of 5 mg/dL and a percentage of intra-subject variation of 1.19.

The Shapiro-Wilk test was used to determine the normality of data distribution. Also, analysis of covariance (Ancova) test was used to check the significant difference of each of the research variables between the training and control groups. The significance level was considered for all calculations ($P<0.05$). All statistical operations were performed using SPSS version 24 software.

3. Results

Individual characteristics of subjects (anthropometric characteristics and body composition) are given in Table 2. First, the research data has been described using descriptive statistics including mean and standard deviation. The results of the analysis of covariance between the experimental and control groups in the values of TNF-α, CRP, IL-6, glucose and insulin in the subjects' serum before and after 8 weeks of Pilates training with the use of royal jelly are presented in Table 3. As a result of 8 weeks of Pilates training with royal jelly in obese diabetic women, the levels of TNF-α, CRP, IL-6, glucose and serum insulin decreased significantly ($P<0.05$). The results of Ben Feroni's post hoc test revealed that there was a significant difference in TNF-α and IL-6 between the training + supplement group and the control group ($P = 0.000$). The results of Bonferroni's post hoc test showed that in the CRP variable, this significant difference was related to the supplement group with the exercise + supplement group ($P=.045$), the exercise and exercise + supplement group with the control group ($P=.000$). Also, the results of Bonferroni's post hoc test indicated that there was a significant difference in serum glucose and insulin between the control group and other groups ($P=.000$). As a result of 8 weeks of Pilates training with royal jelly in obese diabetic women, Vo2max values increased significantly ($P<0.05$). The results of Bonferroni's post hoc test showed that in the Vo2max variable, this significant difference was related to the supplement group with the exercise group ($P=.017$) and the exercise group and supplement exercise with the control group ($P=.000$).

Table 2: Average anthropometric characteristics and body composition

Groups		Age (Year)	Height (cm)	Weight (kg)	Body mass index (kg/m^2)
Control	Pre test	۳۱.۲±۴۵.۶۵	۱±۱۶۵.۸۲	۸۴.۶۴ ±03.2	75.۳۰±0.۴۶۹
	Post test			۸۵.۲±۴۱.۰۲	02.31 ±0.۴۸۱
supplement	Pre test	۳۱.۳±۵۴.۴۴	۱۶۴±۱.۰۷	۸۴.۱±۳۶.۸۸	08.31±0.۷۰۶
	Post test			۸۳.۱±۰۵.۹۱	65.30±0.۶۶۶
Exercise	Pre test	۳۲.۳±۱۸.۰۶	۱۶۵±۱.۳۵	۸۵.۲±۶۸.۱۶	60.31±0.۷۵۲
	Post test			۸۴.۱±۲۷.۷۳	70.30±0.۷۰۳
+ Exercise supplement	Pre test	۳۱.۳±۸۱.۰۶	۱۶۴±۱.۷۵	۸۲.۲±۳۴.۰۱	35.30±0.۳۳۵
	Post test			۷۸.۱±۶۰.۷۸	99.28±0.۴۲۰
Significance level	F			Sig=۰.۰۰۰ F=۱۷۱.۰۵	Sig=۰.۰۰۰ F=۱۴۸.۷۹

Table 3: Variables of the research stage before and after eight weeks of Pilates practice and consumption of royal jelly with covariance test

* A significant level (P ≤ 0.05) is considered.

