The Effect of Combination Exercise Training on Cardiovascular Risk Factors (adiponectin, interleukin-6 and homocysteine) in Sedentary Middle aged Men

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ABSTRACT:
Purpose: Cardiovascular diseases are the major cause of mortality in all over the world. We evaluated the effect of eight weeks combined training on some cardiovascular risk factors in sedentary healthy middle aged men. Methods: 24 sedentary healthy, middle aged male subjects (age: 63.4±2.1 years, BMI: 23.1±2.5 kg/m2) participated in this study. Subject were randomly assigned to training (T, n=12) and control (C, n=12) groups. T group, underwent one training session per day, 3 days/week, for 8 weeks. Training program included both aerobic and resistance exercise. Aerobic training consisted in 20 min running on a treadmill at 60-70% HRR for each session. Resistance training consisted in 10 movement stations e.g., leg flex-extension-press, squat, lat pull, chest press, cross arm, biceps-triceps with halter and sit up in 2 sets with 10 repetitions that performed at 70% 1RM. Before and after combined aerobic/resistance training, all subject underwent body fat assessment and cardiopulmonary exercise test (CPX). Venous blood samples were also drawn for determination of plasma levels of adiponectin, interleukin-6 (IL-6) and homocysteine. Data were analyzed using repeated measure test (ANOVA). P<0.05 was considered statistically significant. Result: Plasma levels of IL-6, homocysteine and body fat were significantly reduced and adiponectin was significantly increased in T, whereas all the variables did not change significantly in C. Vo2max increase significantly only in T group (20.41±2.93 vs 23.25±2.00) and in control group (19.91±2.84 vs 20.08±3.20). Conclusion: Combined aerobic/resistance exercise training program by reducing plasma levels of IL-6 and homocysteine and increasing adiponectin levels might result in an improvement cardiovascular risk in middle-aged healthy subject

KEY WORDS: Adiponectin, Homocysteine, Resistance training, Aerobic training

INTRODUCTION
The risk of cardiovascular diseases increases with aging, and the main reason is atherosclerosis. Atherosclerosis is generated by increase in the deposition of triglycerides, cholesterol, and calcium in the coronary arteries, so that, by continuation of deposition of fats, arteries will be blocked, and it leads to heart attacks [1]. Obesity and lack of exercise are the two main factors associated with the risk of cardiovascular diseases [2]. Heart disease is a progressive disease that begins in childhood and in
old age and the leading cause of death in the industrial world [3]. It is anticipated that in 2025, deaths of cardiovascular disease more than any other disease in the world, probably more than any other disease, such as infection, cancer and trauma[4]. Increased lipoprotein with low density (LDL-C) and reduction of lipoprotein with high density (HDL-C) are the risk factors of cardiovascular diseases; however, reports indicate that some people with heart disease have had normal LDL-C and HDL-C. According to conducted researches, the development of cardiovascular diseases could be due to underlying inflammation. In this regard, general inflammation (systemic) plays a central role in the development of atherosclerosis [3, 5].

