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Journal of Sports Physiology and Athletic Conditioning (J.S.P.A.C) 2022; 2 (5): 1-13

Research Article

Effect of Aerobic Training along with Garlic on Oxidative Stress Index in Obese Women with High Blood Pressure

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Abstract

Background: A large amount of evidence shows that oxidative stress plays a central role in hypertension pathophysiology. The aim of this study was to examine effect of aerobic training along with Garlic on oxidative stress index in obese women with high blood pressure.

Materials and Methods: In this clinical trial study, 36 postmenopausal obese women with hypertension were purposefully and accessibly selected from Sari and were simple randomly divided into four groups Control (C), Aerobic Training (AT), Garlic (G) and Aerobic Training +Garlic (ATG). The training groups participated in a progressive aerobic training for eight weeks, three sessions a week (55% to 65% of the reserved heart rate and for 30 to 55 min). The groups of G and ATG were provided 1000 mg of garlic supplement for eight weeks (After breakfast and dinner). Two days before and after the protocol, blood samples were taken in fasting state. SPSS 16.0 software was used for statistical analysis. Data were analyzed using ANCOVA at $p<0.05$.

Results: The results showed that the levels of malondialdehyde (MDA) (P=0.0001) decrease significantly in the experimental groups. Also, superoxide dismutase (SOD) (P=0.001), glutathione peroxidase (GPX) (P=0.000) and catalase (CAT) (P=0.001) in the experimental groups increased significantly compared to the C group. The amount of SOD, GPx and CAT in the ATG group was significantly higher than the AT and G group ($p\leq0/05$).

Conclusion: It seems that AT and G has interactive effects on reducing Oxidative Stress in obese women with high blood pressure.

Keywords:
Aerobic exercise, Garlic, Oxidative Stress, Hypertension, Obese Women

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1. Introduction

Hypertension is an important modifiable risk factor for premature cardiovascular disease (CVD), however, 43% of adult women have hypertension (1). Blood pressure (BP) increases with age in both sexes, but blood pressure is higher in women after middle age than in men. It has also been shown that after menopause, the prevalence of high blood pressure in women increases (2). Oxidative stress has been implicated as a mediator of hypertension, as most studies have shown that individuals with lower blood pressure also have higher levels of endogenous antioxidants such as vitamins E and C (3). Chronic inflammation and oxidative stress are factors in endothelial dysfunction (4) that are directly involved in increasing systemic vascular resistance and thus increasing blood pressure (BP). Oxidative stress is caused by a systemic imbalance between the production of reactive oxygen species (ROS) and antioxidant capacity (5). ROS levels may be increased due to decreased antioxidant enzyme activity in hypertensive individuals, while reduced oxidative stress may reduce blood pressure (6). Exercise training has a positive effect on blood pressure by reducing lipid peroxidation and oxidative stress (7). Previous studies have reported that although a single bout of exercise increases ROS production, long-term exercise can also regulate the expression of antioxidant defense systems (8). Epidemiological studies have also shown that regular physical exercise performed at low to moderate intensity per week is more suitable for middle-aged and elderly people (9).

Therefore, aerobic training (AT) can be a suitable way for these people by improving the state of oxidative stress in obese women with high blood pressure. AT has been shown to be effective in a decrease of the occurrence of ROS-associated hypertension (10) and exerts a protective effect on oxidative damage, the exact mechanism of the effect of exercise training on cardiovascular function is not well known. In this regard, da Silva et al. (2022) showed that 16 weeks of AT was associated with blood pressure improvement in women with hypertension (11). Several other studies have shown that AT is an important non-pharmacological strategy for the prevention and treatment of hypertension (12). AT reduces sympathetic tone, arterial blood pressure, oxidative stress (13) and inflammation (14). However, Ruangthai et al. (2019) showed that endurance exercise does not have a significant effect on plasma MDA (15). On the other hand, studies have shown that some plants also play a role in controlling blood pressure by modulating oxidative stress. Garlic (*Allium satium L.*) is a nutrient that has beneficial cardiovascular properties and effects and contains various compounds including organosulfur compounds, amino acids, vitamins and minerals. Some sulfur-containing compounds are responsible for the healing properties of garlic, including Allicin, S-allyl Cycteine, and Diallyl Disulfide (16). It has been shown that daily consumption of 1200 mg of garlic significantly reduces systolic and diastolic pressure, decreases arterial wall resistance and lowers total cholesterol level (17).

Also, consuming garlic improves the performance of the antioxidant defense system by increasing superoxide dismutase, catalase and glutathione peroxidase (18). By introducing this trend, blood pressure can be lowered in hypertensive patients with antioxidant measures, and it is possible that one of the mechanisms to reduce cardiovascular diseases caused by high blood pressure is performing endurance sports and using antioxidant supplements. Considering the role of sports activities in reducing and adjusting oxidative stress indicators and the role of oxidative stress in the development of blood pressure, as well as the inhibitory effect of garlic on oxidative stress indicators, the researcher assumes that the simultaneous effect of aerobic exercise with garlic consumption has a better effect on oxidative stress in obese women with high blood pressure. Therefore, this study intends to study the simultaneous effect of garlic consumption and AT on oxidative stress in obese women with hypertension.

2. Materials and Methods

Subjects

In this clinical trial study, 36 inactive postmenopausal women with hypertension (blood pressure higher than 140/90 mmHg) and obesity (body mass index ≤ 30) from Sari city (summer 2018) aged 50 to 65 were included in the study. Sampling was done among hypertensive people voluntarily, purposefully and available. After completing the number of subjects for the research, by making a phone call and obtaining their consent, 36 eligible people were randomly divided into four groups Control (C), Training (AT), Garilc (G) and Training+Garilc (ATG). The criteria for entering the study include: confirmation of hypertension by a doctor, absence of other underlying diseases (structural diseases and skeletal muscle abnormalities, no fractures and sprains in different body tissues, no history of any fractures and surgeries in the last 1 year, disease heart and liver problems), not participating in a regular exercise program in the last six months, being allowed to participate in the exercise program with the doctor's opinion and consenting to participate in the study. The exclusion criteria from the study included not taking supplements and not doing exercise, diagnosis of other underlying diseases during the implementation of the protocol, change in the pattern of drug consumption, feeling the risk of exercising or taking supplements, and not having a phone call from the researcher for follow-up. In this study, the subjects also participated in an exercise familiarization program. Subjects were asked not to change their diet during the study period.

Exercise protocol

The AT group trained for 8 weeks (24 sessions, 3 sessions per week). The duration of the activity was 30 to 55 minutes and the intensity of the activity was considered to be 55 to 65% of the maximum reserve heart rate (Table 1) (4). It should be noted that the training program in the gyms was standardized and implemented with safety precautions, and a Polar heart rate monitor was used to control the intensity of the training.

Table 1: Aerobic training protocol for menopausal women with high blood pressure and obesity

week	1	2	3	4	5	6	7	8
Time (minutes)	30	35	40	45	50	50	55	55
Intensity (HR reserve)	55	55	60	60	60	65	65	65

Supplement preparation and consumption

The garlic supplement was purchased under the brand name (Natural Made) made in the United States. 500 mg of the supplement was placed in gelatin capsules. The subjects were asked to take one capsule after breakfast and dinner. The subjects of the C and AT groups also used the placebo (starch capsule) twice a day in the same amount and volume as the G group. Capsules containing garlic supplement and placebo (capsule containing starch) were given to the subjects in a double-blind manner. The dosage (960 mg) has previously been used in hypertensive patients (systolic blood pressure above 140 mmHg) (20).

Blood sampling and laboratory analysis

Two days before and after the training session, blood samples were taken from the brachial vein in a fasting state (12 hours). MDA serum levels were measured based on the reaction with thiobarbituric acid and using a fluorimetric device. SOD was measured using a commercial kit made by RANDOX company, and GPX and CAT levels were measured by spectrophotometric method.

Statistical analysis

Shapiro-Wilk test was used to ensure normal distribution of data and Levin test was used to ensure homogeneity of variances. Descriptive statistics were used to describe the data and draw graphs, and ANCOVA was used to compare the groups in the studied variables. Significant level was considered P≤0.05. All statistical analysis was performed using 26 SPSS software.

3. Results

Table 2 show the mean changes in weight, height, BMI, WHR, systolic blood pressure (SBP) and diastolic blood pressure (DBP) in different groups (Tab 2).

Table 2: Results related to subjects' characteristics and SBP and DBP

		C	AT	G	ATG	P Between-group
Age (years)	Pre-test	59.89 ± 4.45	61.89 ± 4.85	61.89 ± 4.70	62.56 ±4.66	0.652
Height(meters)	Pre-test	1.56 ± 0.049	1.55 ± 0.037	1.55 ± 0.032	1.56 ±0.040	0.840
Weight (kg)	Pre-test	72.78 ± 8.24	74.00 ± 6.22	69.67 ± 7.64	72.33 ±6.42	
	Post-test	72.44 ± 9.04	69.44 ± 4.47	65.56 ± 6.48	66.56 ±6.12	0.009 β
	P within-group	0.700	0.004*	0.001*	0.005*	
BMI (Kg/m2)	Pre-test	29.70 ± 3.99	30.68 ± 3.54	28.96 ± 3.14	29.75 ± 2.89	
	Post-test	29.57 ± 4.28	28.76 ± 2.55	27.27 ± 2.81	27.33 ± 2.05	0.010 β
	P within-group	0.692	0.005*	0.001*	0.006*	
WHR	Pre-test	0.860±0.051	0.878 ± 0.05	0.882 ± 0.11	0.856 ±0.087	
	Post-test	0.922±0.058	0.853 ± 0.067	0.891±0.107	0.893 ±0.093	0.0001 β
	P within-group	0.000	0.061	0.421	0.042	
SBP (mmHg)	Pre-test	140.67±10.05	143.11 ± 9.06	147.11±8.55	144.67 ± 9.24	
	Post-test	141.56 ± 9.36	30.36±131.78[a]	135.78±8.84a	130.22±15.63a	0.0001 β
	P within-group	0.362	0.0001*	0.002*	0.001*	
DBP (mmHg)	Pre-test	84.33 ± 6.40	82.89 ± 5.08	83.22 ± 7.29	84.89 ± 3.98	
	Post-test	85.11 ± 5.13	81.33 ± 2	82.67 ± 5.14	80.44±14.2[a]	0.032 β
	P within-group	0.432	0.270	0.714	0.003*	

* significance of within-group, a: difference with control group β: difference between groups (P≤0.05)

After confirming the normality of the data using the Shapiro-Wilk test; The results of within-group comparison showed a significant decrease in the average MDA levels in groups AT (P=0.008), G (P=0.009) and ATG (P=0.0001) (Fig 1). Data analysis using covariance test showed that there is a significant difference in the amount of MDA changes between different groups (P=0.0001). The results of Benferroni's post hoc test showed that there is a significant difference between group C with AT (P=0.015), G (P=0.007) and ATG (P=0.0001); and also, between ATG group with AT (P=0.018) and G (P=0.042) (Fig 1).

The results of within-group comparison showed a significant increase in the mean levels of GPx in groups AT (P=0.0001), G (P=0.012) and ATG (P=0.001) (Fig 2). Data analysis using covariance test showed that there is a significant difference in the amount of GPx changes between different groups (P=0.0001). The results of Benferroni's post hoc test showed that there is a significant difference between group C with AT (P=0.015009 G (P=0.012) and ATG (P=0.0001); and also, between ATG group with AT (P=0.031) and G (P=0.016) (Fig 2).

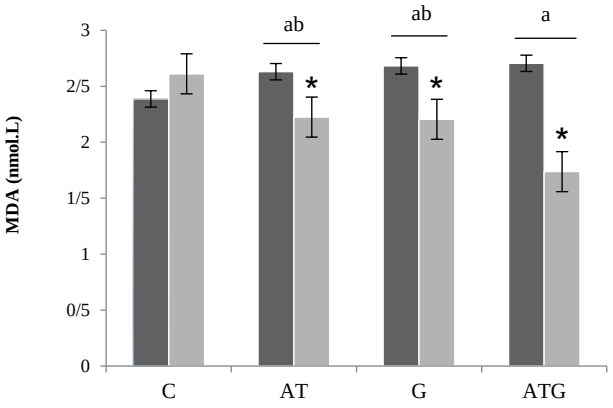

Figure 1: Changes in serum MDA levels in different groups using t test and ANCOVA test (p<0.05 level).

*****Difference with pre-test, a Difference with C group, b Difference with ATG group.

Control (C), Aerobic Training (AT), garlic (G) and Aerobic Training-garlic (ATG).

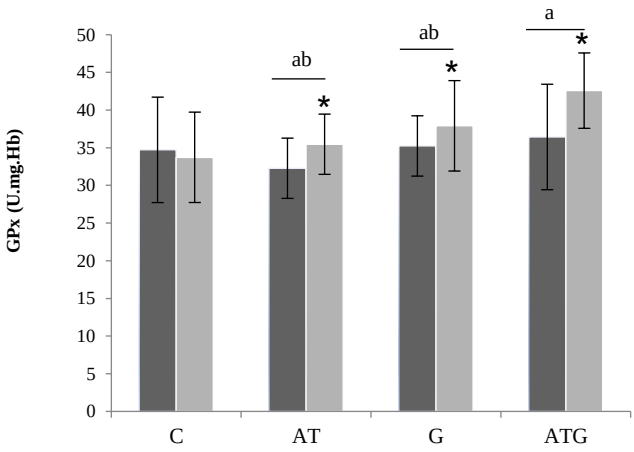

Figure 2: Changes in serum GPx levels in different groups using t test and ANCOVA test (p<0.05 level).

*Difference with pre-test, a Difference with C group, b Difference with ATG group.

Control (C), Aerobic Training (AT), garlic (G) and Aerobic Training-garlic (ATG).

*Difference with pre-test, a Difference with C group, b Difference with ATG group.

Control (C), Aerobic Training (AT), garlic (G) and Aerobic Training-garlic (ATG).

In addition, the results of within-group comparison showed a significant increase in the average SOD levels in AT (P=0.036), G (P=0.031) and ATG (P=0.0001) groups (Fig 3). Data analysis using covariance test showed that there is a significant difference in the amount of SOD changes between different groups (P=0.0001). The results of Benferroni's post hoc test showed that there is a significant difference between group C with AT (P=0.027), G (P=0.045) and ATG (P=0.0001); and also, between ATG group with AT (P=0.042) and G (P=0.023) (Fig 3).

Among the other results of this research, using the within-group test, there was a significant increase in the average CAT levels in groups AT (P=0.015), G (P=0.014) and ATG (P=0.0001) (Fig 4). Data analysis using covariance test showed that there is a significant difference in the amount of CAT changes between different groups (P=0.0001). The results of Benferroni's post hoc test showed that there is a significant difference between group C with AT (P=0.027), G (P=0.022) and ATG (P=0.001); and also, between ATG group with AT (P=0.006) and G (P=0.003) (Fig 4).