Variables		Control	supplement	Exercise	Exercise + supplement	F	Sig	Effect size
TNF-α(pico gram per milliliter)	Pre test Post test	۲۱.۳±۲۳.۲۳ ۲۱.۲±۱۳.۸۲	۲۲.۳±۱۴.۸۴ ۲۱.۳±۱۰.۸۲	۲۱.۴±۳۷.۳۰ ۲۰.۴±۳۲.۶۶	۲۱.۴±۴۰.۰۹ ۱۹.۳±۸۶.۲۰	۴.۲۷	*0.۰۱۱	0.248
CRP) milligrams per liter)	Pre test Post test	۵.۳±۱۳.۰۴ ۵.۳±۱۵.۰۷	۵.۴±۱۱.۰۴ ۴±۵.۳۰	۴.۳±۹۸.۵۱ ۴.۳±۸۲.۷۲	۴.۳±۹۷.۴۲ ۴.۳±۶۶.۱۳	۷.۰۵	*0.۰۰۱	0.352
IL-6 (pico gram per milliliter)	Pre test Post test	25.3±0.۴۱۲ 25.3±0.۳۰۴	21.3±0.۳۱۱ ±۸۲/۲./۲۳۶	±0.۳۷۸ 20.3 41.2±0.۷۱۵	06.3±0.۴۳۳ 35.2±0.۴۸۳	۸.۰۸	*0.۰۰۰	0.383
Glucose(Millimol/liter)	Pre test Post test	76.10±0.۷۲۸ 97.10±0.۷۹۵	55.10±0.۷۵۱ 86.9±0.۷۷۹	74.10±0.۷۶۶ 58.9±0.۷۷۷	14.11±0.۹۱۰ 72.8±0.۷۹۶	۲۰۵.۹۷	*0.۰۰۰	0.941
Insulin (Millimol/liter)	Pre test Post test	35.7±0.۷۷۳ 71.7±0.۳۸۸	16.7±0.۷۴۴ 66.6±0.۷۹۳	36.7±0.۴۴۸ 57.6±0.۵۵۱	47.7±0.۵۳۳ 96.5±0.۵۷۸	۳۰.۸۵	*0.۰۰۰	0.704
Vo2max (M1.kg/ min)	Pre test Post test	۲۷.۱±۸۳.۱۱ ۲۷.۲±۱۱.۹۸	۲۹.۱±۱۵.۶۷ ۲۹.۱±۹۴.۳۵	19.28± ۱.۴۱ ۳۱.۲±۴۵.۶۱	26.28±0.۵۶۶ ۳۰.۱±۵۲.۲۲	۹.۸۶	*0.۰۰۰	0.523

4. Discussion

The results of the present study showed that eight weeks of Pilates training and royal jelly supplementation significantly reduced TNF-α in obese diabetic women. Etemad and Zahli (2018) reported that eight weeks of aerobic training and 500 mg of royal jelly significantly reduced TNF-α in overweight women (14). Moulai et al. (2018) showed that 6 weeks of aerobic training and 1000 mg of royal jelly caused a significant decrease in TNF-α in patients with MS (16), which is consistent with the results of the present study. The possible mechanism related to the findings of the present research is related to the effect of sports activity on the amount of cortisol and catecholamines; So that the results of research indicate that sports activity can affect the amount of cortisol, catecholamines, carbohydrate reserves, and these changes themselves lead to an increase in TNF-α. However, cortisol level was not measured in the current research, which can be one of the limitations of the current research. Researchers reported that the administration of royal jelly inhibits capillary permeability in the acute phase of inflammation and reduces the formation of granulation tissue in the chronic phase of inflammation (17). In the present study, it was seen that royal jelly supplement inhibits pro-inflammatory cytokines (TNF-α and IL-6) and CRP by stimulating macrophages from LPS (lipopolysaccharides) and TNF-γ (interferon gamma). This type of inhibition does not appear to be caused by cytotoxic effects on macrophages; Rather, it is caused by the effects of royal jelly (18). It has also been seen that TNF-α can bind to oromodulin glycan and this binding is inhibited by oligosaccharides.

Because royal jelly contains large amounts of mannose-rich sugar chains, it acts directly on activated macrophages to inhibit the production of pro-inflammatory cytokines (18). Among the pro-inflammatory cytokines, inhibition of TNF-α by royal jelly is more effective. TNF-α is able to stimulate the cascade of pro-inflammatory cytokines. Effective inhibition of TNF-α production also reduces IL-6 production, which may depend on the effective anti-inflammatory action of royal jelly.

