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The Effect of Aerobic Exercise on Homocysteine, C-Reactive Protein and Lipid Profile in Active and Inactive Men

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Abstract
Introduction: A number of studies have reported that regular aerobic exercises can improve cardiovascular biomarkers such as homocysteine, C-reactive protein and lipid profile. The purpose of this study was to investigate the effect of 4 weeks of aerobic exercise on homocysteine, C-reactive protein and lipid profile in active and inactive men.
Methods: in this quasi-experimental study, 26 active (n = 13) and inactive (n = 13) students aged (18- 23) years old participated voluntarily. All subject performed aerobic exercises for 4 weeks, 3 sessions per week and 60 minutes with intensity of 60- 75 % of maximum heart rate reserve per session. Blood sample was taken in the pretest and 24 hours after the last aerobic exercise session in the post- test. Independent sample t- test and paired sample t- test were used to analyze the data ($p \leq 0.05$).
Results: Serum levels of homocysteine (P = 0.001) and low density lipoprotein cholesterol to high density lipoprotein cholesterol (P = 0.003) significantly decreased in the active group compared to the inactive group. However, there were no significant difference in the changes of C-reactive protein (P = 0.12), low density lipoprotein cholesterol (P = 0.56), triglyceride (P = 0.24), total cholesterol (P = 0.21), total cholesterol to high density lipoprotein (P = 0.52) and triglyceride to high density lipoprotein (P = 0.26), between active and inactive group.
Conclusion: It appears that serum levels of homocysteine reduced further after four weeks of exercise in active subjects in comparison with inactive subjects. However, the improvement in the serum levels of C-reactive protein and lipid profiles after four weeks of exercise training were the same in both active and inactive individuals.
Keywords: Exercise, C- Reactive Protein, Homocysteine, Lipid Profile

Introduction

Arteriosclerosis is the most important cause of cardiovascular disease. Atherosclerosis causes the various arteries that go to different parts of the body gradually become hard and narrow and decreases their ability to transmit oxygen and nutrients to the cells of the body. Some factors can accelerate the onset of arteriosclerosis which they increase the chance of developing cardiovascular disease (1). Studies have shown that some people with normal density lipoprotein and high-density lipoprotein have also been affected by cardiovascular disease (2, 3). Therefore, it is important to pay attention to indices that predict the risk of cardiovascular disease with greater accuracy and sensitivity (4). Some of these indices include C-reactive protein (CRP), insulin resistance, homocysteine (Hcy), fibrinogen and lipoprotein A (lp (a)) (5, 6).
CRP is a stable marker of systemic inflammation produced in response to inflammatory cytokines and the alpha-receptor factor (lipoprotein secretion) in the liver (7). Studies have shown that CRP is a strong predictor of cardiovascular disease and has high levels of blood circulating in obese people and metabolic syndrome. Therefore, it seems that reduction of CRP levels is associated with a reduction in cardiovascular