Therefore, in the past decade, researchers paid more attention to inflammatory indicators such as interleukin-6 (IL-6), adiponectin, and homocysteine as independent factors predicting cardiovascular diseases [6-8]. IL-6 is a proinflammatory indicator predicting cardiovascular diseases. The findings show that IL-6 is the regulator of acute phase proteins, anti-inflammatory, and immune inhibitor and may also set the acute response negatively [9]. Adiponectin is one of adipokines that is closely related to obesity and insulin resistance and in this regard, has been of interest to many researchers. The mean value of plasma adiponectin in individuals is 10 micrograms per milliliter [10], and forms about 0.01 percent of the whole plasma proteins [11]. Adiponectin is a protein released from the adipose tissue; in addition, it is a particular adipokine with anti-atherogenic and anti-inflammatory properties, which increases insulin sensitivity [12-13]. Evidence suggests that there is a negative relationship between adiponectin and insulin level and insulin resistance [14]. Compared with other adipokine, the amount of circulating adiponectin in patients with coronary artery disease, obese men with type 2 diabetes, and also obese people is lower; however, its value can be increased through a weight loss program [12-13]. Homocysteine is a by-product during the metabolism of methionine which causes endothelial dysfunction, increased oxidation of LDL, the suppression of nitric oxide function with reducing its post-arterial dilation, the suppression of the oxide synthase, activating platelets, and exerting oxidative stress [15]. It should be noted that there is a close relationship between inflammatory indicators and cardiovascular diseases; furthermore, the reduction of these indicators is associated with decreased cardiovascular disease [16]. Studies have shown that lifestyle modification, weight loss, and increased physical activity has been associated with a reduction in IL-6 and rising of adiponectin [7, 17]. In this regard, i.e. the impact of resistance and aerobic exercises, some researchers have stated that a period of aerobic and resistance exercises can cause significant decrease in the levels of IL-6 [8], while working out 16 weeks of resistance, enduring, and combinatory exercises, despite significant increase in muscle strength, maximum oxygen uptake, and lipid parameters had no effect on levels of interleukin-6 [18] and adiponectin of middle-aged men [19]. Donges et al (2010) show 10 weeks of resistance or aerobic exercise training on interleukin-6 (IL-6) and C-reactive protein (CRP) in 102 sedentary subjects did not reduce IL-6 levels in both groups; however, the resistance and the aerobic groups reduced CRP by 32.8% and 16.1%, respectively[20]. Libardi et al (2012) investigated the effects of 16 wk of resistance training, endurance training, and concurrent training on inflammatory markers, C-reactive protein (CRP), and functional capacity in sedentary middle-age men. They were show that maximal strength was increased after 16 wk, with no differences between RT and CT. VO2peak increased in ET and CT comparing before and after training. There were no significant differences in TNF-α, IL-6, and CRP comparing before and after training[21]. Now, considering the intervention of combinatory exercises on increasing of Adiponectin and reduction of interleukin-6 and homocysteine, few studies have been done and there is some confusion. This study was carried out regarding combinatory exercises in rehabilitation and empowerment of patients, especially middle-aged patients with cardiovascular disease, and restoring them to normal activities.

METHODS

Subjects
In this study 24 middle aged men (age: 60 to 70 yr, Body mass index (BMI): 22 to 25 kg.m2) were selected voluntarily and objectively participate in this study.

Study design
During first stage, individuals were introduced to the concept and ways of co-operating. Important notes were given on subject’s exercises, nutrition, diseases, drug consumption, energy drinks, and drug abuse so that they could take necessary measures to consider them and lack of participation in any exercise program at least two months before taking part in training. Then, in order to make sure that none of volunteers was carrying any of the following diseases including the common cold, influenza, sore throat, coronary sweating, diabetes, kidney and thyroid malfunction, they filled up a self-testifying questionnaire, and after the approval of its standards, the participation and co-operation agreement form, which allowed them to be the subjects of the study, was signed by the qualified volunteers. They were randomly categorized into two groups of 12 individuals: experimental and control.

Anthropometric measures
The body mass index (BMI) was measured by height and weight values as follows: weight (kg)/height (m2) and waist-to-hip ratio (WHR). The body composition was determined by bioelectric impedance using InBody-720 (Biospace, Dogok-dong, South Korea) to study the fat mass (FM), muscle mass (MM) and total body water (TBW). The analysis performed each time under standardized conditions. The body mass
index (BMI) was measured by height and weight values as follows: weight (kg)/height (m2) and waist-to-hip ratio (WHR).

**Height Measurement**

The height of the same person was measured by using an electronic balance with stadiometer (SECA-Germany) to the nearest 0.1 cm[22].

**Blood Pressure Measurement**

The subjects gain admission, after the cardiovascular examination, measurement of blood pressure and electrocardiogram recorded by specialist physicians. The systolic and diastolic BP of participants in the experimental group was measured before and after 6 weeks of exercise training in different circumstances in a sitting position using a mercury sphygmomanometer based on guidelines.

**Measurement of biochemical markers**

The blood samples were taken at 06:00 to 7:00 in the morning after an overnight fast for IL-6, adiponectin and homocysteine levels. For examining the parameters of this study, 5 ml of venous blood obtained at four occasions from each subject, Participants were asked to avoid for 24 hours before all visits intense physically activities like cycling, running and walking lasting more than 15 min. Blood samples in all related studies were collected by venepuncton from forearm vein after at least 15 minutes of sitting at rest or in the supine position. For measurement of serum adiponectin, IL-6 and homocysteine levels were determined using a by ELISA method by using Microplate Reader kits (company Biovendor) from Czech Republic, IL-6 kits (company Biovendor) from Germany and homocysteine kits (company Axis-shield diagnositon) from Germany respectively.