The results of the present study showed that eight weeks of Pilates training and royal jelly supplementation significantly reduced CRP in obese diabetic women. Physical activity reduces interleukin-6 and necrosis factor alpha by reducing fat, leptin, adiponectin and insulin sensitivity, and as a result, reduces CRP (19). Also, the evidence shows that the increase in nitric oxide from the endothelial and the improvement of the function of the endothelial wall reduces systemic and local inflammation, and as a result, reduces the production of inflammatory cytokines from the smooth muscles of the endothelial wall, and their final effect is probably the reduction of the production of the inflammatory index CRP from the liver. (20). The reduction of CRP can be attributed to the effect of royal jelly in addition to exercise. Royal jelly contains important compounds such as proteins, sugars, lipids, amino acids, vitamins and minerals; It also has a wide range of medicinal functions such as antioxidant, anti-edematous, antimicrobial, anti-allergic and anti-tumor properties and protective effects on immune, inflammatory and nervous systems (21). Nora et al. (2020) reported a significant decrease in CRP as a result of eight weeks of endurance training and consumption of royal jelly (100 mg/kg) in the muscle tissue of rats with Alzheimer's disease (22).

The results of the present study showed that eight weeks of Pilates training and royal jelly supplementation significantly reduced IL-6 in obese diabetic women. The long-term increase of IL-6 in type 2 diabetic patients, obesity is related to their inactive lifestyle and may be related to the increase in the number of macrophages located in adipose tissue (23). While exercise causes an acute increase in circulating IL-6 from muscles during contraction, which stimulates the release of glucose from the liver. Therefore, the source of IL-6 in adipose tissue versus skeletal muscle and chronic response versus pulsatile response in healthy individuals and diabetic or obese individuals, respectively, may explain the contradiction related to the role of IL-6 in healthy and diseased humans (24). In other words, it can be said that the sources of IL-6 production in obese diabetics are adipose tissue and in healthy people skeletal muscle. It is well established that insulin resistant and obese subjects with low-grade chronic inflammation recover in concert with a decrease in cytokine levels after interventions such as weight loss (23). However, reducing IL-6 levels may be ineffective in the absence of adipose tissue inflammation (24). Recent studies have shown that the release and abundant production of IL-6 from adipose tissue in obese and overweight people causes a negative regulation of metabolism and may play a role in the development of insulin resistance (25). Research has shown that royal jelly has antimicrobial activity. The ability of royal jelly's antibacterial properties can be attributed to its unique fatty acid. HDA-10 has high antibacterial activity against animal and human pathogens. HDA-10 has been reported to have potential medicinal functions due to its antitumor properties, angiogenesis inhibition, and immunogenetic activities. Inflammatory cytokines such as TNF-α, IL-8, IL-6, IL-1β and TGF-β may cause inflammatory diseases. HDA-10 prevents the production of inflammatory cytokines (26). The results of the present study showed that eight weeks of Pilates training and royal jelly supplementation caused a significant decrease in serum glucose and insulin in obese diabetic women. Studies have shown that consuming royal jelly can improve glucose and insulin levels in diabetic subjects. Askari et al. (2016) reported a significant decrease in serum glucose in type 2 diabetic rats due to the consumption of royal jelly (27). Yilaghi Ashrafi et al. (1400) also reported a significant decrease in glucose and insulin resistance index as a result of eight weeks of intense intermittent exercise and consumption of royal jelly (28), which is in line with the results of the present study. Royal jelly contains large amounts of phenolic compounds from the flavonoid family, the most important of which are quercetin, kaempferol, apigenin, and luteolin. Flavonoids affect diabetes in several ways, these compounds regulate carbohydrate and lipid metabolism and reduce hyperglycemia, dyslipidemia, and insulin resistance, and prevent oxidative stress and inflammatory responses. Flavonoids (especially quercetin) also prevent weight loss in diabetes (29). Therefore, royal jelly with its flavonoid content may have prevented the weight loss of obese diabetic women in the present study. Apigenin and quercetin inhibit the oxidative stress caused by streptozotocin in beta cells, liver and kidney and reduce free radicals (30) Apigenin and kaempferol have hypoglycemic effect in diabetic rats and can reduce fasting glucose. This result was also observed in this study (30).