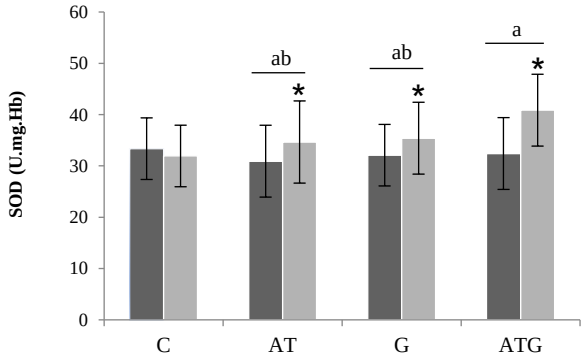

Figure 3: Changes in serum SOD levels in different groups using t test and ANCOVA test (p<0.05 level).

*Difference with pre-test, a Difference with C group, b Difference with ATG group.

Control (C), Aerobic Training (AT), garlic (G) and Aerobic Training-garlic (ATG).

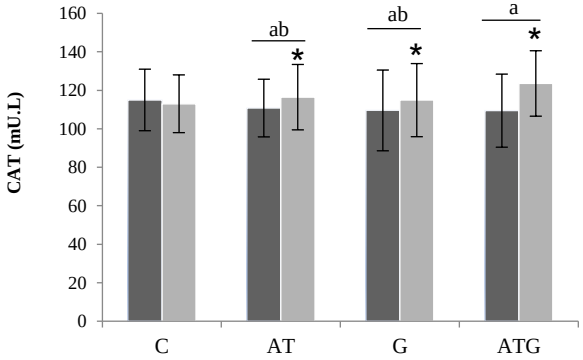

Figure 4: Changes in serum CAT levels in different groups using t test and ANCOVA test (p<0.05 level).

*Difference with pre-test, a Difference with C group, b Difference with ATG group.

Control (C), Aerobic Training (AT), garlic (G) and Aerobic Training-garlic (ATG).

4. Discussion

The results of the present study showed that AT in women with high blood pressure was associated with a decrease in the serum level of MDA and an increase in SOD, GPx and CAT. Oxidative stress plays a key role in the pathophysiology of hypertension (10). The importance of redox imbalance in hypertension has also been demonstrated in many population-based studies. Persistent accumulation of free radicals due to impaired redox homeostasis negatively affects vascular function, thus contributing to the initiation and progression of hypertension (21). Clinical studies of hypertensive patients showed that SBP and DBP were positively correlated with oxidative stress biomarkers and negatively correlated with antioxidant levels. Decreased antioxidant activity (SOD) and increased levels of lipid peroxidation (MDA) may contribute to oxidative stress in human hypertension (22). Naregal et al. (2017) found that plasma MDA levels were significantly increased and SOD antioxidant activity was decreased in elderly hypertensive subjects compared to healthy subjects (23). In addition, some studies have shown that exercise may be able to reduce oxidative damage and increase the clearance of reactive aldehydes in patients with hypertension (23). In line with the present study, Wu et al. (2021) showed that 12 weeks of AT in adult women increased SOD, TCA and decreased MDA (24). Park et al. (2017) also stated that 15 sessions of AT with low to moderate intensity led to positive effects on blood lipid profile, blood pressure, level of blood inflammatory markers and oxidative stress in women over 70 years old (25).

Also, Dantas et al. (2016) showed that exercise increases TAC levels and decreases MDA and mean blood pressure in elderly hypertensive women (26). In the present study, the decrease in MDA concentration was associated with an increase in GPx. GPx, an antioxidant enzyme responsible for the degradation of lipid peroxides, protects the cell membrane against peroxidative damage. Therefore, the decrease in MDA concentration is probably due to the increase in GPx activity (15). Previous studies have shown that AT is effective in reducing ROS and increasing adaptation to oxidative stress by increasing the level of antioxidants in hypertensive subjects (10). It has also been reported that by increasing the frequency of exercise to four days a week or more, markers of oxidative stress improve in people with high blood pressure who participate in walking (27).

Among the other results of the current study, there was a significant decrease in MDA and an increase in SOD, GPx and MDA following the consumption of garlic in obese adult women. In a study, Seo et al. (2012) showed that a 12-week diet of garlic extract combined with regular exercise reduced body weight, body mass index, total cholesterol, LDL-C, malondialdehyde and homocysteine levels reduced cardiovascular risk factors in menopause (28). Ahmadian et al. (2017) found that daily supplementation of 800 mg garlic tablets for four weeks, compared to placebo, resulted in increased TAC concentrations and decreased MDA levels in postmenopausal women with osteoporosis (29).

In agreement with our study, some previous studies reported that garlic supplementation could increase TAC and decrease MDA levels (29, 30). Allicin, diallyl disulfide, and S-allylcysteine derived from garlic contain redox-active sulfhydryl (SH) groups that act as radical scavengers. Also, the mechanism of the antioxidant effect of garlic may be involved in regulating the Nrf2-ARE pathway and increasing the activity of antioxidant enzymes (31). Nrf2 trans-activation promotes the up-regulation of antioxidant genes responsible for the expression of antioxidant enzymes (32). In addition, in vitro studies have shown that garlic can protect endothelial cells against ROS damage by up-regulating cellular inhibitory enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (33). However, in the study of Atkin et al. (2016), no significant effect on oxidative stress markers was observed in the garlic group compared to the placebo group (34). Maybe the difference in the type of subjects caused the difference in the results.

Another finding of the present study was the greater effect of combining AT with garlic on oxidative stress indices in women with high blood pressure. In this regard, Ghyasi et al. (2019) showed in an animal study that six weeks of exercise combined with garlic consumption significantly increased the levels of GPx, SOD, CAT and TAC (35). It was also shown that AT with garlic consumption improves inflammatory indices along with reducing systolic blood pressure and body composition in hypertensive and obese women (14). It seems that in the current research, the combination of exercise and garlic has a synergistic effect and has led to better results than exercise and garlic alone.

Since high blood pressure disease leaves its effects in the long term, perhaps the length of the research period in the present study is another important limitation for the detailed examination of the effects of AT and garlic consumption on this disease. Therefore, it is recommended to use longer periods in subsequent protocols. Also, the gender and age of the subjects was one of the limitations of this study, so the results of this protocol cannot be generalized to other people. Finally, the generalizability of the results is limited due to the small sample size. Therefore, the results should be interpreted with caution.

5.Conclusion

The results of the present study showed that AT and garlic consumption improved oxidative stress in hypertensive and obese women, however, the effect of combining AT and garlic on oxidative stress was better. Therefore, the combination of these two can be used as a strategy to control blood pressure and oxidative damage caused by it in obese hypertensive adult women.

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Author contributions

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

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval the research was conducted with regard to the ethical principles (IR.SSRI.REC.1398.039), and it was registered in the clinical trial system under number IRCT20140415017288N5.

Informed consent Informed consent was obtained from all participants.

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Research Article

Plasma Nesfatin Responses Following a Single Session of Interval Exercise in Young Men: Effects of Glucose, Sucrose and Fructose Intake

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Abstract

Background: Nesfatin is a key regulator of glucose metabolism. The aim of this study was to identify the effect of glucose, sucrose and fructose intake following acute high intensity interval exercise (HIIE) on plasma levels of nesfatin, insulin and glucose in young males.

Materials and Methods: 32 sedentary young males (21.9±2.3 yrs, 77.5±8.9 kg) were assigned into four groups (n= eight per group): glucose, sucrose, fructose and control or water groups. Subjects completed 4×4 min interval running with 90-95% maximal heart rate (HRmax) and 3 min active recovery with 65-70% HRmax between each interval. Blood samples were collected before, immediately after, 30, 60 and 90 minutes after exercise session. Immediately after the second blood sampling, carbohydrate liquids (1.5 g/kg glucose, fructose, sucrose and water) were consumed by the subjects in different groups. The data were analyzed using repeated measures ANOVA test and SPSS-24 software.

Results: Results indicated that there was no significant difference between groups for Nesfatin ($p=0.519$) and glucose ($p=0.062$) levels; but, there was a significant difference between groups for insulin levels ($p<0.001$). Bonferroni multiple comparison corrections as a post hoc test showed a significant difference between water and glucose, water and sucrose, glucose and fructose, and sucrose and fructose groups in 30 and 60 minutes after HIIE ($P<0.05$).

Conclusion: with respect to the present study results, acute carbohydrate supplements (glucose, sucrose and fructose) don't affect nesfatin response following exercise. Therefore, it seems that nesfatin doesn't affect acute exercise-induced metabolic status response to different carbohydrate supplements in healthy subjects.

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1. Introduction

Nesfatin-1 was discovered in 2006 by Oh and Colleagues as a peptide with 82 amino acids, which derived from NEFA/nucleobindin2 (NUCB2) as its precursor. These researchers identified nesfatin-1 in the hypothalamus and suggested that this peptide can exert an anorexigenic effect (1). However, subsequent studies reported that nesfatin-1 is also expressed by different tissue, including pituitary, adipose tissue and pancreas that all of these tissues play important role in regulating energy homeostasis and metabolism (2). Especially, nesfatin-1 influences glucose metabolism and observed that this peptide regulates glucose homeostasis and insulin secretion in rodent (3) and human (4) subjects. Moreover, Nesfatin-1 cause an increase in glucose-stimulated insulin secretion from b-cells by promoting $Ca2^{+}$ influx through L-type calcium channels (5) and regulatory effects of nesfatin-1 on insulin resistance is mediated by inhibition of hepatic glucose production which is associated with increased glucose uptake and also increasing insulin receptors (IRs) (6).

It's suggested that the intravenous injection of nesfatin-1 reduces the blood levels of glucose in hyperglycemic mice (7) and acute subcutaneous infusion of nesfatin-1 reduced food intake in male rats, also a one-day peripheral injection of nesfatin-1 results in increasing physical activity with fat oxidation and simultaneously foot intake was decreased (8), these results represent that nesfatin-1 is a multifunctional peptide. Circulating nesfatin-1 changes in different nutritional and metabolic statuses. it has been shown that there is a correlation between glucose intake and nesfatin-1 changes (4) and suggested that a high dose of glucose consumption is associated with increasing nesfatin-1 gene expression in human islets (9).

In addition to nutrition, blood levels of nesfatin-1 are affected by acute and chronic exercise in human subjects. Exercise is a powerful physiological stimulant that affects the metabolism, oxidation of substances, secretion of hormones and brain neurotransmitters. The range of acute exercise (depending on the type, severity and duration of exercise) can cause short-term suppression of appetite and lower insulin levels (10). Some researchers have examined the effect of acute exercise on nesfatin-1 response. Ganbari Niaki et al. (2010) investigated the effects of acute high intensity and circuit resistance exercise on nesfatin and glucose regulating hormones in male kickboxers but observed no significant changes in nesfatin-1 after two exercise protocols. However, there was a significant change in glucose regulating hormones including, glucagon and cortisol (11). In another study, Mohebbi et al (2015) investigate the effect of exercise in the anaerobic threshold or maximum fatty oxidation intensity on plasma levels of nsafatin-1 and leptin in overweight men. These results indicated that higher intensity exercise resulted in a more significant decrease in nesfatin-1 and leptin compared to exercise with maximum fat oxidation (12). Generally, the effect of acute exercise on nesfatin-1 is controversial. Because of the interaction of glucose metabolism, nesfatin-1 and the effect of acute exercise on nesfatin-1 levels. On the other hand, the aim of the present study was to investigate the acute effect of exercise along with sugars (glucose, fructose and sucrose) supplementations on nesfatin-1 levels in young males.

According to our knowledge is the first study that investigates the acute effect of interval exercise in combination with sugars especially fructose and sucrose supplementation on nesfatin-1 levels. We hypothesized that sugars supplement can affect nesfatin-1 response to acute exercise.

2. Materials and Methods

To perform this study, 32 sedentary healthy young males (the age, weight, height and BMI were 21.9±2.3 yrs, 77.5±8.9 kg, 176.8±1.1 cm, 24.3±2.1 kg/m2 respectively) were recruited for participation in the present study protocol. None of the subjects participated in the regular exercise training program in the last year. One week before performing the exercise protocol, the methodology for conducting the study was explained to the subjects and finally, all of them reviewed and signed Informed consent, and also completed the medical questionnaire. All of the study stages were conducted according to ethical guidelines of the Helsinki Declaration and Ethical approval was obtained from Islamic Azad University, Science and Research Branch, Tehran, Iran. Inclusion criteria for to present study included: non-drug and alcohol addiction, lack of regular exercise activity for at least one year, no history of kidney, liver, cardiovascular diseases, diabetes and any type of injury or physical problem. Since the participants were residents of the university of Mazandaran (Mazandaran, Iran) dormitory, they used the same food as the university self-service. However, the participants were emphasized to consume the same and certain food two days before blood sampling.

The following conditions were required for blood sampling: 1) no use of drugs or supplementation during the course of the study; 2) no change in diet at least two days before the test; 3) avoid heaven physical activity at least one week before the test; 4) no consumption of coffee, banana, cereals and fatty food at least 24 hours before exercise protocol. Finally, participants were randomly assigned into four groups each group consists of eight subjects, including glucose, sucrose, fructose and control groups, who all participated in an acute exercise session. Exercise sessions were performed between 8-11:48 a.m. exercise intervention started with 10 minutes warm-up including running at 70 per cent of maximum heart rate (HRmax) followed by a main acute exercise protocol which conducted as a high-intensity interval exercise (HIIE). HIIE protocol consists of 4×4 minutes' interval running that each interval performed in 90-95% HRmax that conducted on the treadmill and immediately after each intensive four minutes interval, three min active recovery with 65-70% HRmax was performed (13). Immediately after completing the HIIE protocol, Blood samples were collected (second blood sampling after pre-test sampling) and as soon as possible after the second blood sampling, sugary liquids, including 1.5 per kilogram of body weight (g/kg) of glucose, fructose and sucrose consumed by glucose, fructose and sucrose groups respectively. Moreover, subjects in the control group consumed 3.5 ml water per g/kg immediately after the HIIE and blood samples were again collected 30, 60 and 90 minutes after sugar (or water) supplementation.

Briefly, blood samples were collected five times: before, immediately after, 30, 60 and 90 min after the completion exercise protocol. Plasma nesfatin-1 and insulin levels were measured by the ELISA method and glucose levels were determined by a commercial Pars Azmon kit, Tehran, Iran. Data analyzed by SPSS version 24. To determine the normality of data distribution, Kolmogorov-Smirnov (KS) test and for homogeneity of variances, Levene tests were used. Differences between and within groups calculate by repeated-measures ANOVA that was followed up with Bonferroni post-hoc test.

3. Results

The Kolmogorov-Smirnov and the Levene test indicated that the present study data have a normal and appropriate distribution (P> 0.05). Inter-group results for insulin levels showed a significant difference between groups (p< 0.001). Bonferroni post-hoc test for insulin levels indicated that there was a significant difference between the glucose group with water group in 30 min (p= 0.002) and 60 min after HIIE (p= 0.008); also significant differences between the glucose group with the fructose group in 30 min (p= 0.01) and 60 min after HIIE (p= 0.04) were observed. In addition, Bonferroni post-hoc test indicated that there is a significant difference between the sucrose group and with water group in 30 min (p= 0.001) and 60 min after HIIE (p= 0.001). Moreover, there was shown a significant difference between the sucrose group with the fructose group in 30 min (p= 0.003) and 60 min after HIIE (p= 0.02). In summarizing, insulin levels significantly increased in glucose and sucrose groups in comparison to water and fructose groups 30 minutes and 60 minutes after HIIE completion. Intra-group results of insulin for different groups showed that there was a significant difference only in the glucose and sucrose group. According to the present study results, insulin levels of glucose and sucrose groups significantly increased in 30 minutes and 60 minutes after HIIE in comparison to before and immediately after HIIE stages (p< 0.05) (Fig 1).

