The Effect of Six Weeks of High Intensity Interval Training (HIIT) on Plasmatic Levels of Cellular Adhesion Molecules (ICAM-1) and Lipid Profile in Young Overweight Women

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ABSTRACT: The purpose of this study was to determine the effect of six weeks of high intensity interval training (HIIT) on plasmatic levels of cellular adhesion molecules (ICAM-1) and lipid profile in young overweight women. Sixteen young overweight girls voluntarily participated in this study and were randomly divided into two groups: Control group (n=8, age=18.87±.83yr, height =165.25±4.59cm, weight = 70.25±5.38kg) and: Experimental group (n=8, age=18.62±.55yr, height = 162.43±4.85cm, weight = 69.75±5.09kg) groups. Experimental group participated in three HIIT sessions per week for 6 weeks. Each session consisted of four to six repeats of sprint running over a 20 m distance with maximum speed and 20–30 seconds of recovery. Fasting blood samples were collected one day before and after exercise protocol. Data were analyzed using independent t and Pearson correlation coefficient tests. The results showed that six weeks of HIIT leads to significant reduction (P = 0.05) of plasmatic levels of ICAM-1 and also improvement of serum lipid profile in young overweight women in the experimental group. Furthermore, Pearson correlation tests showed that there is a moderate correlation between ICAM-1 and lipid profile. Results show that performing HIIT leads to improvement in the levels of cellular adhesion molecules and lipid profiles. Hence, it appears that in terms of time efficiency HIIT is an effective factor for preventing and improving chronic cardiovascular diseases and also improvement of lipids profile in young overweight women.

Keywords: High intensity interval training, ICAM-1, Lipid profiles

INTRODUCTION

In recent years relationship between inflammation and atherosclerosis has been reported in many studies. According to most reports the spread of cardiovascular diseases has an inflammatory background and overall inflammation has a central role in the spread of atherosclerosis (Bauer & Snow, 2003; Blake & Ridker, 2001). Inflammation is a physiological response to different stimuli such as infection, tissue wounds and body trauma (Vojgany, 2004), which is accompanied by leukocyte aggregation in infection spots, arterial extension and increasing vascular permeability. This process is indicated by changes in plasma proteins (Larty, 2004). There are several risk factors leading to cardiovascular events including inappropriate diet, lack of physical activity, low aerobic readiness, obesity and overweight, high blood pressure, and abnormal lipid profile (Ridker et al, 2002). Studies on mechanisms relating obesity to cardiovascular and metabolic diseases, recommend the increase of fat bulk as the most effective factor (Samaras and Elrick, 2006).

Some of inflammatory markers which can predict cardiovascular diseases are fibrinogen, Haptoglobin, cytokines, Serum Amyloid A, C-Reactive Protein, Inter-Cellular Adhesion Molecule 1, Vascular Cell Adhesion Molecule 1, selectins, and Integrins (Blake et al, 2001; Ali zadeh et al, 2004). On the other hand, increase in cellular adhesion molecules in obese patients plays an important role in the spread of disorder or Atherosclerosis (Bosanska et al, 2010). Lipid profile has been used for a long time to identify individuals who are in danger of cardiovascular events. In a study on 27939 healthy women at the age of 54.7 it was indicated that almost half of all cardiovascular events occurred in those women whose LDL-C levels were lower than 130 mg/dL. This shows that one must seek other indicators to identify endangered individuals. Cellular Adhesion Molecule (sICAM-1) is a new indicator which increases vascular inflammation and may possess higher sensitivity and precision than lipid profile (Blake et al, 2001; Witkowska, 2005).
Adhesion molecules are glycoprotein receptor which appear on different surfaces of cells and not only exist on cell’s external membrane but also penetrate the membrane and enter cytoplasm. Adhesion molecules are important in directing leukocyte and also their departure from blood flow towards ganglionic tissues and etc., especially the sites of infection and inflammation. Some of these molecules are soluble in plasma and their presence indicate the level of blood vessel disorder. The existence of vessel disorder is the beginning of a stage of vascular changes final result of which is atherosclerosis with all unfavorable consequences (Blann, 2003).

On the other hand, physical activity by improving fat/glucose metabolism, insulin resistance and high pressure will stop cardiovascular risk factors. Although, there is a certain optimum length and intensity of physical activity to stop each risk factor, however, the ability of a high-intensity or short-term physical activity to stop or prevent cardiovascular risk factors is not known much. Thus, optimum length and intensity of physical exercise for preventing cardiovascular risk factors, and relationship between effects of physical activity on those factors, need more explanation in future.

Despite many health benefits of endurance exercise, many adults do not participate in such activities due to lack of time as an important obstacle. Therefor, the study of an alternative physical activity with similar metabolic compatibility without significant time commitment is needed. One of physical activity protocols which recently has gained much interest among sport physiology researchers, is high intensity interval training (HIIT). HIIT includes intervals of very high intensity sport activity with low intensity active resting intervals (Trapp et al, 2008).