**Exercise interventions**

**Aerobic training**

The aerobic training program included 3 sessions/week, 60 min/session. The exercise interventions were either 20-min aerobic exercise on a treadmill at 60 to 70% heart rate reserve (HRR) ±10 beats/min. The intensity of the aerobic training according to the heart rate reserve (HRR), every single middle aged man was respectively measured based on Karvonen equation (220-age-resting heart rate) (1) and was also controlled during exercise by a heart rate monitor (made in Finland–Polar)[23]; thus, every two weeks, 5% was added to the intensity of aerobic activity. The aerobic activity included a 10 minutes warm-up such as stretching, exercise and running, 30 to 45 minutes main training exercises and 10 minutes cool-down exercises. The subjects were also advised to refuse any other exercise during the six-week program[24].

**Heart rate measurement**

Heart rate (HR) was measured every 5 minutes with, the aim of keeping it between 60% and 70% of heart rate. The participant was encouraged to increase intensity if the HR was less than expected, or to decrease the intensity if it was more than what was expected.

**Resistance training**

The resistance protocol included: resistance training by overload with 70% of 1-RM that each of movements performed in 2 sets with 10 repetitions. Rest intervals between repetitions and sets were 30seconds. The intensity of the resistance training was determined using by maximum repetition equation (2). The resistance training included 10 movement stations like a circular. For instance, leg flexion, leg extension, leg press, squat, lat pull, chest press, cross arm, biceps with halter, and triceps with halter and sit up. At the end of each exercise session, activities like jogging, walking and stretching were done for 10 minutes to return the body to its normal condition[25]. While the control group was prevented from any sport activity and just participated in pre- and post-testing.

Equation (1): Target heart rate= (%60 or %70+ (((220- age) - Resting heart rate)) + Resting heart rate

Equation (2): One maximum repetition = displaced weight (kilograms)/ (0/0278 x number of repetition to exhaustion) – 1/0278

**Statistical analysis**

All statistical analyses were performed with SPSS version 20 Descriptive statistics including means and SEs for the outcome variables of interest were computed. The average and standard deviation of data were calculated after checking the data distribution normalcy using Shapiro–Wilk test and Homogeneity of variance method. The comparison of means within and between means groups repeated measure test was used. Statistical significance was assigned at P < 0.05 for all analysis.

**STATISTICAL RESULTS**

**Characteristics of the subjects and changes in body composition**

The characteristics of the subjects are shown in Table 1. According to the (Table 2), Body mass index (p=0.008) and body fat percent (p=0.02) significantly decreased.

The resultant changes in Adiponectin, IL-6 and Homocysteine at rest levels after long-term exercise training

The changes in resting adiponectin levels and some cardiovascular risk factors of subjects in response to exercise training are shown in Table 2. According to (Table 2), our results show a significant reduction in IL-6 and homocysteine were observed in experimental group (p=0.001 and P=0.001). Adiponectin (20.27%, p<0.016) significantly increased after 8-weeks exercise training. A significant increase of maximal oxygen consumption (Vo2max) values was observed after eight weeks exercise training compared to that of before it. On the other hand, there were no significant changes in control group. Results showed a variance between group such as: adiponectin, IL-6, homocysteine, Body fat percent and Vo2max is significant (P<0.05).
DISCUSSION

The aim of this study was to examine the effect of eight weeks combined training on levels of IL-6, adiponectin and homocysteine in sedentary healthy middle aged men. In this study, eight weeks combined training exercise had a meaningful impact on interleukin-6 levels. The findings from the present study are consistent with those reported in the literature [8, 26] and this finding was not supported by different studies [18, 27-28]. It seems that the reason for differences in the results of findings is the discrepancy in age, race, exercise programs, and diet of subjects. Since IL-6 is a proinflammatory cytokines secreted by adipose tissue, and circulating levels are directly related to the amount of fat in the blood [1], the effect of exercise and physical activity on the production of IL-6 depends greatly on intensity, duration and body muscles’ mass [29]. Intracellular concentrations of muscle glycogen is an important stimulus for IL-6. In other words, IL-6 also acts as a cytokine which is sensitive to the glycogen resources [30]. Produced IL by the contracted muscle often rises up in short-term extreme sports [31]. This increase is due to the effects of exercise on adipose tissue and also the increase in lipolysis and fat oxidation [32] on glycogen hemostasis in liver [8] and its anti-inflammatory impact. Increased IL-6 in this condition, through increase in IL-10 and decrease of TNF-α, can have an inhibitory effect on the activity of T regulatory cells [33]. Studies have shown that IL-6 levels also increases during intense exercises associated with inflammation and tissue damage [34]. Furthermore, because the concentration of IL-6 is related to the fuel reserves of muscles, especially glycogen, long-term activities can cause the drainage in the reserves and reduction of interleukin-6 [30].