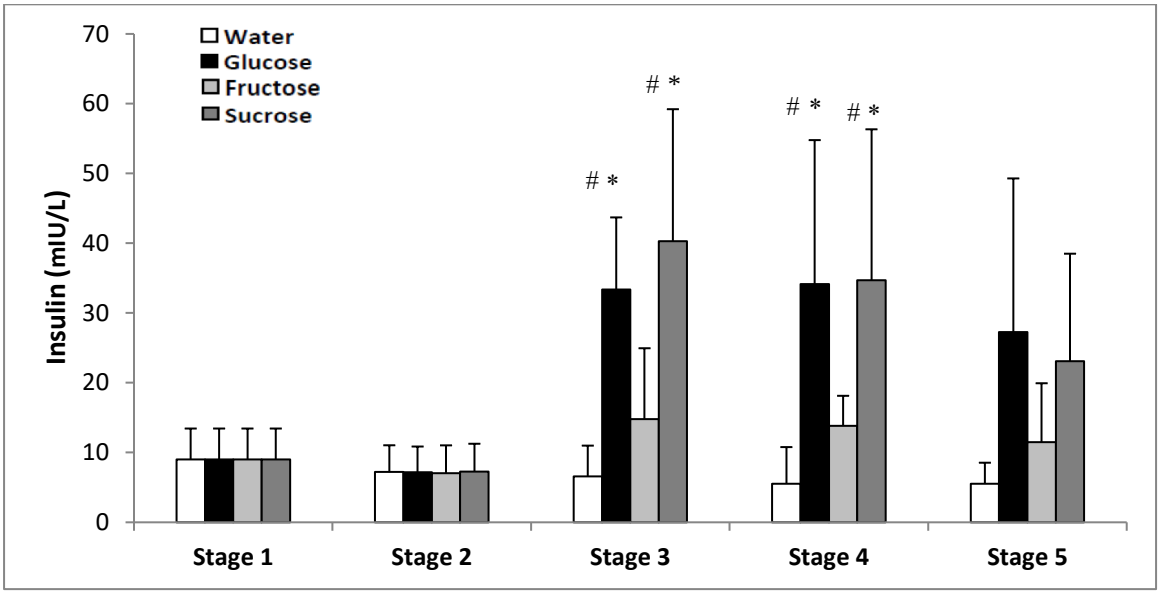

Figure 1: Changes in insulin levels. * Indicated a significant increase in comparison to water and fructose groups. # Indicated a significant difference in blood sampling time in glucose and sucrose groups: significant insulin increases in glucose and sucrose groups in 30 min and 60 min after HIIE in comparison to before and immediately after HIIE. Stage1: before HITE, stage2: immediately after HIIE, stage3: 30 min after HIIE, stage4: 60 min after HIIE, and stage5: 90 minutes after HIIE.

Inter-group results of nesfatin levels showed that there was no significant difference between different groups (p= 0.519). Regarding intra-group differences, didn't observe a significant difference between any stages of blood sampling (p > 0.05, Fig.2).

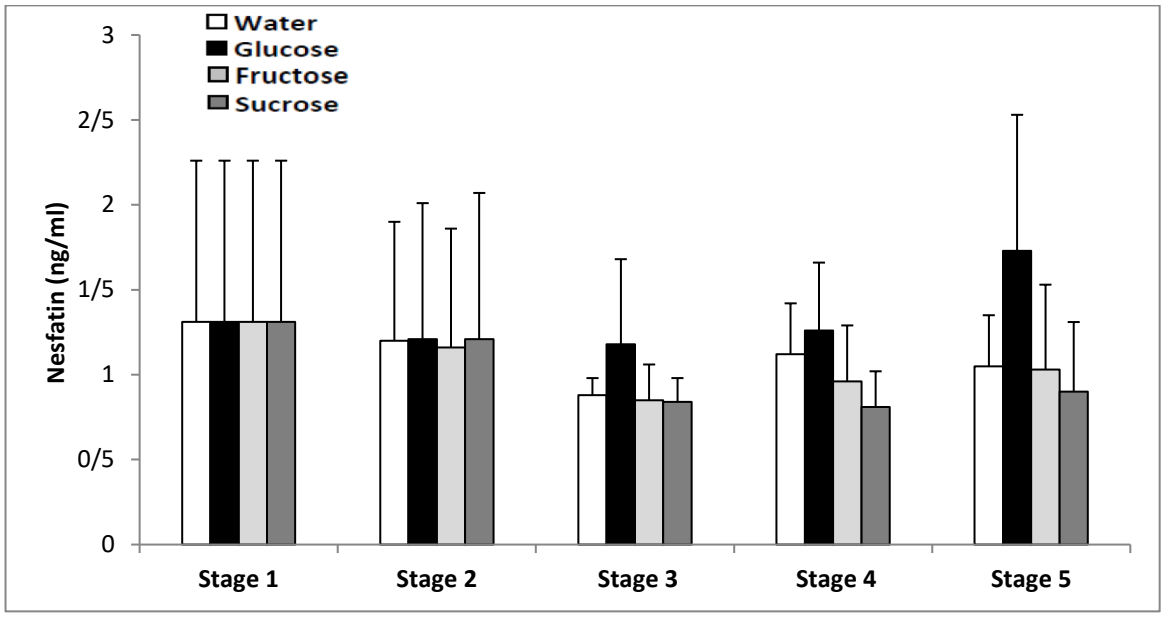

Figure 2: Changes in Nesfatin levels

Inter-group analysis of glucose levels showed no significant difference between different groups (p= 0.062). Within groups, results indicated that glucose levels significantly increased 30 minutes after HIIE in comparison to immediately after HIIE in the glucose group. There are no significant changes between other stages in a different group (p> 0.05, Fig. 3).

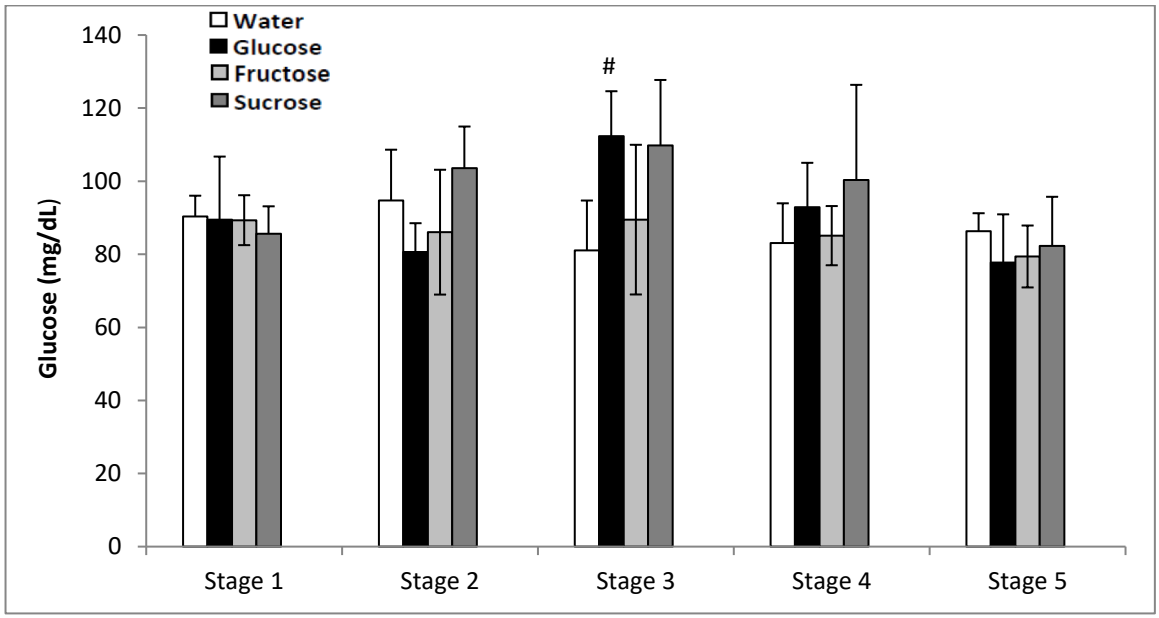

Figure 3: Changes in glucose levels. # Indicated a significant difference in glucose levels between immediately after and 30 minutes after HIIE stages in the glucose group.

4. Discussion

The aim of the present study was to investigate the effect of carbohydrate supplementation (glucose, sucrose, fructose or water) after acute high intensity interval exercise on plasma levels of nesfatin, glucose and insulin in sedentary healthy males. The main finding in the present study is that carbohydrate supplementations don't affect nesfatin response to HIIE protocol. Nesfatin-1 has a widely distribution and different organ, including stomach, pancreas, pituitary and adipose tissue remarkably express and secreted nesfatin-1 which indicates this peptide can influence feeding or another metabolic status (14, 15). In a hunger state, a selective reduction in nucleosidein 2/nsafatin-1 mRNA expression has been reported in the ventricular lateral nuclei of the mice but observed that re-feeding cause returns nesfatin-1 expression to the initial levels (1). It was shown that intravaginal injection of nucleobudinine-2 /nsafatin-1 to male Wistar rats reduced food consumption for 6 hours after the injection in a dose-dependent manner (16).

In the present study, it was observed that nesfatin levels don't change immediately after exercise and also, nesfatin levels remain unchanged after the consumption of glucose, sucrose and fructose supplementation. To explain these findings, we should note the intensity and duration of exercise. In agreement with our findings, Ghanbari-Niaki et al (2010) indicated that acute anaerobic interval exercise or circuit resistance exercise session (20 min) doesn't affect plasma levels of nesfatin-1 in males' boxers (11). These results indicated that acute high-intensity exercise can't change circulating levels of nesfatin after exercise. Ghanbari-Niaki et al. (2010) suggested that probably long-term exercise is needed for observing significant changes in nesfatin-1 levels.

Present study HIIE protocol duration (25 min) is similar to Ghanbari-Niaki et al conducted exercise and we also don't observe a significant change in plasma nesfatin. It has been shown that intense and prolonged exercise can result in the depletion of most body's energy resources such as glycogen. Depletion energy sources of the body cause an increase in appetite-related peptides such as ghrelin and Agrp. On the other hand, it can decrease anti-appetite peptides such as nesfatin, leptin and insulin (11, 12, 17, and 18). In this study, a relative decline in plasma levels of nesfatin was observed immediately after exercise that statically wasn't significant. These results can be attributed to the different intensity and duration of the HIIE protocol, conducted in the present study in comparison to other researchers. To support the importance of exercise duration effectiveness on nesfatin-1 response to acute exercise, Mohebbi et al (2012) reported that one session cycling on a computer-controlled Ergometer until reaching higher than 800 kcal significantly decreased plasma levels of nesfatin-1 in overweight males, but an exercise in fat max intensity doesn't affect nesfatin-1 (12). These researchers concluded that the controversial result between different intensities is the consequence of differing substrate utilization during the two-exercise trial. In support of this idea, observed that glucose homeostasis results in nesfatin-1 secretion into the blood circulation (19). Oral administration of glucose is associated with a significant increase in basal nesfatin-1 levels than the saline-treated control group (4).

In contrast, throughout the day fasting decrease nesfatin-1 levels in plasma. Our result indicated that glucose administration cause increases nesfatin levels in comparison to other groups, although this increase wasn't significant that can attribute to low doses of glucose. In this way, Riva et al, 2011 have proven that a high dose of glucose consumption can elevate nesfatin-1 expression in human islets, but a lower dose doesn't exert a significant effect (9). Increasing the dose of consuming sugars after exercise, quickly restores the lost resources such as glycogen and results in further increases of the anti-appetite hormones in the after-exercise hours (20, 21). However, to our knowledge is the first research that investigates the effect of carbohydrate supplementation on nesfatin changes after acute high-intensity exercise. Regarding about long-term effect of high-intensity interval training (HIIT), Ahmadizad et al (2015) reported that six weeks of HIIT significantly increased nesfatin levels in sedentary overweight men. However, these researchers indicated that a 6-week moderate intensity interval exercise training doesn't change nesfatin-1 levels (22). These findings supported the impact of exercise on nesfatin changes after the exercise training period. Unfortunately, in the present study, we don't examine the chronic effect of high intensity exercise alone or with carbohydrate supplementations on nesfatin changes.

In addition to nesfatin-1 result, the present study has shown that sucrose and glucose increase the insulin levels in 30 and 60 minutes after acute exercise sessions compared to water and fructose groups. These differences between carbohydrate supplements are related to the nature of the supplement, as well as the absorption pathway of glucose and sucrose. Since the glucose is absorbed directly through its transporters (GLUTs), and because glucose transporters increase after exercise and in fact, exercise has an insulin-like effect, the increased insulin in the glucose group is lower in comparison to the sucrose group immediately after the HIIE protocol. On the other hand, it has been shown that sucrose and fructose first should be glucose-digested to be absorbed, or fructose should go to the liver to be absorbed (23, 24).

۵. Conclusion

In conclusion, it seems that different carbohydrate supplementations (sucrose, glucose, and fructose) immediately after high-intensity exercise can't affect nesfatin levels. These results can be related to the dose of glucose consumption and duration and intensity of exercise. With respect to a relative increase in nesfatin levels after glucose ingestion in the glucose group, an increase in the dose of glucose or probably duration of exercise can be associated with further changes in nesfatin levels.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest relevant to this article.

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

Informed consent Informed consent was obtained from all participants.

Author contributions

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

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Research Article

The Effect of One Bout High Intensity Interval Exercise (HIIE) On Serum Levels of Decorin And IGF-I In Active Young Men

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Abstract

Background: Some researchers attributed the positive effects of exercise training on secretion of different myokines from skeletal muscles. Acute exercise lead to changes in gene expression and phosphorylation that stimulates muscular adaptation. However, one bout exercise isn't adequate to change muscle phenotype and phenotypic adaptation to training consists of aggregation stimulation of one bout exercise sessions Decorin is new discovered myokine that its changes in response to exercise such as high intensity interval exercise (HIIE) is unknown. It seems that Decorin has effects on skeletal muscle hypertrophy. Therefore, the aim of present study was investigated the effect of one bout high intensity interval exercise (HIIE) on serum levels of decorin and IGF-I in active young male.

Materials and Methods: For this purpose, 10 active young males with mean age of 25.4±2.36 voluntary participated in this study. The subjects took part in HIIE protocol in 10 am and after 3 hours. HIIE protocol consisted of four minutes' intervals with 90-95 percent of maximum heart rate that between each interval, 3-minute active recovery with 60-70 percent of maximum heart rate performed. The subjects heart rate during HIIE protocol was monitored by polar belt. Blood samples were collected immediately after exercise, subsequently IGF-I and decorin levels were measured by ELISA method. In order to data analyzed, SPSS software version 24 and paired t test were used and significantly level was considered $p<0.05$.