risk and other chronic obesity-related diseases (including diabetes and cancer) (8). Homocysteine is a small amino acid containing an amount of sulfur synthesized from methionine, which requires certain vitamins such as B12, B6, and B6 to metabolize. Hence, it can produce peroxide and hydrogen superoxide to the inner stratum of the arteries, platelet aggregation, the amount of coagulation factors that make the formation of the clot easier, and preventing the spread of small arteries leads to their blockade and can affect the vessels (9). Several factors affect total serum homocysteine concentrations such as genetics, smoking, hypertension, creatinine levels, total cholesterol and serum protein, and nutritional factors such as vitamin B6, B12 deficiency (10). Researches have indicated that increased levels of serum homocysteine are associated with increased risk of ischemic heart disease and stroke (11- 14). In recent studies, the effect of exercise on new and traditional cardiovascular parameters has been studied and interesting results have been reported; in this regard, there are different results about the effect of aerobic exercise on the serum CRP. In a study conducted on 102 males and 100 inactive women aged 40- 75, Christian *et al.* (2008) concluded that 12 months of mild aerobic exercise (six sessions per week for 60 minutes, equivalent to 60- 85 % of maximal oxygen consumption) does not change the levels of the CRP (16). In a study conducted on male and female professional wrestlers, Habnrowsniack and Achowki (2009) reported that strength and speed exercises in professional wrestlers significantly reduced the levels of CRP and Hcy (17). Mora *et al.* (2006) in a study entitled "The relationship between physical activity and body mass index (BMI) with new cardiovascular and traditional biomarkers in women", reported that lower levels of physical activity and increased body mass index are directly related with independently increased total cholesterol, triglycerides glyceride, and Low-density lipoprotein (LDL-C) and inflammatory indices (CRP and fibrinogen) (18) while, Christopher *et al.* (2006) reported a lack of correlation between physical activity and inflammatory indices and stated that continuous endurance exercises have no significant effect on new cardiovascular parameters (TNF-a and CRP) (19). Goldhamer *et al.* (2005) studied the effects of 45 minutes of aerobic exercise with an intensity of 70- 80% of maximum heart rate for 3 days a week in coronary artery disease patients and reported that aerobic exercise caused a significant reduction in the levels of TNF-a, CRP, Interleukin (IL-6) (20). Homocysteine and CRP are considered as potent predictors of cardiovascular disease. Although most of these risk factors are acting independently and even more potent than the traditional risk factors of atherosclerosis (6). Therefore, the purpose of current study was to investigate the effect of aerobic exercise on homocysteine, C- reactive protein and lipid profile in active and inactive men.

Methods

This research was a quasi-experimental method in which two active and inactive groups were compared with the pre-test and post-test design. The statistical sample of this study included 26 people (13 people) professional footballer in the active group and 13 students in the inactive group who were selected from a target group through a selective and accessible sampling after the diagnosis and clinical examination. The inclusion criteria included being healthy based on the health questionnaire, no drug consumption, being non-smoker, and not having any coronary artery disease, renal failure and hypothyroidism. In the first stage, people became familiar with the nature and manner of cooperation with the implementation of the research.. Subjects participated voluntarily in the research based on the research conditions and signed the consent form. The subjects' age ranged from 18 to 23 years old. The height of the subjects was measured by Seca with 5 mm sensitivity, the hip and waist circumference were measured by meter strip (Mabis / Japan) with a sensitivity of 5 mm, body fat percentage and weight were measured by the impedance bioelectric device (model / IOI353) with sensitivity of 100 grams. The body mass index (Kg / m2) was determined by dividing body weight by height/meter squared. To determine the waist to hip ratio of the subjects, the researcher measured the waist circumference with a meter strip at the lowest point (between the lower end of the chest and navel) in cm and the hip circumference (in the wider area, on the cap) in cm measured from the lumbar to the hip circumference. All measurements were made when the subjects refused to eat and drink four hours before the test, and even the bladder, stomach and intestine were evacuated. The subjects were allowed to enter the project after the measurement of blood pressure and electrocardiogram recording and cardiovascular examination by a specialist physician. Blood samples were taken after 12 hours of fasting and in two stages to evaluate the biochemical variables, i.e., before the beginning of the exercise protocol and after 4 weeks of aerobic exercise. In the first stage, blood samples were taken from the left arm vein in the sitting position and at rest, and the samples were immediately sent to the laboratory to be analyzed by a specialist. Blood samples were isolated and centrifuged one hour after blood sampling at 2,700 rpm for 10 to 15 minutes. After completing the exercise period (4 weeks) and 24 hours after the last exercise session, blood samples were taken from the subjects the same as the first stage. The serum was stored in a freezer at a temperature of -20 ° C. All biochemical measures were performed by an autoanalyzer instrument. For the CRP, the method used to measure hsCRP was an amplified meter immunoturbid method for measuring two-point photometric measurements. In this method, the CRP present in the sample with a polyclonal antibody sensitive to human CRP, coded for latex particles, forms a complex and creates opacity. The amount of opacity created has a direct relation with the amount of CRP contained in the subject sample. The kits used were manufactured by Pars Azmoun Co. (Tehran-Iran). This kit is designed to measure CRP in the range of 0.1 to 20 mg / L, and the minimum amount of hsCRP measurable is 0.1 mg / L. Serum Hcy values were measured using a special kit and ELISA method (21). Before binding to the protein Hcy immune system, Hcy was first released and then enzymatically converted to adenosine l-homocysteine (SAH). Total serum cholesterol was measured by enzymatic colorimetric method and in the presence of cholesterol estradiol and cholesterol oxidase. Serum triglyceride levels were determined by enzymatic colorimetry and in the presence of glycerol phosphate oxidase. Serum HDL level was determined by enzymatic method after precipitation of all other lipoproteins containing Apo B by phosphotangestric acid and magnesium chloride solution (22). The regular exercise in this study was a specialist exercise program for professional male football players, held for 4 weeks, three days per week. The exercises were held in the afternoon (from 15:00 to 16:30), including general warmup for 10 minutes (walking, gentle running, stretching and jumping). For each person, ten minutes of private warming up included short and fast starts, roundabouts and balls, and 10 to 15 m start-ups. Then, the main exercise sessions lasted for 45 to 60 minutes with an intensity of 60- 75 % of the maximum heart rate reserve. At the end of each exercise session, 10 minutes of cooling down (slow running, walking and tensile movements) was performed to let the body return to its original state. The intensity of exercise was controlled using a Polar TB machine (19). Independent sample t- test and paired sample t- test were used to analyze the data ($p \leq 0.05$).