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variations (M±SD)</th>
<th>Age (years)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>Body Mass Index (kg·m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td></td>
<td>64.2±2.2</td>
<td>1.73±0.31</td>
<td>72.2±7.6</td>
<td>24.5±2.4</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>62.7±2.1</td>
<td>1.72±0.36</td>
<td>71.3±3.2</td>
<td>24.2±1.1</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group(s)</th>
<th>Pre-test M±SD*</th>
<th>Post-test M±SD*</th>
<th>Variations</th>
<th>Within groups</th>
<th>Between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>Exercise</td>
<td>1.48±0.14</td>
<td>1.78±0.21</td>
<td>0.016 †</td>
<td>0.0001 †</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.45±0.17</td>
<td>1.43±0.16</td>
<td>0.633</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (μg/ml)</td>
<td>Exercise</td>
<td>4.27±0.25</td>
<td>3.8±0.21</td>
<td>0.001 †</td>
<td>0.005 †</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.43±0.25</td>
<td>4.32±0.25</td>
<td>0.280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homocysteine (mm/l)</td>
<td>Exercise</td>
<td>20.91±2.50</td>
<td>18.16±2.20</td>
<td>0.001 †</td>
<td>0.01 †</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20.66±2.77</td>
<td>21.25±3.07</td>
<td>0.69</td>
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<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Exercise</td>
<td>23.12±2.52</td>
<td>23.3±1.92</td>
<td>0.008 †</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23.08±2.67</td>
<td>23.04±2.41</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body fat percent (%)</td>
<td>Exercise</td>
<td>34.00±3.88</td>
<td>30.75±3.49</td>
<td>0.02 †</td>
<td>0.03 †</td>
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<tr>
<td></td>
<td>Control</td>
<td>35.50±3.75</td>
<td>34.25±3.86</td>
<td>0.283</td>
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<td></td>
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<tr>
<td>VO₂max (ml/kg/min)</td>
<td>Exercise</td>
<td>20.41±2.93</td>
<td>23.25±2.00</td>
<td>0.008 †</td>
<td>0.008 †</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>19.91±2.84</td>
<td>20.08±3.20</td>
<td>0.88</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data presented as mean ± standard deviation †The mean difference is significant at the 0.05 level
The result of this study showed significant increase in adiponectin concentration after the long-term training in middle aged men. Our result corroborates the reports of Balducci et al. [35] and Peeri et al.[36]. While, this finding was not supported by Oberbach et al. [37] and Klimcakova et al.[38]. Interestingly, a similar study by Balducci et al. [35] observed a significant decrease in interleukin-6, resistin and Leptin, whereas adiponectin increased in the high-intensity aerobic or aerobic-resistance group compared to Control group. Whereas, Klimcakova et al.[38] reported that there is no change in plasma levels of adiponectin 3 months of exercise training compared to that of before it. In the meantime, numerous studies have shown that adiponectin has a strong positive correlation with insulin sensitivity [39]. The effect of adiponectin on glucose is one of the important mechanisms by which the adiponectin hormone with negative regulation of key enzymes of gluconeogenesis process (Pyruvate, phosphoethanolamine, and carboxy kinase glucose-6-phosphatase) prevents the production of glucose by liver, and increases insulin sensitivity [40]. Adiponectin also has a significant effect on intracellular function of insulin, and it has been shown that low concentration of plasma adiponectin causes the reduction of tyrosine phosphorylation and insulin receptors of muscle cells; in addition, it can be the starting point for diabetes, especially in older people [41].