Results: The results indicated that decorin and IGF-I increased significantly after exercise ($p\leq0/05$).

Conclusion: It seems that some HIIT-induced adaptations partly are related to increase in decorin levels.

Keywords:
Myokine, Decorin, High Intensity Interval Exercise

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1. Introduction

About 30% of adults in all of the word have sedentary life style. This life style is related to some disorders like obesity, type 2 diabetes, hypertension, coronary heart disease and other health problems. So, physical fitness parameters are in concern in scientific and clinical centers because of their importance in health (1). World Health Organization (WHO) and other international organizations have suggested 150 min physical activity program with medium intensity or 75 min physical activity with high intensity per week in order to get health benefits from exercise. However, many of people don't have activity at this level and one the main problems to participate in physical activity programs is lack of time. Thus, many of researchers suggest participation in High Intensity Interval Training (HIIT), that consists of intervals with 10 sec to 4 min with intensity above 85% maximal heart rate and it can be a proper solution to time lack(2). The main advantages of participating in HIIT program are: we can do them in a short period, they don't need to equipment and they are joyful. In comparison with traditional endurance training HIIT has greater cardiometabolic effects in less time. These findings are reported in healthy and obese people (3). Acute exercise lead to changes in gene expression and phosphorylation that stimulates muscular adaptation. However, one bout exercise isn't adequate to change muscle phenotype and phenotypic adaptation to training consists of aggregation stimulation of one bout exercise sessions (4).

Many researches have been reported that skeletal muscle can secrete peptides during physical activity that they named these myokines and it seems that myokines attribute in many of positive effects of exercise. According to this, we can define myokines as proteins with small molecoules that are secreted by stimulated muscle. These skeletal muscle derived factors cab be induced with muscle(5). Different types of myokines are identified. At first, transgenic expression of PGC1-α in skeletal muscle as a key regulatory factor lead to identification of Irisin and Meteorin- like factor as a myokines that regulated with physical activity and both of them are associated with stimulation of energy usage and brown adipose tissue in rats. Primary findings in human subjects reported secretion of IL-6, IL-8, IL-10, IL-15 from skeletal muscle. On the other hand, researchers observe increasing in gene expression and level of proteins like ANGPTL4[1], BDNF, CTGF[1], CYR61[1] and Fractalkine in skeletal muscle after acute physical activity. Decorin is other myokine that regulated with physical activity and play a key role in proliferation of human skeletal muscle cells (6). Decorin (NM-133507) is an extracellular proteoglycan and consist of 359 amino acids that can be found in many tissues (7). It is a core protein consist on 10 sequence full of leucine in central domain and has a glycosaminoglycan domain on amino terminus (8). Today, Decorin recognized as a multi-functional and multi directional signaling molecoule and not only controls tumor qrowth and activity but also has many functions as: inflammatory responses and Keratinocyte function (9).

Studies show that Decorin moderates growth factors function, tyrosine kinase receptors, angiogenesis, tissue deformation, bacterial infection and cardiovascular disease (10). Researchers reported that Decorin can increase proliferation and differentiation of myogenic cells via inhibit myostatin. Therefore, Decorin was suggested as a new molecule in myostatin pathway. Myostatin and Decorin are produced concurrent by skeletal muscle. On the other hand, it seems that Decorin can increase Follistatin activity and all of them supported the key role of Decorin on muscle growth and hypertrophy and prevent of muscle atrophy (11). Decorin expresses and secretes due to muscle contraction and its circulatory levels increase after acute physical exercise in human. Furthermore, its expression increases in skeletal muscle of human and rats after training. Over expression of Decorin is related to increase of muscle hypertrophy and at the same time ubiquitin ligases (MURF-1 and Atrogin-1) that involve in atrophy path ways down regulates (12). Decorin attach to IGF-IR and limit endothelial cells apoptosis (13).

IGF-1 is an anabolic growth factor that stimulates skeletal protein synthesis, proliferation and differentiation of satellite cells. IGF-1 has antiapoptic effects on muscle cells, suppresses proteolysis and inhibits ubiquitin-proteasome pathways (14). IGF-1 is an unique because it has receptor on all of cells and tissues (15). Despite this, Decorine roles and effect of different exercise programs on its circulatory levels don't determine and information about its relation with IGF-1 isn't clear. Thus in this research we investigated the effect of one bout HIIE on serum level of Decorin and IGF-1 in active young men.

2. Materials and Methods

This study was a semi- experimental research and it's design was ….. group with pretest and post test. Subjects were active young men that 10 person selected based on inclusion criteria. Inclusion criteria were: sexuality (man), age between 22-30, activity about 3 sessions per week in 6 months, don't have any cardiovascular disease, do not use supplements in next 6 months, have ability to perform program, normal weight.

Exclusion criteria was: can't complete exercise protocol.

At the first day subject received information about project and signed consent letter, then they familiar with exercise protocol. We want them to don't have any heavy activity 48 hours before protocol. They attempted in 10 a.m. and they had their last meal 3 hours before. At first anthropometric measurements were done. Palsma samples collected before and after protocol. Subjects warmed up about 10 min before program.

Exercise protocol

The high intensity interval exercise (HIIE) exercise protocol consisted of 4 interval with 4 min duration and 90-95% of maximal heart rate intensity. The rest between intervals was 3 min active rest with 60-70% maximal heart rate intensity. Subjects heart rate during activity monitored with polar heart rate monitor and HIIE protocol performed on treadmill.

Samples and data collection: plasma samples collected before and after exercise protocol. Chemiluminescent Immunoassay (CLIA) is being used to measure plasma level of IGF-1 and Decorin levels measured with ELIZA method.

Statistics: Shapirowilk test was used to evaluate normal distribution of data and then paired sample t_test was used to find differences between pre test and post test. All data analysis were done with SPSS version 24.

3. Results

Subjects descriptive characteristics was shown in table1. Decorin and IGF-1 levels in pretest and post test were shown on table2.

Table 1: subjects demographic data

variable	mean±SD
Age(year)	25.4±2.36
Weight(kg)	74.4±7.66
Height(cm)	173.3±9.75

Table 2: changes in Decorin and IGF-1 in pretest and post test

Variable	pretest	Posttest	Changes percent
Decorin(ng.ml)	2.18±0.77	3.11±0.94	+42.66
IGF-1(ng.ml)	182.4±56.95	191.1±65.31	+4.76

Table 3: paired sample t-test results

	Changes mean	SD	t	df	p-value
Decorin	0.929	1.01443	-2.896	9	0.018*
IGF-1	8.7	9.847	-2.794	9	0.021*

Data analysis show that plasma levels of decoroin had significant changes after one bout exercise (t=-2.896, df=9, P<0.05). IGF-1 changes after exercise were significant too (t=-2.794, df=9, P<0.05).

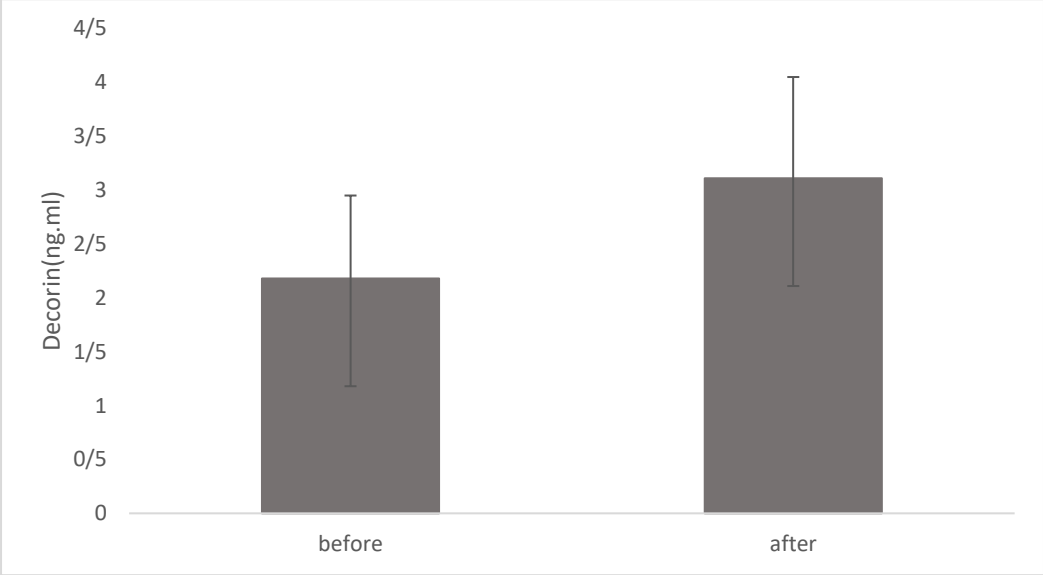

Chart 1: Decorin changes

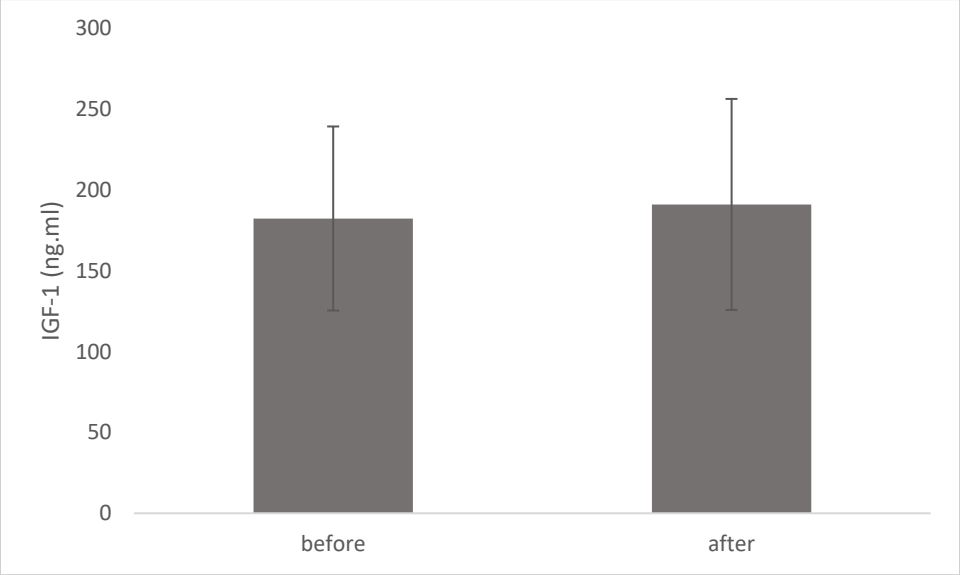

Chart 2: IGF-1 changes

4. Discussion

Our results show that Decorin increased significantly immediately after HIIE (P=0.018) and IGF-1 increased after exercise session significantly too(P=0.021). Based on this results, it seems that some of adaptations to HIIT maybe be related to Decorin changes. Kanzleiter et al. (2014) showed that muscular transcription of Decorin increased after one bout exercise and it associated with increased plasma levels of decorin (12). Although, it seems that acute exercise session can increase Decorin the effect of training is unknown. On the other hand, sampling time is a important factore, because Decorin levels decrease to primary levels about one hour after exercise. Also, exercise intensity and duration have main effect on Decorin responses. Researchers suggested that Decorin has effect on muscle hypertrophy but their underline mechanisms are unknown(19).

Meckel et al.(2011) reported increasing in IGF-1 levels after one bout exercise (19) but in other researches increase in IGF-1 was not significant. The response of IGF-1 to one bout exercise depends on many factors like: exercise protocol, exercise duration and intensity, subjects' physical fitness in base line, sexuality and others. Exercise recruitments systemic IGF-1 from liver and local IGF-1 from muscle. Growth hormone is a main stimulator for production of IGF-1 in liver, but this process needs a long time(21). Findings showed that increase of IGF-1 after exercise is independent to GH and it seems that relates to muscular IGF-1 (21).

۵. Conclusion

Our finding showed that one bout HIIE increased Decorin and IGF-1 significantly. Due to this findings it seems that Decorin can effect on hypertrophy process after exercise.

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Author contributions

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

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

Informed consent Informed consent was obtained from all participants.

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Research Article

The effect of two methods of aerobic and combined training on biomechanics of blood in middle-aged 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-posttest 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:
Aerobic and Combined Exercise Training, biomechanics of blood, blood pressure, blood flow

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1. Introduction

Nowadays, cardiovascular disease (CVD) is considered 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 will probably be the cause of 33% of all deaths in 2030(4). deaths all over the world. 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 vague nerve, which indicates a malfunction in the ventricles. It is on the left side of the heart (5). An inability after the occurrence of these diseases, clinical treatments, and high treatment costs at different (6) ages have caused much research to be carried out in order to develop effective solutions to prevent and improve it. 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) in such a way that 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 or middle-aged, with the age range is 40-65 years (9). Considering that atherosclerosis or the accumulation of lipid deposits begins in childhood and increases in older ages, and with the narrowing of blood vessels (10, 11) and subsequently, disruption of blood supply to the heart, brain, and other peripheral organs, it leads to heart attack, stroke, and ischemia. becomes the lower limb (12, 13).

This can justify the increase in peripheral vascular occlusive diseases along with aging (14). In other words, changes in atherosclerosis progress with increasing age (15) and ultimately lead to clinical problems and death (16). Therefore, increasing age and gender (more men than women) seem to be uncontrollable risk factors in the development of CVD (17). In addition, aging is associated with an increasing decrease in the levels of maximal oxygen consumption (VO2max) (18), functional capacity (FC) (19), and a decrease in ejection fraction (EF) (20) in the heart. VO2max is the index of maximum cardiorespiratory performance, aerobic fitness, and how the heart system works, which decreases with age and its decrease is a risk factor in CVD mortality is known. 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 left ventricular function (20). When the muscle strength of the heart decreases so much that the decrease in EF reaches less than 40%, we will see 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) is associated 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 preventing the 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 the blood flow on the wall Arterial and finally 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, logically, 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 resulting from 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). Khorram Del 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 sports activity 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 the intensity 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 to achieve its protective benefits. (27, 33). Therefore, although endurance and aerobic exercises by improving cardiovascular fitness bring many health benefits to the elderly (18), the 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 the lifestyle and controlling modifiable risk factors (12). Moderate intensity continues training (7) are considered as 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, the mortality rate due to CVD can be further reduced. 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 in ischemic heart patients (7) and after CABG, there is a decrease in muscle mass and strength, followed by a decrease VO2peak and subsequent decrease in FC and quality of life (37), it is believed that by increasing muscle strength we will achieve performance optimization in this segment (7). Therefore, to increase muscle strength and subsequently 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 pointed out the effects of different methods of cardiac rehabilitation programs (combined (7) and aerobic (7, 36)) on the FC (7) of cardiovascular patients. However, few studies have investigated the effects of combined and aerobic exercises in Middle-aged men who have undergone 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-test and post-test research design, the influence measurement model (scientific-comparative) and applied type, from within the statistical population of 2648 middle-aged heart patients 40 to 65 years old (W.H.O.) who underwent coronary artery bypass grafting (968) in Tehran Heart Center Hospital, and among 382 male coronary artery bypass grafts Kroner, 68 middle-aged people who were referred to the rehabilitation center of Tehran Heart Center Hospital two to three weeks after their operation, constituted the subjects of the present study. Subjects were randomly selected into three groups:1) Aerobic exercise training (20 people), Combined exercise training (20 people), and control group (28 people) were included. 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, 68 subjects were introduced to the imaging center for pre-examination one day before the start of the training programs. Before starting the test, the patients were explained the purpose of the research and then the consent forms for the research were completed by the patients.