With its strong antioxidant properties, royal jelly fights against reactive oxygen species such as hydroxyl radicals and superoxide anions and significantly reduces lipid peroxidation and increases antioxidants in the pancreatic tissue of type 2 diabetic patients, which according to According to the mentioned materials, some of these effects are probably due to the presence of flavonoids in royal jelly. The hypoglycemic effect of royal jelly can also be attributed to the vitamins in it (31). Studies have shown that vitamins B, C, D, E, biotin and niacin are abundantly found in royal jelly. Vitamin C reduces the level of serum glucose in type 2 diabetes (31) and in many chemical reactions, it competitively replaces glucose and prevents the glycosylation of proteins, especially hemoglobin and lipoproteins. Vitamins B1, B6, B12, D and E, biotin and niacin also strengthen the function of beta cells and by stimulating the production of glycogen and inhibiting gluconeogenesis, it reduces the glucose level in patients with diabetes. Therefore, part of the role of royal jelly in reducing glucose and insulin can be attributed to the vitamin compounds in it (32).

The results of the present study showed that eight weeks of Pilates training and royal jelly supplementation caused a significant increase in Vo2max of obese diabetic women. Regular physical activities lead to an increase in plasma volume, which increases venous return to the heart and ventricular preload, and as a result, the stroke volume increases for a certain intensity of exercise. An increase in stroke volume at a similar heart rate increases output, blood flow to skeletal muscles increases, and muscle tissue oxygen is provided to a greater extent than before, as a result, maximum oxygen consumption increases.

Antioxidant supplements also increase oxygen supply to the brain, reduce fatigue, prevent blood clots and blood vessels from clogging, for this reason it improves blood flow and oxygen supply to the skeletal muscles, and probably increases the body's oxygen consumption. (33).

Conclusion

In general, it can be said that eight weeks of pilates training and royal jelly supplementation significantly reduced TNF-α, CRP, IL-6, glucose and serum insulin in obese diabetic women. The findings of this research showed the beneficial role of Pilates exercises and the use of royal jelly supplements on the level of inflammation and blood sugar control in obese women with type 2 diabetes. Also, according to the results of the research, it can be said that the use of two intervention methods, regular exercise and consumption of royal jelly together, will make blood sugar control more effective in the form of a reduction in serum glucose and insulin in these people. The existence of a significant difference in TNF-α, CRP, and IL-6 indicates the effective inhibitory role of pilates exercises and consumption of royal jelly in the production of pro-inflammatory cytokines and CRP from stimulated macrophages. Therefore, performing Pilates exercises along with royal jelly supplement is recommended as an effective method in treating diabetes and reducing inflammation for obese diabetic women.

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Author contributions

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

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Conflict of interest None declared.

Ethical approval The Ethics Committee of Islamic Azad University, East Tehran Branch approved the study.

Informed consent Informed consent was obtained from all participants.

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Research Article

The effect of five weeks of aerobic training with royal jelly consumption on glycemic indices in multiple sclerosis rats

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Abstract

Background: Although the beneficial role of exercise and antioxidants in multiple sclerosis (MS) has been reported, the interactive effect of aerobic training (AT) and royal jelly (RJ) consumption is still not well known. Therefore, the aim of the present study was to investigate the effect of five weeks of ET and RJ consumption on glycemic indices of MS rats.

Materials and Methods: In this experimental trial, 49 female Sprague-Dawley rats of EAE model weighing 180-210 gr were divided into seven groups based on their motor disability, including (1) experimental autoimmune encephalomyelitis (EAE), (2) sham (Sh), (3) 50 mg /kg of royal jelly consumption (RJ50), (4) 100 mg /kg of royal jelly consumption (RJ100), (5) aerobic training (AT), (6) AT+RJ50, and (7) AT+RJ100. In order to investigate the effects of EAE on the variables, 7 healthy rats were included in the healthy control group (HC). AT was performed for five weeks, four sessions per week at a speed of 11-15 m/min for 30 minutes, and RJ was injected intraperitoneally with the determined dose daily. To analyze the findings, one-way analysis of variance and Tukey's *post hoc* test were used ($P \leq 0.05$).

Results: Insulin levels in the RJ50, RJ100, ET, ET+RJ50 and ET+RJ100 groups were significantly lower than the EAE group (P=0.01).