Thus, in case of effects of exercise on adiponectin concentration, the process can be followed by a reduction of insulin resistance, which can be an appropriate and cost-effective solution to prevent possible disease of diabetes in the elderly people. Evidence shows changes in blood levels of adiponectin are inversely related to body fat mass [42-43], and its positive changes are in relation to weight loss and increased muscle mass. Hence, the reason for the change of adiponectin levels in subjects of this research, followed by intervention of eight weeks of combinatorial exercise program, can be caused by an impact of exercise on the amount of fat mass. Even though the exact path in which adiponectin causes the oxidation of fatty acids has remained unknown, it is indicated that it is probably related to changes in gene expression of adiponectin in adipose tissue. Adipose tissue can detect energy balance and lipid content as energy storage and accordingly amend adiponectin gene expression [44].

Much of the fatty acids, needed by the working muscles, are provided through 3-4 times increase of lipolysis triglycerides of fat tissue. Physical activity with moderate intensity doubles the amount of blood flow to fat tissue and causes 10-fold or greater increase in blood flow to active muscles of the body that in the opinion of many scholars, reduces body fat and improves body composition because of imbalance between energy intake and consumption. Furthermore, negative caloric balance may result in increased concentrations of adiponectin and reduced plasma leptin levels after prolonged exercise activities [45-46]. Since the concentration of plasma fatty acids in adiponectin secretion is a positive regulatory impact, it is likely that exercise, by increasing lipolysis in adipose tissue, increases plasma levels of fatty acids and glycerol and helps an increase in adiponectin in intercellular space [47]. However, physical training, through increase of glucose uptake by peripheral tissues in response to: acidosis, lactate accumulation, increase in sympathoadrenal input, energy costs, and glycogen depletion and glycolysis inhibition can improve insulin sensitivity and lead to an increase in adiponectin [44].

The results of this study show a significant reduction in homocysteine was observed in sedentary healthy middle aged men. In this research, the reduce in the level of homocysteine after adjusting to the combined activity was similar to the results of a number of previous studies[48]. On the other hand, there are studies that obtained different results from ours [6, 49]. Hejazi et al. [48] investigated the effects of eight weeks of aerobic exercise training (3 sessions per week, 60 minutes per session) with the intensity of 50 to 75 Vo2max on thirty sedentary middle-aged women. They observed significant decreases homocysteine levels, hs-CRP and fibrinogen in the experimental group compared to control. While, Bambaeichi et al. [49] observed exercise cannot reduce plasma homocysteine as risk factors for cardiovascular disease.

The reason for different results can be the difference in exercise protocols, duration, intensity, type of exercise, and of course person’s fitness level [50]. Another difference is that in long and heavy exercises, protein metabolism and blood levels of certain amino acid change and cause the reduction in the concentration of methionine amino acid. Decreased availability of methionine will also increase the production of homocysteine and leads to the accumulation of homocysteine. In these severe and long-term exercises, transmission mechanism of protein increases homocysteine concentration during the exercise [51]. On the other hand, regular exercise increases the need of metabolic reactions to renovate and repair the muscle tissue. By increasing demand for energy production and protein synthesis, concentration of methionine, which is an amino acid, is reduced. Therefore, homocysteine, which is a by-product of methionine metabolism, will be reduced [51]. Moreover, regular exercise can decrease oxygen shortage, followed by reduction of the dependence of body to phosphazene system early in the exercise. Since the production of creatine in the body is done during the methyl transfer reactions, in which methionine by converting to homocysteine leads to the synthesis of creatine, thus, reduction of the need to produce creatine in the body is associated with homocysteine reduction [1]. Therefore, one of the other possible reasons for the reduction of homocysteine levels could be the decline in dependence on phosphazene system during oxygen shortage and reduction of oxidative stress[52]. In general, it can be said that the eight-week combinatorial exercise through increasing aerobic capacity, decline in glucose and cardiovascular risk factors, including IL-6, homocysteine, and increased adiponectin are effective to improve cardiovascular health and reduce the risk of atherosclerotic disease. Therefore, it is suggested that combinatorial exercises must be applied in order to prevent adverse effects caused by
the increased prevalence of atherosclerosis and can be considered a crucial part in the lifestyle of middle-aged people.

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REFERENCES

COMBINATION EXERCISE TRAINING ON CARDIOVASCULAR RISK FACTORS