Then the patients started 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 Heart Rate Reserve, which was calculated according to Karonen'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 training group (70% aerobic and 30% resistance) first exercised for 40 minutes according to the protocol of aerobic exercise and then did resistance exercise twice a week for 20 minutes with four thigh adductor machines. They did Seated chest press, 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 noted that during the training period, the relevant officials constantly checked the heart rate and the training pressure to prevent excessive pressure and not harm the patient in case of possible training pressure on the patient. 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 ejection fraction, the VIVD3 echocardiograph machine made by General Electric of America was used, and to determine the levels of functional capacity and maximum oxygen consumption, the 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 arteries graft patients either refused to continue this research due to personal reasons, or due to death, repeated MI and hospitalization, or absence of more than two sessions, the researcher excluded them from continuing the research.

Statistical Analyses

In order 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, using Levene test and in order to investigate the effectiveness of aerobic and combined exercise methods on selected variables of heart function, using the one-way MANOVA test, and in order to determine the location of differences and comparison between groups in the groups, using Bonferoni Post hoc Test at the level $P \leq 0.05$ significance 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' land variables (Average± standard deviation) in the research groups.

Background variables	Groups		
	Aerobic training	**Combined training**	**Control**
Age (Year)	٥٥±٦/٦٧١	٥٤/٧٦±٧/٠٣١	٥٨/١١±٥/١٠٩
Weight (Kg)	٧٩/٤٤±٦/٨٠٧	٧٦/٤٤±٧/٥٧٨	٧٥/٥٠±٨/٤١٧
height (Cm)	١٧٤/٣٢±٥/٨٢٢	١٧١/٤٠±٤/٠٥٢	١٧١/٧٢±٥/٣٨٩
resting heart rate (thud / minutes)	٨٠/٢٠±١٣/٣٩٨	٨٣/٣٢±١١/٥١٧	٧٨/٥٦±١٠/٢٨٥
Maximum heart rate)thud / minutes)	١٣١/٦٠±١٤/٤٣١	١٢٢/٨٨±١٢/٩٦٢	١٢٨/٣٣±١٦/٧٣٣

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 ANOVA, the effect of the group 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 blood flow velocity variables in the systolic phase, the blood flow intensity in the systolic phase did not have a significant group effect, meaning 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 velocity and intensity variables in the diastolic phase, the results of which are reported in Table 4-10.

Table 4-9: Summary of one-way MANOVA test results related to selected blood biomechanical values

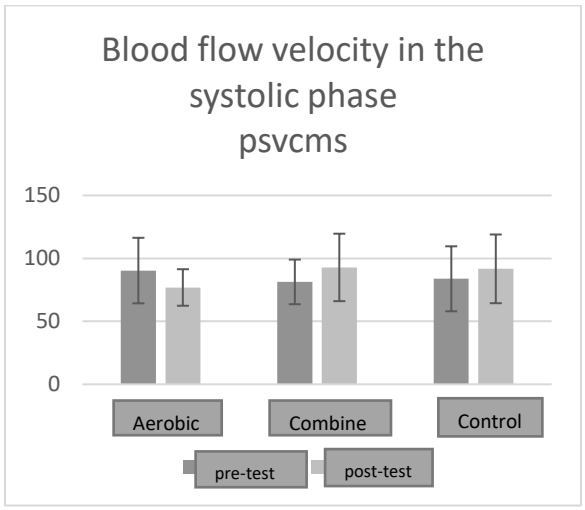
		Source of changes	sum of squares (SS)	degrees of freedom	mean square (MS)	Amount P	Value P	Effect size
Blood flow velocity in the systolic phase psvcms	Pre-test	Group	۲۰۷/۸۵۸	۲	۱۰۳/۹۲۹	۰/۱۹۶	۰/۸۲۲	۰/۰۰۶
		Error	۳٤٤٦٤/۲۹۲	٦٥	٥۳۰/۲۲۰			
	post-test	Group	۲٥۰٦/۳٦٥	۲	۱۲٥۳/۱۸۲	۲/٥۱٥	۰/۰۸۹	۰/۰۷۲
		Error						
Blood flow velocity in the diastolic phase Edvcms	Pre-test	Group	۱۱۹/۷۹٤	۲	٥۹/۸۹۷	۲/٤۱۷	۰/۰۹۷	۰/۰٦۹
		Error	۱٦۱۰/۷۳۸	٦٥	۲٤/۷۸۱			
	post-test	Group	۷۹٤/٦٥۸	۲	۳۹۷/۳۲۹	۱۳/٤٦۹	۰/۰۰۰	۰/۲۹۳
		Error	۱۹۱۷/٤۰۲	٦٥	۲۹/٤۹۸			
Blood flow intensity in the systolic phase psmm	Pre-test	Group	٦/۸۳۰	۲	۳/٤۱٥	۱/۱۲٥	۰/۳۳۱	۰/۰۳۳
		Error	۱۹۷/۲٥۸	٦٥	۳/۰۳٥			
	post-test	Group	۱۲/۳۱۳	۲	٦/۱٥٦	۱/٥۰٦	۰/۲۲۹	۰/۰٤٤
		Error	۲٦٥/۷٤٥	٦٥	٤/۰۸۸			
Blood flow intensity in the diastolic phase	Pre-test	Group	۰/۰۲۰	۲	۰/۰۱۰	۰/٦٤۰	۰/٥۳۱	۰/۰۱۹
		Error	۰/۹۹۲	۰/٦٥	۰/۰۱٥			
	post-test	Group	۰/۰٦٥	۲	۰/۰۳۳	۳/۹۰۱	۰/۰۲٥	۰/۱۰۷
		Error	۰/٥٤٥	٦٥	۰/۰۰۸			

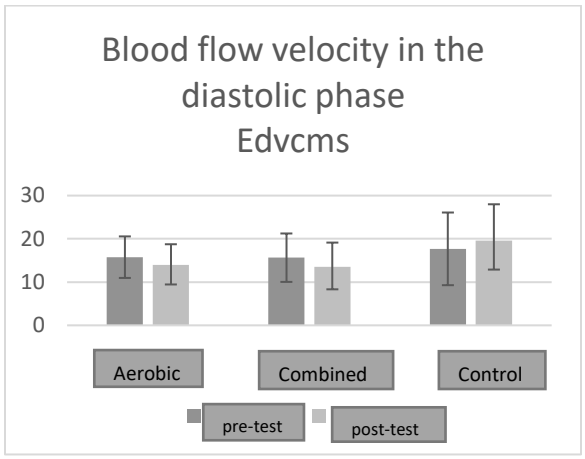
Table 4-10: Summary of post-test results for paired comparisons of blood flow speed and intensity in systolic and diastolic phases.

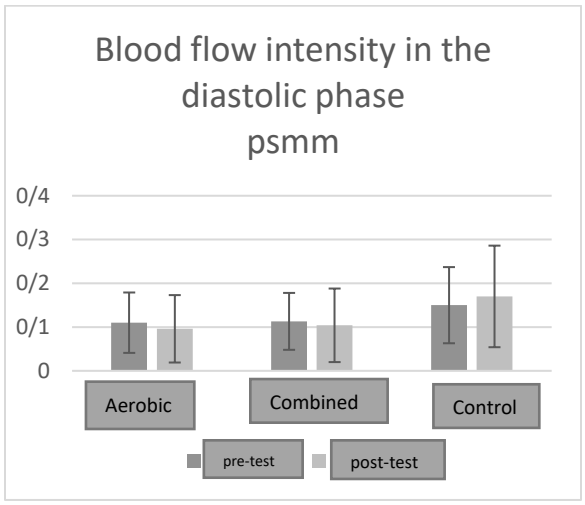
Variable	Time	Group		Mean Difference	Amount P
Blood flow velocity in the systolic phase Edvcms	Pre-test	Aerobic training	Combined training	۱/۳۷٤	۰/۹۹۸
			Control	-۲/۰۰۹	۰/۵۸۹
		Combined training	Control	-۳/۳۸۳	۰/۹٤
	post-test	Aerobic training	Combined training	۰/۷۳٤	۱/۰۰۰
			Control	-۷/۳٤۹	۰/۰۰۰
		Combined training	Control	۰/۷۳٤	۱/۰۰۰
Blood flow intensity in the diastolic phase PDMM	Pre-test	Aerobic training	Combined training	۰/۰۰۲	۱/۰۰۰
			Control	-۰/۰۳۹	۰/۹۱۹
		Combined training	Control	۰/۰۰۲	۱/۰۰۰
	post-test	Aerobic training	Combined training	۰/۰۰۸	۱/۰۰۰
			Control	۰/۰۷٤	۰/۰۳۳
		Combined training	Control	-۰/۰٦٦	۰/۰۷۱

According to the results of Table 4-10, 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 pair of groups in the pre-test, and in the post-test, there was only a significant difference between the aerobic training group and the control group.


Blood flow velocity in the systolic phase
psvcms
150
100
50
0
Aerobic
Combine
Control
pre-test
post-test


Blood flow velocity in the diastolic phase
Edvcms
30
20
10
0
Aerobic
Combined
Control
pre-test
post-test


Blood flow intensity in the diastolic phase
psmm
0/4
0/3
0/2
0/1
0
Aerobic
Combined
Control
pre-test
post-test

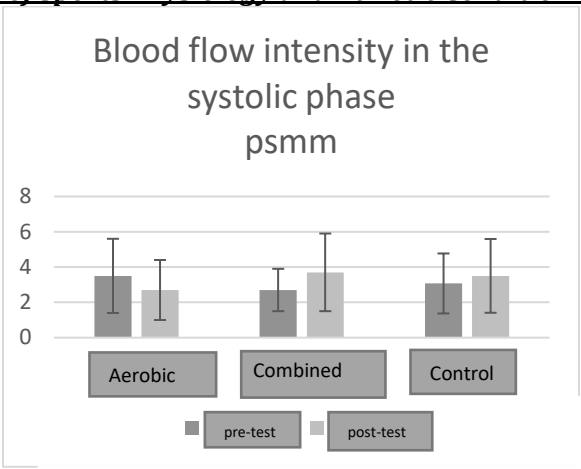


Chart 1: Chart of changes in blood flow speed and intensity in systolic and diastolic phases from pre-test to post-test in the studied groups

4. Discussion

This research aimed to investigate the effect of two types of aerobic and combined exercise (aerobic and resistance) on selected blood biomechanical variables (speed and intensity of blood flow in the systolic and diastolic phases) in middle-aged male patients after CABG surgery. The effect of cardiac rehabilitation exercises on selected blood biomechanical variables in cardiovascular patients were studied. The results of the research showed that in the systolic phase, there was no difference between the speed and intensity of blood flow in the groups under study. Blood flow velocity increased in the combined exercise group from pre-test to post-test, but decreased in the aerobic exercise group. In the diastolic phase, there was a significant difference in blood flow speed and intensity between the groups under study, and the aerobic training group had the greatest effect on the speed and intensity of blood flow compared to the combined training group and the control group, although there were differences between the two groups of combined training and aerobic training.

There was no sign. The results of this part of the research were in line with the research results of Xing et al. (2020) who reported improvements in blood biomechanics due to combined and aerobic exercises. Cardiac patients can increase their capacity and physical strength by performing regular sports activities and thus improve their health and quality of life (39). The results of the findings confirm that due to the occurrence of arterial atherosclerosis, the structure of the vessel wall and the nature of its components are changed and become hard and thick. The results of the research with the results of the studies of Green et al. (2017) agreed with the blood flow rate (40). Besides, with the development of atherosclerosis of the artery, the blood flow changes from a uniform state to a turbulent flow. The mechanical forces acting on the vessel wall, including the shear stress of the blood flow on the vessel lumen and the peripheral stress caused by blood pressure, will change the pattern and increase the severity of the disease with the progress of atherosclerosis injuries (41).

Studies have shown that the effects of sports rehabilitation on atherosclerotic patients include morphological changes that lead to improved blood flow (42). The results of the present study also showed that aerobic exercises can affect the turbulent flow of blood. 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. The research results were consistent with the research results of Maiorana et al. (2001), by studying eight weeks of a combination of aerobic and resistance training on type 2 diabetic patients, concluded that these exercises increased the intensity of their brachial artery blood flow (43), The results of the present study also showed that the aerobic training group had a greater effect on blood biomechanical indices, although the differences were not significant compared to the combined training group. It seems that it is necessary to check the durability of the effects of exercises over a longer period, which is one of the limitations of the present study, so it is suggested that in future studies, subjects should be subjected to longer periods after implementing the exercise protocols. be monitored by researchers so that the effects of cardiac rehabilitation programs can be examined more precisely.

۵. Conclusion

In general, the results obtained from the present study showed that performing eight weeks of aerobic and combined exercise programs can increase the intensity and speed of blood flow 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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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest in publishing this article.

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

Informed consent Informed consent was obtained from all participants

Author contributions

Conceptualization: GH.R., H.S., Y.S.; Methodology: GH.R., H.S., Y.S.; Software: GH.R.; 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.; 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: H.S.; Project administration: GH.R.; Funding acquisition: GH.R., H.S., Y.S.

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Research Article

Influence of B-Hydroxy-B-Methyl Butyrate Supplementation on Strength, Muscle and Liver-Damage Indices Induced by Dual Pyramid Resistance Training in Beginner Bodybuilders

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Abstract

Background: It has been suggested that ingesting supplements decrease muscle soreness, increase muscle strength, hypertrophy, and health-related indices. This study investigated the influence of 8-week β-Hydroxy-β-Methyl butyrate (HMB) supplementation combined with a dual pyramid resistance exercise program on strength, muscle, and liver-damage indices (ALT, AST, CK, and LDH) and body mass index (BMI) in beginner bodybuilders.

Materials and Methods: A total of 40 beginner bodybuilders was randomized to an intervention group (resistance training + HMB supplementation) and a control group (resistance training + placebo) for 8 weeks. Blood samples and body measurements were taken at baseline and after the end of the intervention. The subjects were evaluated for BMI, 1 repetition maximum (1RM) bench press and leg press prior to and after the training intervention. In addition, blood samples were obtained before and after 8-week resistance training to evaluate creatine kinase (CK), lactate dehydrogenase (LD), aspartate aminotransferase (AST), and alanine aminotransferase (Johnson et al.) responses.

Results: After 8 weeks of HMB ingestion, serum levels of CK ($p<0.001$), LDH ($p<0.014$), and ALT ($p<0.009$) of participants in the experimental group significantly decreased compared to the placebo group. Furthermore, The HMB group showed greater gains compared with the placebo group in 1RM leg and bench press ($p<0.001$). Regarding BMI and AST serum levels, there were no significant differences between groups.

Conclusion: The results of the present study showed that HMB supplementation may attenuate the resistance exercise-induced muscle and liver damage indices and have beneficial effects on muscle strength.