Results

The results of paired sample t- test in Table 2 showed that in active group after four weeks of aerobic training, serum levels of homocysteine (P = 0.0001), CRP (P = 0.024), low density lipoprotein cholesterol (023 (P = 0.02), high density lipoprotein cholesterol (P = 0.023), total cholesterol (P = 0.002), low density lipoprotein cholesterol to high density lipoprotein cholesterol (P = 0.006), total cholesterol to high density lipoprotein cholesterol (P = 0.001), significantly decreased in the post- test compared to the pre- test. However, there were no significant difference in changes of triglyceride (P = 0.99) and triglyceride to high density lipoprotein cholesterol (P = 0.52) in the post- test compared to the pre- test. Also, the results of paired sample t- test in Table 2 showed that in the inactive group after four weeks of aerobic training, serum levels of low density lipoprotein cholesterol (P = 0.006), high density lipoprotein cholesterol (P = 0.042), total cholesterol (P = 0.015), low density lipoprotein cholesterol to high density lipoprotein cholesterol (P = 0.004), total cholesterol to high density lipoprotein cholesterol (P = 0.005), and triglyceride in the post- test have decreased in comparison with the pre- test. However, there were no significant difference in the changes of high density lipoprotein cholesterol (P = 0.35), homocysteine (P = 0.58), CRP (P = 0.95) and triglyceride (P= 0.202) in the post- test compared to the pre- test. The results of independent t-test in Table 2 showed that serum homocysteine (P = 0.001), low density lipoprotein cholesterol to high density lipoprotein cholesterol (P = 0.003) in the active group were significantly decreased compared to the inactive group. However, there were no significant difference in the changes in CRP (P = 0.12), low density lipoprotein cholesterol (P = 0.56), triglyceride (P = 0.24), total cholesterol (0.21) = P), total cholesterol to high density lipoprotein cholesterol (P = 0.52) and triglyceride to lipoprotein cholesterol (P = 0.26) between active and inactive groups.

Discussion

In this study, 4 weeks of aerobic exercise program significantly reduced cardiovascular biomarkers of active and inactive men, which is consistent with the findings of Dastani *et al.* (2013). They showed a reduction in serum CRP levels after 8 weeks of aerobic exercise 3 times a week with an intensity of 50-60 percent of maximum heart rate in postmenopausal women; also the findings of Mani *et al.* (2013) showed a significant reduction in CRP after exercise. Many *et al.* (2013) evaluated the effect of aerobic exercise on inflammatory indices in obese adolescents and showed no significant change in the CRP levels after aerobic exercise for 180 minutes per week with an intensity of 40 to 55 percent of maximum oxygen consumption (24).