Conclusion: It seems that aerobic training combined with royal jelly consumption has a synergistic and favorable effect on improving glycemic indices of MS rats.

Keywords:
Training, Royal Jelly, Glycemic Indices, Multiple Sclerosis.

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1. Introduction

Multiple sclerosis (MS) is an inflammatory disease in the central nervous system, and having this disease is associated with metabolic disorders such as obesity (1). Despite the multifactorial nature of MS disease, studies show that weight gain, metabolic disorders, central and peripheral immune system disorders, and axonal demyelination are associated with the incidence of cardiac disorders in MS patients (1,2). Since the central nervous system controls the vessels and internal organs of the body through the sympathetic and parasympathetic system, there seems to be a strong connection between the nervous system and immune cells; because the disorders of the nervous system due to peripheral disruptions can lead to the reaction of the inflammatory system through the increase of free radicals, and by increasing the circulating levels of circulating pro-inflammatory proteins such as C-reactive protein, interleukin 1 (IL-1), interferon gamma (IFN-γ) lead to damage to the vagus nerves, the adrenergic system, as well as the beta-adrenergic receptor (β-AR) (3,4). Subsequently, the systemic increase of inflammation through disruption in peripheral endothelial is associated with disruption in nitric oxide (NO) levels and ultimately causes disruption in low-density lipoprotein (LDL), cholesterol (Chol) and a decrease in high-density lipoprotein (HDL) levels (4,5). On the other hand, studies show that various synthetic drugs have been developed today to moderate the destructive effects of disorders associated with experimental autoimmune encephalomyelitis (EAE); but despite the advances, these drugs are always associated with various side effects in patients (6).

Therefore, due to the restrictions of the research on human patients, researchers use EAE animal modeling to improve treatment and find the best way to reduce and treat the complications of neuroimmune system deficiency diseases (7). Researchers believe that changing lifestyle from inactive to active lifestyle is one of the non-invasive methods to improve neurotrophins, immune system function and improve quality of life (8). It seems that exercise training improves the metabolism of energy substrates, improves the function of the immune system, reduces inflammation and improves cardiovascular function and finally improves the quality of life of these patients by creating different adaptations (9). Researchers believe that in addition to exercises, proper diet, and the use of natural antioxidants lead to the modulation of immune system function and reduction of inflammation in patients with nervous system disorder (10). One of these natural antioxidants is royal jelly (RJ), which is secreted by the submandibular glands of honey bees and is recommended to patients due to its antioxidant, anti-inflammatory, neurotrophin-improving properties in the nervous system (11). Also, because of its anti-inflammatory and antioxidant properties, RJ improves fat profile and reduces CRP in overweight elderly people (12). Therefore, the potential of RJ in improving the fat profile and improving neurotrophins can be a favorable method in the prevention or treatment of nervous and metabolic system diseases. According to noted sentences present study aimed to investigate the effect of five weeks of aerobic training (AT) along with the consumption of two different doses of RJ on glycemic indices of MS rats.

2. Materials and Methods

In this experimental study with a post-test design along with a control group, 58 female Sprague-Dawley rats with an approximate age of 9 ± 2 weeks, and an approximate weight of 200 ± 20 grams were prepared and transferred to the Animal Physiology Laboratory of the Islamic Azad University, Marvdasht Branch. The samples were kept in the laboratory for one week to adapt to the environment. It is worth mentioning that during the research period, all the ethical principles of working with laboratory animals were observed in compatibility with the Helsinki Agreement and under the supervision of the University Biomedical Ethics Committee. During the whole research period, all standard conditions including 12-12 hours of light-darkness, approximate humidity of 55-60%, and the standard temperature of 22-24° C were observed. Also, during the research protocol, animals had free access to water and special food for rats. In addition, to keep the samples, washable cages and sterile grated soil were used to absorb the urine and moisture in the cages.