Keywords:
β-Hydroxy-β-Methyl butyrate, muscle strength, liver-damage markers, muscle-damage indices, dual pyramid resistance training

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1. Introduction

High-intensity resistance training appears essential for improving physical fitness factors, particularly muscular strength and hypertrophy, and is a common form of activity recommended for various healthy age groups and patients (1, 2). Double pyramid resistance training, a combination of the pyramid and reverse pyramid system, uses the maximum number of muscle fibers because they are under the most possible load (3). Repeatedly exposing resistance training exerts mechanical overloads on the body and can stimulate the tissue to adapt with further overload. Muscle and connective tissue damage may occur due to resistance exercises. As a result, bodybuilders need much time to recovery to be able to increase their training load and improve their performance (4).

Creatine kinase (CK) and lactate dehydrogenase (LDH) are two indirect skeletal muscle damage indices. CK is a dimeric globular protein that buffers ATP and ADP concentrations by catalyzing the exchange of phosphate bonds between phosphocreatine and ADP produced during muscular contraction. LDH is an enzyme that catalyzes the conversion of pyruvate to lactate during anaerobic glycolysis (4). Following long-term and strenuous exercises, serum levels of CK and LDH enzymes can dramatically increase (5, 6).

Meanwhile, it has been observed that resistance training may lead to elevations of aspartate aminotransferase (AST) and alanine aminotransferase (7). AST and ALT enzymes are hugely found in the liver. AST is also abundant in other tissues such as the heart, kidneys, skeletal muscle, and red blood cells; but, ALT concentrations in skeletal muscle are low (8).

In fact, an increase in serum level of AST and ALT indicates the entry of muscle and liver enzymes into the bloodstream (6). In addition to this, ALT activation has a high correlation with body mass index (BMI), while AST has a slight one to this variable (6, 9).

It has been suggested that ingesting supplements decrease muscle soreness, increase muscle strength, hypertrophy, and health-related indices (10-12). Over the past 25 years, one dietary supplement that has drawn specific attention in sport is β-hydroxy-β-methyl butyrate (HMB) (10, 13). Beneficial effects of HMB – a metabolite of leucine and 2-ketoisocaproic acid – may be correlated with its anti-catabolic action, influence on the de novo synthesis of cholesterol, increased insulin-like growth factor 1 transcription, the stimulation of the mTOR kinase pathway, or the ubiquitin-proteasome system and caspase activity (10, 14).

There have been several studies to investigate the impacts of the ingestion of HMB in combination with resistance training in terms of improving strength, and damage markers. Asjodi and Izadi (2019), Standley et al. (2017), van Someren et al. (2005) and Panton et al (15) reported that HMB ingestion (3 g/day) during 3–8 weeks of resistance training decreased CK and LDH, lowered inflammatory biomarkers, and improved body composition; However, Arazi et al.(2015), Kirby et al.(2010), Lamboley et al (2007), and Shirato et al. (2016) were found no significant differences between HMB and placebo groups regarding blood lipids, muscle damage and body composition.

In general, muscle fibers damage and inflammatory responses due to intense and eccentric exercises reduce athlete's performance in subsequent sessions; so, it is essential to prevent injuries in the athletes for further efforts. To best our knowledge, the long-term effects of the combination of HMB ingestions and resistance training on liver-damage indices have not been reported yet in previous studies. According to the previous contradictory results and the increase in HMB consumption among resistance and competitive athletes, the aim of the present study was to investigate the influence of HMB supplementation with dual pyramid resistance exercises on muscle and liver damage enzymes, muscle strength and BMI in beginner bodybuilders.

2. Materials and Methods

This randomized, double-blind, placebo-controlled trial with pre and post-test design approved by the Ethics Committee of Azad University of Rasht (IR.IAU.RASHT.REC.1399). The trial has been registered in the Iranian Registry of Clinical Trials (IRCT) as IRCT20180503039517N4.

Participants

The study population consisted of 53 beginner males (aged 19–29) bodybuilders who were members of a sports club in Lahijan City, Iran. Inclusion criteria were: 1) a maximum of 3 months involving in resistance exercise, 2) no smoking, 3) no history of cardiorespiratory, musculoskeletal, neurological, hormonal or orthopedic disorders, 4) no change in daily diet during the study, and 5) no ingestion of androgenic and energetic supplements in the past 1 months. Exclusion criteria included 1) absence for more than two sessions through intervention, 2) consumption of additional supplementation during the study, and 3) suddenly being disease.

Randomization and study groups

The sample comprised 40 amateur bodybuilders who were voluntarily recruited and then randomly divided into two groups: HMB supplementation+ resistance training (n=20) and placebo+ resistance training (n=20). A priori calculations of statistical power using G ∗ Power indicated that this sample size was appropriate to satisfy power at or above 80% (16). A researcher not involved with data collection was responsible for randomization and group allocation (using a computer-generated randomization sequence). Participants signed informed consent and were completely voluntary to withdraw at any stage of research. They were screened by a physician to be eligible to participate in exercise training. None of them received any muscle-building supplements before.

All subjects completed a familiarization session with equipment, resistance training room, and proper strength tests one week prior to the initiation of the resistance training intervention.

For HMB supplementation group, HMB capsules containing 1 g of HMB per capsule were taken (Optimum Nutrition, Inc., USA): One capsule was ingested 3 times a day before breakfast, lunch, and supper (HMB: 3 g/day) on non-training days. Moreover, on training days, participants took three capsules of the assigned preparation per day in three doses as follows: before breakfast and supper, and 30 minutes before training, as well. Each serving of placebo contained the same amount of polydextrose (17). It should be reminded that both HMB and placebo capsules were the same in size and appearance.

Resistance training involved an eight-week dual pyramid pattern program (3 sessions per week). Each session consisted of a 10 minutes warm-up, 65 minutes of main exercises, and 5 minutes cool down. The main part of training program included the 8 exercises in each session (8 sets with a break of 5 minutes in the first 2 weeks, 4 minutes in the third and fourth weeks and 3 minutes in the last 4 weeks) (18) (resistance program was shown in table 1 in detail). They were closely supervised by a trained exercise specialist to assure performing techniques properly. During this pretest and posttest scheduling food intake was controlled by an investigator by means of an interview. All participants completed training sessions in full.

Table 1: Main dual pyramid resistance training program

<table>
<tr><td>Program activity</td><td>duration</td><td colspan="2">Content</td></tr>
<tr><td>Warm up</td><td>10 min</td><td colspan="2">jogging and stretching (focusing on the muscle groups that were about to be trained)</td></tr>
<tr><td rowspan="9">Main dual pyramid resistance training program</td><td rowspan="9">65 min</td><td>movements</td><td>Set = reps × load</td></tr>
<tr><td rowspan="8">bench press, leg press, hamstring curl, knee extension, cable biceps curl and triceps extension</td><td>1= 4 × 80% 1RM</td></tr>
<tr><td>2= 3 × 85% 1RM</td></tr>
<tr><td>3= 2 × 90% 1RM</td></tr>
<tr><td>4= 1 × 95% 1RM</td></tr>
<tr><td>5= 1 × 95% 1RM</td></tr>
<tr><td>6= 2 × 90% 1RM</td></tr>
<tr><td>7= 3 × 85% 1RM</td></tr>
<tr><td>8= 4 × 80% 1RM</td></tr>
<tr><td>Cool down</td><td>5 min</td><td colspan="2">The same exercises as warm up</td></tr>
</table>

Measures

Baseline anthropometric and physical fitness assessment

Forty-eight hours before and after the intervention, height, weight, BMI were measured by dividing body weight (kg) by the square of the height (m2) of the subject. For the upper and lower body strength, bench and leg press exercises were measured by free weights (Mobarez Company, Tehran, Iran) as mentioned before (19). One RM was assessed in knee extension, hamstring curl, leg press, bench press, cable biceps curl, and triceps pushdown exercises. Briefly, the participants performed a warm-up which consisted of jogging, static and dynamic stretching and exercises. Two to three trials separated by 2-3 minutes of rest were used to determine the individuals' 1RM for each resistance exercise. In these sessions, a weight that could be lifted maximally to fatigue after 2-10 repetitions was used to calculate 1RM according to the formula proposed by Brzycki (20).

Laboratory investigations

All laboratory measurements were performed one day before and two days after the intervention period. Trained and experienced phlebotomists collected ten-milliliter venous blood samples in a seated position after 12 -h overnight fasting between 7:30–9 a.m. The serum was separated and stored at -80°C until analysis. In both stages, the serum level of CK, LDH, ALT, and AST enzymes were measured by an enzymatic colorimetric method (Pars Azmoun, Tehran, Iran).

Statistical Analyses

The normality of all distributions was determined by Shapiro-Wilk test, a paired sample t-test was used to calculate the statistical significance of the difference between pre and post measurements within each group. Independent t-test was also used to compare the differences in change scores (post-test minus pre-test scores for each subject) between intervention and control groups. To calculate an effect size Cohen's d was used with the parameter; $d \leq 0.2$ considered small, > 0.8 large, and between these values moderate (21). Standard statistical procedures were selected for the calculation of means, standard deviations and 95% confidence intervals. Statistical significance was set at $p< 0.05$. All analyses were conducted using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA).

3. Results

In total, 40 beginner bodybuilders (mean age 22.85 ± 2.98 years) were recruited for this study. In order to examine the differences between the two groups in baseline scores, we performed an independent t-test. At baseline, no significant difference was found between the intervention and control groups regarding the participants' demographic features (age, height, weight, and BMI) and research variables (leg press, bench press, BMI, CPK, LDH, ALT and AST values) ($p > 0.05$) (see Table 2).

Table 2: Baseline Measurements

	HMB (n=20) Mean ± SD	Placebo (n=20) Mean ± SD	p-value*
Baseline demographics			
Age (year)	22.45±2.91	23.45±3.15	0.140
Height (m)	1.74±0.07	1.71±0.074	0.834
Wight (kg)	73.9±9.7	70.6±7.67	0.240
BMI (kg/m^2)	25.18±2.1	24.03±2.52	0.126

*p Values were derived using two-tailed, independent-samples t-tests.

In order to investigate the effect of dual pyramid resistance training combined with HMB or placebo on research variables, the independent t-test was conducted to compare mean changes (Δ) of the variable values between the HMB and placebo groups (See table 3).

Table 3: Comparison of research variable scores and mean changes (Δ) of the aquatic and control groups.

Variables	HMB group (n=20)			Placebo group (n=20)					
	Pre Mean (SD)	Post Mean (SD)	Δ Mean (SD)	Pre Mean (SD)	Post Mean (SD)	Δ Mean (SD)	ES [%95CI]	t	p
BMI (kg/m^2)	25.18 (2.1)	24.92 (2.07)	0.25 (0.83)	24.03 (2.52)	24.15 (2.24)	0.11 (1.08)	0.14 [-0.24,0.99]	1.22	0.228
Leg Press	158.6 (6.23)	173.50 (9.31)	-14.9 (6.73)	158.95 (7.65)	163.00 (7.55)	4.05 (0.75)	2.26 [-13.91, -7.78]	-7.15	0.001
Bench Press	53.25 (5.39)	61.6 (5.73)	-8.35 (4.09)	51.00 (5.74)	55.75 (5.63)	4.75 (0.96)	0.141 [-5.55, -1.64]	3.82	0.001
CPK (U/L)	256.1 (89.26)	190.45 (76.15)	65.65 (52.58)	253.1 (72.3)	244.95 (72.60)	8.15 (4.30)	1.54 [33.61, -81.38]	4.87	0.001
LDH (U/L)	349.05 (64.07)	317.9 (61.21)	31.15 (35.1)	331.8 (67.6)	321.95 (68.08)	9.85 (3.95)	0.85 [5.29, -37.30]	2.69	0.014
ALT (mg/dl)	25.80 (9.44)	19.20 (4.33)	6.60 (8.81)	26.95 (8.59)	26.10 (8.44)	0.85 (1.18)	0.91 [1.72, -9.77]	2.89	0.009
AST (mg/dl)	22.85 (4.72)	18.00 (3.78)	4.85 (4.25)	21.85 (5.54)	17.90 (3.83)	0.11 (0.74)	1.55 [-1.65,3.45]	0.713	0.48

Paired samples t-tests were used to compare within groups changes. After 8 weeks exercise, the HMB group showed statistically increase for bench press 1RM record (improvement =8.35, effect size = 0.151, 95% CI [6.43, 10.26], t (19) =9.12, p<.001), and the leg press record (improvement =14.90, effect size = 0.093, 95% CI [11.74, 18.05], t (19) =9.89, p<.001). Moreover, the HMB group showed significant decrease for CK (improvement= -65.60, effect size = 0.25, 95% CI [-90.26, -41.03], t (19) =-5.58, p<.001), LDH (improvement= - 65.60, ES = 0.089, 95% CI [-47.59, -14.70], t (19) =-3.96, p<.001), ALT (improvement= -6.60, effect size = 0.25, 95% CI [-10.72, -2.47], t (19) =-3.35, p<.003), and AST values (improvement= -4.85, effect size = 0.20, 95% CI [-6.84, -2.85], t (19) =-5.09, p<.001).

On the other hand, After 8 weeks exercise, The placebo group showed statistically increase for bench press record (improvement =4.75, effect size = 0.09, 95% CI [4.29, 5.20], t (19) =21.97, p<.001), and leg press record (improvement =0.025, effect size = 0.093, 95% CI [3.69, 4.40], t (19) =23.85, p<.001). Moreover, the placebo group showed significant decreases for CK (improvement= -8.15, effect size = 0.032, 95% CI [-10.16, -6.13], t (19) =-8.46, p<.001), LDH (improvement= -8.15, ES = 0.029, 95% CI [-11.69, -8.00], , t (19) =-11.15, p<.001), ALT (improvement= -0.85, effect size = 0.031, 95% CI [-1.40, -0.29], t (19) =-3.21, p<.005), and AST values (improvement= -3.95, effect size = 0.180, 95% CI [-5.68, -2.21], t (19) =-4.76, p<.001). BMI was compared in both groups before and after intervention. The results indicated no significant change in BMI in HMB (p>0.189) and placebo (p>0.628) groups.

4. Discussion

The present study aimed to examine the effect of HMB supplementation on liver and muscle damage markers, BMI, and muscle strength after 8 weeks of dual pyramid resistance training in amateur bodybuilders. Our results demonstrated that there was a significant difference in CK and LDH serum levels, between two groups, following 8 weeks of resistance training. In relation to the pre-investigation value, after HMB supplementation and placebo, the changes in CK and LDH were significant; however, the HMB supplementation group indicated greater meaningful changes.

Our results are consistent with those of some researchers (10, 22-24). Asjodi et al investigated the effects of 8 weeks HMB supplementation (3 g/day) on body composition, CK, and LDH following exercise. Results revealed that the values of CK and LDH in the HMB group were significantly lower compared to the placebo group. Durkalek-Michaleski and Jeszka, also reported that HMB ingestion (3 g/day) during 12 weeks of resistance training decreased CK and LDH activity. Furthermore, Nissen et al. and van Someren et al., following HMB supplementation, found a lower activity of CK and/or LDH in the blood of examined individuals.