Table 1. Characteristics of participating subjects

	Variables (Mean ± Standard deviation)					
Groups	**Age (year)**	**Height (cm)**	**Weight (kg)**	**Body Mass Index (kg / m2)**	**Body Fat Percentage (%)**	**Waist to Hip Ratio (Percent)**
Active (13)	18.38±0.50	177.23±4.67	56.7±7.7	20.9±2.1	10.8±3.7	0.6 ±0.5
Inactive (13)	21.07±1.55	173.53±4.11	69.3±6.7	24.9±4.0	19.0±7.9	0.7±0.6

Table 2. Comparing the paired sample t- test and dependent sample t- test variance changes of chemical factors and cardiovascular biomarkers

Variable	Group	Time	M±SD	Paird sample t-test	Independent Samples t-test
Homocysteine (MI / L)	Active	Pre- test	23.69±2.6	0.0001	0.001
		Post- tets	21.69±1.7		
	Inactive	Pre- test	23.15±2.6	0.58	
		Post- tets	23.00±3.05		
CRP (ng/ml)	Active	Pre- test	2463.69±191.9	0.024	0.12
		Post- tets	2461.84±191.77		
	Inactive	Pre- test	2458.07±253.17	0.95	
		Post- tets	2465.92±245.90		
Low-density lipoprotein cholesterol (Mg / dl)	Active	Pre- test	93.6±8.7	0.23	0.56
		Post- tets	89.9±5.9		
	Inactive	Pre- test	128.0±30.5	0.006	
		Post- tets	117.0±27.3		
High-density lipoprotein cholesterol (Mg / dl)	Active	Pre- test	41.2±6.3	0.23	0.24
		Post- tets	43.3±5.6		
	Inactive	Pre- test	37.9±3.9	0.42	
		Post- tets	38.9±3.7		
Triglyceride (mg / dl)	Active	Pre- test	51.9±14.2	0.99	0.24
		Post- tets	51.9±13.0		
	Inactive	Pre- test	97.1±57.4	0.20	
		Post- tets	107.3±52.5		
Total cholesterol (mg / dl)	Active	Pre- test	145.2±9.8	0.002	0.21
		Post- tets	140.2±9.2		
	Inactive	Pre- test	185.4±37.1	0.15	
		Post- tets	175.8±33.9		
Low-density lipoprotein cholesterol ratio to high-density lipoprotein cholesterol (mg / dl)	Active	Pre- test	2.3±0.3	0.006	0.003
		Post- tets	2.1±0.2		
	Inactive	Pre- test	3.4±0.9	0.004	
		Post- tets	3.0±0.7		
Total cholesterol ratio to high-density lipoprotein cholesterol (mg / dl)	Active	Pre- test	3.5±0.4	0.001	0.52
		Post- tets	3.2±0.3		
	Inactive	Pre- test	4.9±1.2	0.005	
		Post- tets	4.5±0.9		
Triglyceride ratio to high-density lipoprotein cholesterol (mg / dl)	Active	Pre- test	1.2±0.4	0.52	0.26
		Post- tets	1.2±0.4		
	Inactive	Pre- test	2.6±1.6	0.35	
		Post- tets	2.8±1.5		