Induction of experimental autoimmune encephalomyelitis (EAE)

In order to induce EAE in this research, 20 guinea pigs were prepared at the same time as rats and transferred to the laboratory. Three days later, the guinea pigs were anesthesized with ketamine and xylazine at a dose of 20 mg/kg and 55 mg/kg, respectively. After anesthesia, the spinal cord of guinea pigs was carefully extracted and used as an antigen. Thus, first, the guinea pig spinal cord was placed in a liquid nitrogen tank immediately after extraction and was crushed after a few minutes. Next, to homogenize, guinea pig spinal cord was mixed with an equal amount of normal saline and dissolved for five minutes at room temperature.

Then the homogenous solution obtained was mixed with an equal proportion of complete Freund's Adjuvant (Compound Freund's Adjuvant = CFA) and was dissolved for 10 minutes to turn into a white and uniform solution. This solution, comprising 400 microliters of antigen solution and Freund's complete adjuvant, which finally became a suspension, was then injected subcutaneously after anesthesia in the area next to the spinal cord of rats. Also, 100 microliters of the suspension was injected into the leg pad area of each animal with a No. 25 needle to 50 rats. One week after the injection, the first signs of EAE induction emerged to follow up the condition of the disease. Thus, in order to classify the disease, the following scaling was considered in animals: 0: no disease, 1: tail movement disorder, 2: tail paralysis, 3: gait disorder, 4: one-leg paralysis, 5: paralysis of both legs, 6: paralysis of all four legs and hands, and 7: death It is worth mentioning that one rat died at this stage due to the severity of the disease (13,14).

Grouping and research design

Given the disease scales and homogenization, 49 rats with EAE were divided into seven groups of seven animals, including: (1) EAE control, (2) Sh, (3) consumption of 50 mg/kg of royal jelly (RJ50), (4) consumption of 100 mg/kg of royal jelly (RJ100), (5) aerobic training (AT), (6) AT+RJ50 and (7) AT+RJ100 were divided. It is also worth mentioning that seven healthy rats were included in the healthy control group (HC) to investigate the effects of EAE induction on the research variables.

Endurance aerobic training protocol

To perform endurance training 10 days after EAE induction, rats were first introduced to the treadmill for 5 to 25 minutes every day for a week at a speed of 6 m/min and an incline of 11 degrees. Next, they did endurance training every day at a speed of 11 m/min for 30 minutes for 5 weeks (15,16).

Consumption of royal jelly

To consume royal jelly at doses of 100 and 50 mg / kg during five weeks, the daily required royal jelly prepared from Marvdasht Agricultural Jihad Center was dissolved in normal saline and was then injected peritoneally into rats (17).

Dissection

48 hours after the last training session, rats were anesthetized using a combination of ketamine and xylazine in a 12-hour fasting state. After ensuring complete anesthesia, 4-5 cc of blood was taken directly from the heart tissue of the samples using a 5 cc Supa syringe made in Iran. It is worth mentioning that to separate the serum from the plasma, the samples were left at room temperature for 2 hours to clot. Next, the blood samples were centrifuged for 10 minutes in an eight-channel centrifuge manufactured by Behdad Company in Iran at 13,000 revolutions per minute to separate serum and plasma. These serum samples were kept at a temperature of -21° C until the time of measurement.

Method of measuring glycemic indices

To measure glucose the kit of Pars Azmoun company with mg/dL scale, and to measure insulin, the kit of Pars Azmoun company with µIU/mL scale were used.

Statistical analysis

The Shapiro-Wilk , one-way analysis of variance test along with Tukey's *post-hoc* tests were used for statistical analysis of data (P≤0.05).

3. Results

The results of one-way analysis of variance showed a significant difference in the levels of glucose (P=0.001 and F=6.41) and insulin (P=0.001 and F=11.01) in the research groups. The results showed that blood glucose levels in the EAE group were significantly higher than the HC group (P=0.001), but no significant difference was observed in the Sh and EAE groups (P=0.99). However, in the ET+RJ50 (P=0.02) and ET+RJ100 (P=0.01) groups, the levels were significantly lower than the Sh group (D-1). Insulin levels in the EAE group were significantly higher than the HC group (P=0.001); However, no significant difference was observed in the Sh and EAE (P=0.99) groups. Insulin levels in the RJ50 (P=0.003), RJ100 (P=0.002), ET (P=0.005), ET+RJ50 (P=0.001) and ET+RJ100 (P=0.001) groups were significantly lower than the EAE group (D-2).