In contrast, there is evidence of no change in the serum levels of muscle damage parameters following HMB supplementation. Shirato et al and Wilson et al suggested that the ingestion of HMB does not have a role to reduce muscle damage markers after resistance exercise. The discrepancy between this study and previous research may be attributed to the length of supplementation prior to the eccentric exercise. In this study, subjects ingested the HMB supplement 30 minutes before the exercise bout.

However, the duration to take the supplement was 60 minutes prior to the eccentric exercise trial in Wilson's study. In addition, it may be due to various supplementary periods in two studies (7 days before and 4 days after one special eccentric exercise in Shirato's study versus 24 sessions in 8-week supplementation (3 meals per day) in our study.

The other findings of our study revealed that there was a significant difference between the two groups in the serum level of ALT, after 8 weeks of resistance training, but we could not observe a significant difference in AST serum level between the groups. In relation to the pre-investigation value, after HMB supplementation and placebo, the changes in ALT and AST were significant.

To our knowledge, this is the first study to investigate the effects of long-term HMB supplementation on serum levels of liver enzymes following 8 weeks of resistance exercise. Only one study examined the effects of short-term HMB supplementation on serum AST and ALT after intense resistance exercise in untrained male students (25). Contrary to our results, Saki et al concluded that consumption of HMB before exercise did not have significant effects on serum AST and ALT. It should be noted that different lengths of supplementation (6 days before weight training) and shorter approach of the intervention (a single session of exercise) could be the possible reason for the contradiction between the results of the present study and the mentioned study.

It has been shown that intense resistance training resulted in profound increases in the muscle and liver function parameters, which may cause increased levels of CK and LDH, as well as in AST and ALT levels (8, 25). The beneficial effects of HMB combined with resistance training have been reported in several studies. It has been demonstrated that the utilization of HBM has the ability to debilitate muscle damage indices and improve recovery (2, 22, 26). One explanation for the positive results of HMB supplementation relates to the fact that HMB is a precursor of cholesterol amalgamation in skeletal muscle. This has prompted the theory that HMB would raise post-training cholesterol synthesis, along these lines quickening the repair of training-induced muscle damage. This, in turn, would permit for increased training loads finally leading to elevated hypertrophic responses (27).

Finally, the present results addressed that there was a significant difference in muscle strength values (1RM leg press and bench press), between two groups, after 8 weeks of resistance training. In relation to the pre-investigation value, after HMB supplementation and placebo, the changes in upper and lower extremity muscle strength were significant; however, the HMB supplementation group indicated greater meaningful changes. Moreover, no significant differences were observed in BMI between HMB and placebo groups, after resistance training.

Lamboley et al 's result (28) is not agreeing with our results. They could not observe a significant effect of HMB on BMI in the active college students. To explain the difference between the findings, it should be noted that the type of training program (aerobic program) and the supplementation period (5 weeks) were different.

On the other hand, Asjodi et al, Asadi et al and Panton et al reported that HMB supplementation significantly improved muscle strength and BMI, which are in line with our findings. Two mechanisms for HMB action have been proposed. The first depends on the perception that HMB can slow or restrain muscle proteolysis that is increased during exercise. It can also improve fatty acid oxidation and cause increases in fat-free mass, and enhances the biochemical mechanisms necessary for protein synthesis (22, 29). The second probable mechanism concerns HMB being an antecedent of muscle-cell cholesterol. The expansion in muscle hypertrophy during weight training may bring about a local shortage in cholesterol in the muscle cell, which might result in insufficient cholesterol for membrane synthesis and consequently slower cell growth or sub-optimally functioning cell membranes. Providing basic measures of cholesterol precursors in muscle would permit the muscle to maintain and synthesize new muscle plasma membranes (30). The present study has certain limitations. First, the study included only beginner bodybuilders; so, the results might not be generalizable to intermediate or elite athletes. Second, the population in this study were young (19 to 29 years), which may not be suitable for popularizing the results to other age groups.

۵. Conclusion

According to the findings of the present study, it can be concluded that 8-week ingestion of HMB may have a positive effect on muscle damage markers after the dual pyramid resistance exercise of amateur bodybuilders. Additionally, HMB consumption combined with resistance exercises could induce meaningful increases in muscle strength.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest in publishing this article.

Ethical approval This article was derived from a master's thesis on Exercise Physiology with the project code: 981480 and the ethical code: IR.IAU.RASHT.REC.1399, Ethics Committee of Azad University of Rasht, Rasht, Iran. This study was also registered in the Iranian Registry of Clinical Trials with the code: IRCT20180503039517N4.

Informed consent Informed consent was obtained from all participants

Author contributions

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

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Research Article

The effect of 8-week aerobic training and green tea consumption on adropin and lipid profiles of overweight-obese women

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Abstract

Background: Aerobic training and green tea consumption affect fat metabolism via a change in several elements. Adropin is a unique hormone, which is related to fat metabolism. This study was carried out to evaluate the effect of eight weeks of aerobic training and green tea consumption on adropin and lipid profiles of overweight-obese women.

Materials and Methods: 32 overweight-obese non-athletes (age: 20-40 years) were included in this study and divided into four groups (n=8 each group): 1) green tea and exercise, 2) green tea, 3) exercise, and 4) control. The exercise training program was three days a week, 60% to 70% of the maximum heart rate each session, for 8 weeks. The green tea supplement dose was one 500 mg capsule of green tea, consumed three days a week for 8 weeks. BMI as well as serum levels of adropin, total cholesterol, HDL, and LDL, were determined before and after the interventions. The collected data were analyzed via covariance test at a significance level of $P<0.05$.

Results: 8 weeks of aerobic training and tea consumption had no significant effect on adropine, cholesterol, and triglyceride levels in overweight-obese women ($p>0.05$). However, 8 weeks of aerobic training and green tea supplementation had a significant beneficial effect on the LDL, HDL, BMI, and weight of overweight-obese women ($p<0.05$).

Conclusion: 8 weeks of aerobic training and consumption of green tea significantly improved the weight, BMI, HDL, and LDL of the participants.

Keywords:
Adropin, Aerobic training, Lipid profile, Green tea

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1. Introduction

Obesity is the main risk factor for many common diseases in the world, including diabetes, cardiovascular diseases, high blood pressure, metabolic disorders, and various types of cancers(1, 2). In fact, inactivity may be a more effective factor in the development of obesity than overeating. To lose weight, a negative balance of energy is needed, which is obtained by reducing the intake of calories and increasing the intake of calories. In this regard, some researchers consider inactivity to be more important than the intake of calories and characterize laxity and neglect of physical activities. First, fat people know(3). The more activity a person does, the more energy he spends daily, and the faster obesity will disappear (4). Therefore, forced muscle activity is often considered a necessary part of the treatment of obesity, and due to the high risk of obesity, interventions that help to reduce or maintain weight are of particular importance (5). Sports activity leads to weight loss and improvement of subjects' health and blood factors related to obesity(6, 7).

Regular aerobic exercises such as walking, jogging, swimming, and bicycling can have a positive effect on the amount of fat and lipoproteins (8-10). Green tea has received much attention due to its beneficial effects on health. Green tea is an important source of flavonoids. Green tea contains a group of polyphenolic flavonoid compounds called catechins, and among catechins, epigallocatechin gallate (EGCG) is a strong antioxidant in laboratory conditions and the most common and abundant polyphenol in tea. is green EGCG prevents the activity of the catechol methyltransferase (COMT) enzyme, which reduces noradrenaline, and by regulating sympathetic activity and lipolysis, it increases energy consumption, fat oxidation, and decreases body fat mass(11).

In addition to its effects on fat metabolism, green tea causes an increase in adropin and as a result can have effects on glucose action and insulin resistance (11). Due to the close relationship between obesity and the components of metabolic syndrome with the functions of some peptides, many researchers tried to understand their functions and investigate the effect of various interventions on these mediators that regulate energy homeostasis. One of these peptide hormones is adropin (12) Regulating the metabolism of carbohydrates, lipids and fats are one of the effects of adropin. Increasing the amount of circulating adropin reduces insulin resistance and glucose intolerance, which occurs in response to metabolic stress (13). Research results show that exercise stimulates the increase of adropin levels, which may be related to the effect of exercise on arterial stiffness and obesity in obese adults (14) according to the background of research conducted in the field of obesity and overweight. Effective indicators on lipid profile including adropin, therefore, in this study, the effect of eight weeks of aerobic exercise and green tea consumption on adropin and lipid profile of overweight-obese women was investigated.

2. Materials and Methods

In this research, the statistical population consists of 32 overweight-obese non-athletic women (age: 20-40 years) who, after completing the consent form, were randomly divided into four groups of 8 people, including the first group of green tea consumption and exercise, the group the second group was green tea consumption, the third was the exercise group and the fourth was the control group. It is worth mentioning that all the people used in the study entered the research by filling out the personal information questionnaire, medical records, and physical activity evaluation questionnaire. The main criteria considered for people to enter the research were: 1) having perfect health and no history of illness 2) body mass index between 25 and 35 3) not using drugs and supplements effective in the research results ٤- not performing regular exercises Sports or having a training history. Before starting the exercise program, the subjects' anthropometric indices were measured. Measurement of anthropometric indicators included weight, height, and BMI, which was done with minimal clothes and no shoes. The height and weight of people were measured while standing, respectively, using a wall-mounted height gauge (with an accuracy of 5.5 cm) and a digital scale (with an accuracy of 1.5 cm). BMI is calculated by dividing body weight (kilograms) by the square of height (square meters). The ethical considerations considered in this research were to fully explain the objectives of the research to the participants, to assure them of the confidentiality of the information, and to leave the research for personal reasons.

Blood sampling

To check the biochemical variables, blood was taken in two stages, 48 hours before the start of training (first week), and the eighth week (48 hours after the last training session). Subjects were advised to refrain from sports activities 24 hours before blood sampling and eat a light diet the night before blood sampling. To measure LDL, HDL, and triglyceride, was done using the quantitative detection kit of Pars Azmoun Iran and the photometric method. Total cholesterol was measured by the enzymatic method and Pars Azmoun kit. To measure the amount of adropin, it was calculated using the sandwich ELISA method and using the adropin ELISA kit (Cat No: E3231Hu) with an assay range of 5 to 100 ng/L and a sensitivity of 2.49 ng/L.

Exercise protocol

A week before the start of the research, the subjects first participate in a familiarization session and get acquainted with the correct way to perform the exercise. The training program was three days a week for 8 weeks. The schedule of each session includes 10 minutes of warm-up, 20 minutes of aerobic exercise (running and local movements), and 5 minutes of cooling down, which continued with an intensity of 60% to 70% of the maximum heart rate (15).

Consumption of green tea

In this research, to increase the accuracy of the work and ensure the consumption of the determined dose, instead of brewing green tea, herbal tablets with a certain amount of catechin were used. Each 500 mg tablet of green tea contained 300 mg of catechin. These tablets were obtained. The green tea supplement dose was one 500 mg capsule of green tea for three days a week. And one form was given to the subjects, which had to be consumed by the subjects three days a week, after having lunch (16).

Statistical Analysis

To analyze the data, the normality of the data was checked using the Kolmogorov-Smirnov statistical test. After the normality of the data was confirmed by the test, analysis of covariance and Tukey's post hoc test were used to compare the average variables between the research groups. All statistical information was analyzed by SPSS version 24 statistical software at a significant level ($P<0.05$).

3. Results

According to Table 1. The results of the analysis of covariance did not show significant differences in the levels of adropin (p=0.48), cholesterol (p=0.43), and triglyceride (p=0.77) in the four research groups. But the results of covariance analysis showed a significant difference in the levels of HDL (p=0.002), LDL (p=0.006), BMI (p=0.02), and weight (p=0.02) of the participants.

Table 1: Comparison of variables in four groups.

Variable	Review time	Control		Green tea		Aerobic		Aerobic + Green tea		Sig
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Adropin	pretest	103.37	18.83	109	32.51	116	50.21	117.50	29.15	0.48
	post-test	101.62	16.32	109.25	31.21	119.75	47.30	121.12	29.36	
Cholesterol	pretest	195.87	6.95	195.75	9.05	191.75	13.85	191.87	9.28	0.43
	post-test	195.12	4.58	193	7.70	191.12	13.44	187.75	9.48	
TG	pretest	121.75	27.47	117.25	31.20	123.87	28.11	123.87	25.04	0.77
	post-test	119.25	25.67	111.12	29.80	123	28.20	122.25	23.34	
BMI	pretest	30.30	2.88	30.10	3.36	29.49	3.19	30.15	3.71	0.02
	post-test	30.19	2.83	29.41*	3.47	28.58*	3.34	28.62*	4.15	
HDL	pretest	39.25	7.02	37.875	4.48	36.12	4.38	34.87	4.99	0.002
	post-test	38.25	6.62	39.75*	5.06	39.75*	5.14	38*	5.04	
LDL	pretest	121.37	19.61	118.50	20.07	125.87	20.82	130.62	19.41	0.006
	post-test	119.37	15.46	116.50*	20.12	119.37*	21.13	121.62*	24.51	
Weight	pretest	78.75	7.95	77.62	9.06	77.50	7.07	77.75	7.04	0.02
	post-test	79.50	8.29	75.68*	9.19	74.75*	7.68	72.98*	7.61	

4. Discussion

The results of the present study showed that eight weeks of aerobic exercise and consumption of green tea had no significant effect on the levels of adropin, cholesterol, and triglycerides in overweight-obese women, however, eight weeks of aerobic exercise and consumption of green tea supplements had a significant beneficial effect on the LDL, HDL, BMI and the weight of overweight-obese women.

In a study conducted by Fujie et al. (2017) to investigate the relationship between the level of adropin and the effect of aerobic exercise on arterial stiffness and obesity in adults (14), serum adropin levels in healthy, overweight, and obese adults had a negative relationship with arterial stiffness and He had abdominal visceral fat. After an 8-week exercise program, serum adropine levels increased in obese adults, which was inconsistent with the results of this study. On the other hand, in the study of Alizadeh et al. (2018), aerobic activity with an intensity proportional to the maximum fat oxidation had a significant effect on adropin and insulin resistance in overweight women in insulin factors and insulin resistance, but no significant changes were observed for glucose and adropin (17). It is possible that due to the effect of the fasting state on the increase of adropine or insufficient duration and intensity of exercise training, no significant changes in the level of adropine were observed in this study.

In the research of Amir Hossein Haghighi et al., eight weeks of aerobic exercise and consumption of green tea in medium and high doses caused a significant decrease in body weight, body mass index, body fat percentage, waist circumference, and a significant increase in maximum oxygen consumption, but between two doses No significant difference was observed in consumption (18). which is consistent with the present study. Probably, the combination of exercise training and consumption of green tea leads to weight loss and body mass index. Due to the caffeine present in it, green tea can increase epinephrine, and along with exercise, it has a greater effect on fat burning, which leads to weight loss (19). Also, green tea can increase a person's basic metabolism, which leads to losing more weight. In the research of Fathei et al. (2016), the mean total cholesterol levels in the green tea group, aerobic and combined exercise, the average triglyceride in the green tea and aerobic exercise group, the mean low-density lipoprotein in the green tea and combined group, and the mean high-density lipoprotein Above, there were significant changes only in the aerobic exercise group in the post-test phase compared to the pre-test (20). In the present study, consumption of green tea along with aerobic exercise resulted in no change in total cholesterol level, a decrease in LDL, and an increase in HDL.