Sig level p<0.05

Therefore, it can be said that at least two months of regular exercise with different intensities can reduce CRP and decrease the risk of atherogenesis. One of the pathophysiologic mechanisms of inflammation is the production of cytokines in response to stimuli such as oxidized LDL, macrophages with atherosclerotic plaque and other risk factors such as homocysteine. Laboratory studies have shown that compounds of these cytokines stimulate the production of CRP, and regular exercise can reduce oxidized LDL, homocysteine, and subsequently reduce serum CRP levels (25). Physical activity can reduce CRP by decreasing the production of cytokines in adipose tissue, muscle and mononuclear cells. It can also indirectly reduce CRP by increasing insulin sensitivity and improving endothelial function (26). The reason for the difference in the results of studies can be the differences in exercise programs such as weight loss (27), diet, exercise design (28) and the type of subjects (29). There are still many controversies regarding the mechanisms by which regular exercise can improve the level of CRP. The results of this study showed that 4 weeks of aerobic exercise significantly reduced the CRP in active men. These results are consistent with the findings of Videsagar *et al.* (2013) (21). However, it is not consistent with the results of Albright *et al.* (2006) (22). Videsagar *et al.* (2013) in a study comparing the effect of three different exercise intensities on the level of CRP in healthy young people, found that after 8 weeks of exercise, CRP level significantly decreased in all three groups. Albright *et al.* (2006) showed that CRP increased in response to a moderate and severe aerobic exercise session (22). In several studies that examine the relationship between weight loss and reduction of inflammation (CRP), the reported results indicate that the reduction in fat mass is a determining factor in reducing the reactive protein. It has been suggested that to produce anti-inflammatory effects, at least 3.5 pounds of weight loss is necessary. Therefore, it seems that, weight loss has probably been observed in the experimental group only, which led to a decrease in the CRP. Findings also suggest that reducing the CRP is associated with the improvement of symptoms of metabolic syndrome, including half-side of blood lipids (23). The mechanism of reduction of CRP following weight loss is not clear. On the other hand, physical activity has been shown to reduce the penetration of macrophages into adipose tissue. Therefore, it is believed that exercise by reducing fat mass and less penetration of macrophages leads to less inflammatory factors produced by adipose tissue (24, 25). The results of this study showed that four weeks of aerobic exercise caused a significant decrease in total cholesterol, low-density lipoprotein cholesterol to high-density lipoprotein, triglyceride to high-density lipoprotein cholesterol and total cholesterol to high-density lipoprotein cholesterol in active men and high-density lipoprotein cholesterol increased significantly in both active and inactive men. The results are consistent with the findings of Eliot *et al.* (2002) (26). In some studies, the levels of LDL, TC, and TG decreased and HDL levels were significantly increased, but in this study, only the TG level was significantly decreased, thus, the results were consistent with the results of this study. According to Eliot *et al.* (2002), eight weeks of resistance exercise with an intensity of 80% of ten maximum repetition did not significantly change the lipid profiles and body composition of menopausal women. The researchers concluded that a low-intensity exercise program with a low duration to produce significant changes in blood lipid levels was not enough. There was no significant change in waist-hip to ratio and fat percentage, and its results are consistent with the present study (26). Quillard *et al.* (2001) stated that regular endurance exercise significantly reduced TG and significantly increased HDL (27). The duration of exercise can affect the change in fat profile. It is possible that by increasing the number of exercise sessions per week (more than 3 sessions per week) or increasing the duration of each session (more than 60 minutes of activity), optimal changes, or better, HDL and decreased LDL. Low exercise sessions may not affect the levels of these variables, but can reduce the percentage of lipids and blood pressure and increase aerobic power (according to the findings of this study) (28). In addition to physical activity, levels of thyroid and sex hormones also affect blood cholesterol and lipoproteins (29). Therefore, it is likely that the non-change of these factors can be attributed to thyroid and sex hormones. Physical activity and exercise, especially aerobic activities, significantly increase the levels of HDL in the blood, which is due to the activation of lipoprotein lipase and lecithin cholesterol, asyl transferase enzymes and decreased activity of the liver lipase enzyme (30). Although the activity of these enzymes was not investigated in this study, it seems that there may be a significant change in the level of HDL in the function of these enzymes. Regular endurance exercises increase the expression of the gene and the function of lipolysis enzymes (31). TG is the most important source of energy during endurance physical activity. Lipoprotein lipase is a TG degrading enzyme that releases free fatty acids from TG to provide energy during aerobic activity. Therefore, there is a high relationship between lipoprotein lipase enzyme activity and TG clearance. Therefore, it can be concluded that due to aerobic exercises and increased lipoprotein lipase enzyme activity, blood TG levels have decreased for energy production (32).

Conclusion

According to the results of the present study, it can be concluded that serum levels of homocysteine after four weeks of exercise in active individuals are more likely to decrease than inactive people .However, the improvement of serum levels of CRP and lipid profile in active and inactive subjects are the same after four weeks of aerobic exercise.

Ethical issues

Not applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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