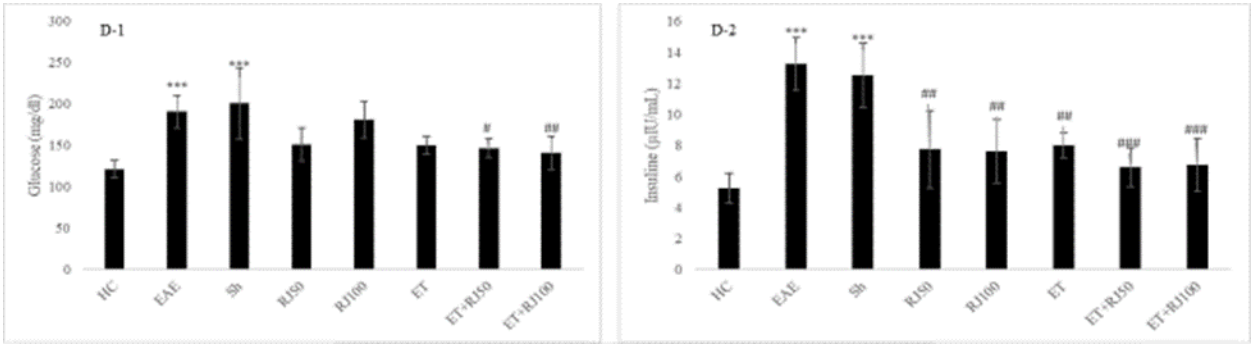

Figure 1: Levels of glycemic indices in research groups: Blood glucose (D-1) and insulin (D-2) in the research groups rats

***(P=0.001), significant change compared to the HC group

#(P=0.05), ## (P=0.01) significant change compared to the Sh and EAE group

4. Discussion

The results of the present study showed that blood glucose levels in the ET+RJ50 and ET+RJ100 groups were significantly lower than the Sh group. Insulin levels in the RJ50, RJ100, ET, ET+RJ50 and ET+RJ100 groups were significantly lower than the EAE group. In an study, swimming training reduced LDL, VLDL, TG, cholesterol and insulin resistance levels in diabetic rats (18). Also, in the field of metabolic indicators in patients with MS, researchers showed that three weeks of aerobic training reduced fatty acids, total cholesterol, and TG levels. In addition to this, the results of this study showed that following exercise training, fatty acid oxidation increased, and this improved metabolism and physical performance in MS patients (19). Eight weeks of aerobic training decreased total cholesterol, LDL, TG, VLDL and body fat percentage in women with MS (20). Also, in a previous study, the results showed that aerobic training improved weight loss, visceral fat weight, and aerobic capacity in an EAE model (21).

In a pilot study, researchers showed that 12 weeks of moderate-intensity endurance training reduced LDL, VLDL, and intermediate-density lipoprotein particle count in patients with MS, however no significant change was reported following high intensity endurance training. Also, 12 weeks of moderate-intensity training improved the blood glucose of these patients (22). In a study, consumption of 100 mg/kg of RJ led to a decrease in visceral fat weight, improved aerobic capacity and caloric intake in the EAE model, and the interactive effect of training and RJ with a dose of 100 mg/kg was far more favorable than training or RJ alone (21). In addition, RJ consumption led to improvement of adipokines, reduction of CRP, reduction of LDL, increase of HDL and increase of total antioxidant capacity in overweight elderly people (12). In another study, researchers showed that daily consumption of 1000 mg of RJ increased HDL, decreased TG, decreased hs-CRP and total cholesterol in women with type 2 diabetes (23). Studies show that doing aerobic training and taking RJ, depending on the dosage, duration of treatment, intensity, type and duration of training can lead to the improvement of metabolic indicators.

Conclusion

According to findings of present study it appears that aerobic training combined with royal jelly consumption has a synergistic and favorable effect on improving glycemic indices of MS rats.

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

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