In the research of Cardoso et al. (2013), it was found that compared to other groups (exercise, control, and green tea), consumption of green tea along with resistance training increases resting metabolism, increases net body weight, increases strength and decreases body fat percentage. waist circumference and triglyceride reserves (21).

In the present study, aerobic exercise along with the use of green tea was not associated with a decrease in triglyceride levels. The reason for this discrepancy could be the different exercise protocols in the two studies. In the research of Ichinose et al. (2011), the effect of aerobic exercise and consumption of green tea extract on substrate metabolism during exercise in healthy male subjects decreased the ratio of respiratory exchange and increased the use of fats during exercise (22). In the research of Maki et al. (2009), it was found that a drink containing catechin caused a significant decrease in the concentration of free fatty acids and serum TG (23).

In the study of Baharloo et al. (2014), after 12 weeks of aerobic training in the experimental group, weight, body mass index, waist circumference, the ratio of waist circumference to hip circumference and cholesterol, LDL cholesterol, C-reactive protein, and thyroid stimulating hormone levels were significantly reduced. No significant change was observed in triglyceride levels (24). The longer follow-up period in this study compared to the current study could be one of the reasons for some discrepancies.

۵. Conclusion

In summary, the results of the present study showed that eight weeks of aerobic exercise and consumption of green tea significantly improved the weight, BMI, HDL, and LDL of overweight-obese women compared to other groups. Based on this, eight weeks of exercise and consumption of green tea seem to be an effective strategy for weight loss.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval the research was conducted concerning ethical principles.

Informed consent was obtained from all participants

Informed consent Informed consent was obtained from all participants.

Author contributions

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

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Research Article

The Effect of 8 Weeks of High-Intensity Interval Training on the Expression of Lipasin in Diabetic Rats

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Abstract

Background: Diabetes is a metabolic disorder recognized as one of the most common diseases in the world. The disease has also increased dramatically in Iran.Today, there are many ways to treat diabetes, one of which is the increase in the level of pancreatic beta cells. The increase in these cells is done in several ways Several studies have demonstrated that the lipasin or betatrophin gene, a liver-expressed peptide hormone, increases the proliferation of beta cells, and that overexpression of this gene can increase the number of beta cells.

Materials and Methods: The study was conducted on 16 Wistar rats with a mean weight of 160 ± 10. They were induced by diabetes (seven months of diabetes mellitus was caused in rats). They then were divided into two groups of 6: Control (C) and High-Intensity Interval Training (HIIT). Eight weeks of exercise training was conducted on rats.The qRT-PCR technique was used to investigate changes in lipasin expression. An Independent t-test was used for data analysis, and Pearson correlation was used to determine the correlation between lipasin expression and insulin resistance index ($P < 0.05$).

Results: The results showed that expression of lipasin gene in the liver of rats in the training group was significantly higher than the control group rats after 8 weeks of training; Insulin resistance index of plasma, plasma insulin and plasma glucose decreased considerably after eight weeks of HIIT. Between lipasin expression and insulin resistance index in rats with type 2 diabetes in the training group, a consider correlation has been observed.

Conclusion: This study showed that an 8-week HIIT training period, with increased lipasin expression, could increase beta cells and also recover type 2 diabetes, which had been destroyed by these cells, and as a result of this increase in Insulin secretion and there is a way to prevent the disease.

Keywords:
HIIT training, insulin resistance, Lipasin, Type 2 diabetes

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1. Introduction

The prevalence of type 2 diabetes worldwide is rising rapidly due to the proliferation of this disease, insulin resistance, resulting in the destruction of the pancreatic beta cells and eventually disappearing, resulting in insulin not being secreted.

The pancreatic cells release insulin in two periods in this manner. In the first phase, insulin is released immediately in reaction to a rise in blood glucose, while in the second phase, newly created vesicles are released slowly and independently of blood glucose (2).

As long as stem cells continue to proliferate, the phenotype and duplication of beta cells in humans will be impossible (5). Beta cells are a vital receptor for blood glucose and release a little amount of insulin to manage glucose and energy balance (4).

Recent reports of a liver secreted protein have been responsible for liver signaling to beta cells. This protein is the same as betatrophin/ lipasin, which is the liver's expression in the pancreas and the function of the secreted protein in the pancreas. Lipasin It is an unknown gene that is called c19 or F80 in humans and GM6484 in rats, but several different names are used for this protein, including RIFL (Refeeding Induced Fat and Liver), ANGPTL8 (Angiopoietin-Like Protein 8), and betatrophin (7). It is a new protein but a member of the ANGPTL protein family, secreted from the liver. It participates in triglyceride metabolism, is a regulator of lipid metabolism (8), reduces TG (triglyceride) barriers by increasing serum TG content (4), and its levels are sensitive to food consumption (8), which means that it is stimulated by eating food and stopped by hunger. Most importantly, increases pancreatic β-cell proliferation (7).

Betatrophin/lipasin has recently received attention in preventing diabetes and as a β-cell regenerator, it has a potential role in type 2 diabetes (9). How insulin is returned to the cell by the pancreatic β-cell mass is incredibly beneficial for all people with diabetes (type 1 and 2), and the renewal of functional β-cell mass is valuable in that it is a crucial target for the prevention of diabetes (4).

Exercise is an essential part of preventing diabetes, primarily type 2 diabetes. Its benefits include improving physical fitness, preventing and reducing fat, improving the fat status and metabolic control of blood sugar, reducing the risk of coronary heart disease, and mental and social benefits: stress reduction and the possibility of reducing or eliminating diabetes medications for type 2 diabetes (1).

This study aimed to investigate the effect of eight weeks of High Intensity Interval training (HIIT) on lipasin expression in liver tissue in type 2 diabetic rats.

2. Materials and Methods

This study was performed on 16 Male Wistar rats with a mean weight of 160 ± 10 g. After induction of diabetes (feeding rats for seven months with food containing 30% animal fat and 25% fructose), rats were randomly divided into two groups, control, and HIIT. The training program was run for 8 weeks and 5 sessions per week on the treadmill running. Exercise with two periods (two-minute) of intense activity (80-90% of maximum oxygen intake) in the first week, with four replicates in the fourth week and at the end of the eighth week; between any two high-intensity alternatives, two minutes of Low Intensity regression (30% Maximum Oxygen intake).

The rats of the training group were placed on the treadmill for one week to familiarize themselves with the training protocol, with the utmost precision and calmness and at a low and uniform speed, and from the first week of training until the eighth week, they performed the corresponding protocol five days a week. They did it for eight weeks. The velocity maximum oxygen consumption (VO2max) was measured on the sixth day every two weeks. Due to the absence of availability to a direct instrument, such as a respiratory gas analysis equipment, an indirect technique with high precision was utilized (pilots have been conducted).

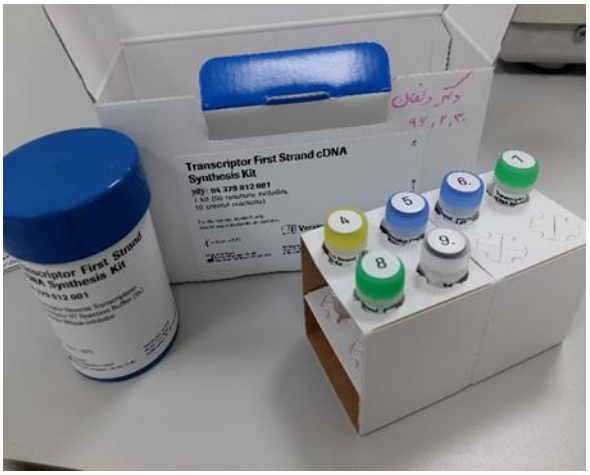
After three minutes of warming up at a speed of five meters per minute, the speed of the treadmill was increased once every two minutes by 4 meters per minute, the maximum speed was the maximum when the rats could not run at a constant speed for at least 1.3 minutes and Immediately after that, they were not able to run by increasing the speed (the incline of the treadmill was 0 degrees).

The control group did not participate in any sports activity program, but they were placed next to the treadmill to make them adapt to the environment and equalize the conditions with other rats.

In the HIIT training protocol, at the beginning, there was a three-minute warm-up with an intensity of 30 % of VO2max. After the warm-up, there was a high-intensity interval, with an intensity of 80 % of VO2max in the first week and 90 % of VO2max from the second week to the end of the eight week.

Low-intensity intervals were at 30 % of VO2max; The number of high-intensity interval was two repetitions in the first week, three repetitions in the second, third, and fourth weeks, and four repetitions from the fifth to the eight week. The high-intensity interval was two minutes and the low-intensity interval was two minutes. At the end, three minutes of cooling was done with an intensity of 30 % of VO2max. The incline of the treadmill was set to 0 degrees during the whole time of the exercise protocol.

Twenty-four hours after the last training session, the animals were sacrificed, and the lipasin gene expression from liver tissue was measured using the qPCR Real-Time method. An Independent t-test was used for data analysis, and Pearson correlation was used to determine the correlation between lipasin expression and insulin resistance index ($P < 0.05$).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3. Results

The results showed that after eight weeks of training, the expression of the lipasin gene expression in the liver of the rats in the training group was significantly higher than in the control group (P = 0.037); also, a significant negative correlation was observed between the expression of the lipasin gene and the insulin resistance index in the training group was compared to the control group (r = -0.568, P = 0.037).

In this study, the changes in the expression of the lipasin gene with the intervention of eight weeks of HIIT exercise as a preventive method for type 2 diabetic rats, by measuring plasma insulin, plasma glucose, insulin resistance index and expression of the lipasin gene in the liver of rats. The results of research indicate that HIIT workouts considerably enhance the expression of the lipasin gene compared to the control group, and that this increase has a good effect on the prevention of diabetic rats with the purpose of accelerating their recovery. Therefore, future study must investigate the impact of various training techniques in this area.

* Indicates the significance of the mean difference at the level of P<0.05

Sig.	standard error	Average group difference	Variable
0.016	0.34038	-0.92567*	HIIT, C

* Significant

Pearson Correlation Test Results

* Indicates the significance of the mean difference at the level of P<0.05

Sig.	Correlation with insulin resistance index	Variable
0.014	-0.568*	lipasin

* Significant

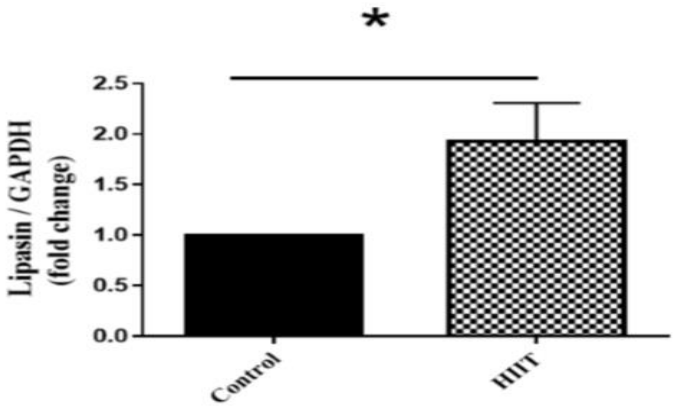

Figure 1: Comparison of significant levels of lipase expression in severe periodic exercise group (HIIT) and control group

* Significant

4. Discussion

By raising the expression of the lipasin gene, it appears that eight weeks of HIIT can increase the beta cells in diabetes patients and may be a beneficial non-pharmacologic intervention for reducing the disease's symptoms and consequences. Some studies have documented the therapeutic effect of this gene, and this therapeutic effect in diabetic patients will occur due to increased beta cell mass due to the overexpression of this gene.

A study on the claim was made by Daniel Espes et al. 2015. In this study, betatrophin in diabetics as an enhancer of beta cell proliferation is a target drug for the treatment of this disease (1). Collaborative studies have been conducted by Chen J et al. (2) that have been shown to improve glucose tolerance and insulin uptake, and studies conducted by Jonase Ah et al. (3), Lynnel et al (4), Chen et al. (5) and controversial reviews with this discussion have been made by Cox Aaron R et al. (6) and Victoria G et al. which have shown no increase and control of beta cells in the over expressive effect of this gene (7).

Yi et al. 2013, conducted a study on the betatrophin hormone that controls beta-cell proliferation. As a result of this study, the expression of betatrophin in the liver promotes beta-cell proliferation, promotes beta-cell mass development, and improves glucose tolerance. Therefore, betatrophin therapy can replace several insulin-producing cells instead of insulin injections in diabetics (8). Other investigations were conducted on Jonathan Ah's research on serum betatrophin in 2014, and as a consequence of this study, betatrophin will offer promise for the treatment of type 2 diabetes (3). Additionally, Zhu et al. did a study on the new insights of betatrophin about the therapy of diabetes and lipid metabolism. Showed that beta cells are known to respond to betatrophin stimulation, which modulates the mechanism of action between the liver and the fat by pancreas, and provides a way to treat diabetes with this approach (9).

There are many studies in line with this research, all of which have been conducted on serum lipasin in different conditions (pregnant women, children, obese people, etc.). The researches of Mohamed Abu-Farha et al., Shimin Wu et al., Chang-chiang chen et al., Natalia W et al., Onur Erol et al., Thomas Ebert et al., Yamada H et al., (10,12 ,11,13,14,15).

In study conducted by M Abu-farha et al., the opposite result was observed and there was no significant correlation between serum betatrophin/lipasin and insulin resistance index. Of course, these studies were conducted on serum lipasin and cannot be generalized to our research problem.

In a research conducted in 2017 by M Cahova et al., the findings of this research showed that the expression of the lipasin gene in white adipose tissue in Wistar rats has no significant correlation with insulin resistance, and this result is contrary to the current research and the reason Inconsistency is that measurements were made in adipose tissue (16).

In some studies, they also looked at the difference between lipasin gene concentration in obese and diabetic people. As a result of these investigations, it was shown that the serum betatrophin concentration is increasing in obese people, but no significant increase was seen in type 2 diabetics, and the reason for this inconsistency can be seen in the measurement of serum betatrophin (17).

Additionally, studies have been done by Cox Aaron R and colleagues (6) and Victoria G and colleagues (7) who have proven the lack of increase and control of beta cells due to the overexpression of this gene and the reason for this inconsistency These studies have been conducted on healthy people or rats.

۵. Conclusion

The results showed that expression of lipasin gene in the liver of rats in the training group was significantly higher than the control group rats after 8 weeks of training; Insulin resistance index of plasma, plasma insulin and plasma glucose decreased significantly after 8 weeks of HIIT, and between lipasin expression and insulin resistance index in rats with type 2 diabetes in the training group, Significant correlation has been observed. This study showed that an 8-week HIIT training period, with increased lipasin expression, could increase beta cells and also recovered in type 2 diabetes, which had been destroyed by these cells, and as a result of this increase Insulin secretion and there is a way to prevention the disease.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest in publishing this article.

Ethical approval the research was conducted with regard to the ethical principles (IR.SBMU.RETECH.REC.1395.883)

Informed consent Informed consent was obtained from all participants.

Author contributions

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

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