Previous studies showed that six weeks of HIIT led to metabolic compatibilities in adults similar to those of traditional endurance training (Burgomaster et al, 2008). Also, previous studies showed that performing HIIT will increase the capacity for fat oxidation as well as the activity of mitochondrion anzymes (Talanian et al, 2007). Recently, it was also reported that stable state moderate-intensity physical activities for 30 minutes in most of the days of week lead to no decrease or less decrease in body fat compared to HIIT, which indicates high compatibility of HIIT for increasing fat oxidation and decreasing adipose tissue (Bosanska et al, 2010). On the other hand, high amount of traditional aerobic sport activities decrease the risk of metabolic and cardiovascular diseases, but needs a lot of time (Babraj, 2009). Therefor, considering high capability of this training method in decreasing body fat, its time effectiveness and also the lack of similar studies on the effects of HIIT on changes in adhesion molecules, this study seems to be necessary.

METHODOLOGY

Participants

This is a semi-experimental study. Sixteen obese female students voluntarily participated in this study. First, necessary information about how the study will be carried out and its stages was given to subjects. Then, using questionnaires information about the level of physical activity and health status of subjects was collected and finally they declared their written agreement to participate in the program. Subjects were randomly divided into two groups, experimental group (n = 8) and control group (n=8). None of the subjects have practiced high intensity interval training at least during six month before the program. Preliminary assessments including measurement of height, body weight, body fat and body mass index (BMI) were done two weeks before the start of exercises. BMI was calculated by dividing weight (in Kilograms) by height (in Meters) squared, and to measure body fat percentage three spot methods (triceps, iliac crest and thigh) was deployed using Harpenden caliper (Table 1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n =8)</th>
<th>Post-test</th>
<th>Experimental group (n = 8)</th>
<th>Post-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18.87±0.83</td>
<td>-</td>
<td>18.62±0.74</td>
<td>-</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.25±4.59</td>
<td>-</td>
<td>162.34±4.85</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.25±5.38</td>
<td>70.68±5.74</td>
<td>69.75±5.09</td>
<td>68.81±5.26</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>25.66±0.77</td>
<td>25.84±0.82</td>
<td>26.42±1.23</td>
<td>26.05±1.16</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>34.01±2.25</td>
<td>34.16±2.34</td>
<td>35.08±1.88</td>
<td>33.10±1.93</td>
</tr>
</tbody>
</table>

Training protocol

Experimental group subjects performed training protocol in a 20 m distance marked by three cones three times a week for six weeks as follows (fig. 1). At start, subjects sprinted with maximum speed from start point (cone 1) to cone 2 (path A), then sprinted 20 meters toward cone 3 (path B) and finally ran towards start point (cone 1) with maximum speed (path C) to complete their 40 meters distance. They repeated this course to complete 30 seconds training protocol, then after 30 seconds of resting, they repeated the protocol. Training was extended by increasing the number of repeats of 30 second protocols from four times in first and second week to five times in third and fourth weeks and six times in fifth and sixth weeks. Subjects did warm-up for five
minutes just before each session of training protocol and also of cool-down for five minutes after each session. Training protocol included a 40m - maximal shuttle run test, which is a valid test for assessing anaerobic performance (Glaister, 2009). In six weeks of training protocol, subjects in control group, had no regular sport training.

LABORATORY METHODS AND ANALYTE MEASUREMENTS

24 hours before the first session and 48 hours before the last session, 10 cc fasting (8:30 a.m) blood samples of all subjects were collected from their cephalic (untecubital) veins. Blood samples were immediately poured in tubes containing Ethylenediaminetetraacetic Acid. Then, they were centrifugated at 3000 rpm for 10 minutes at 4º C. Obtained plasma was stored at -80º C for later measurements. Plasma ICAM-1 level evaluation was carried out with Elisa reader method using kits of Boster Co. with sensitivity lower than 10 pg/mL. To evaluate lipid profile levels, lipid profile kits made by Pars Azmoon Co. were used and Elisa reader Hyperion model was applied.

STATISTICAL ANALYSIS METHODS

Collected statistical data was analyzed using SPSS 18. The Kolmogorov-Smirnove test was used for assessing normality of data and since it demonstrated the normality of our data, we used parametric tests. Then, to study inter-group changes, independent samples t-test was applied and Pearson correlation coefficient test was used for determining correlation between variables. All tests were carried out at significance level of α = 0.05. Furthermore, to determine changes in plasma volume, we used Dill and Costill equation (Dill & Costill, 1974).

\%
\[ \% \text{pv} = \left( \frac{HB1}{HB2} \times \frac{100-HCT2}{100-HCT1} - 1 \right) \times 100 \]

Where HB1 is pretest Hemoglobin, HB2 is post-test hemoglobin, HCT1 is pretest hematocrit, HCT2 is post-test hematocrit, and \%pv is change in plasma volume.

Research findings

Research results showed that, after training intervention levels of ACAM-1 had a signicant decrease from 63.12±15.35 to 51.37±14.50 ( P = 0.05), and also six weeks of HIIt led to an improvement in serum volumes of lipid profile in obese young females, although it was not statistically significant. Furthermore, results of Pearson correlation tests demonstrated that there is a correlation between ICAM-1 and total cholesterol after the training, however, there is no significance correlation between other lipid profiles. After training intervention, 19.61 percent increase was observed for average VO2max (from 30.03±4.83 to 35.92±4.10) in experimental group. Independent t- test between experimental and control groups showed that, after six weeks of HIIT, plasma volumes in experimental group have increased significantly compared to control group (P = 0.02) (Tables 2, 3, 4) (Fig 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>avg-standard deviation</th>
<th>t-value</th>
<th>P-value</th>
<th>Degree of freedom</th>
<th>Change percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1 (pic/ml)</td>
<td>Control</td>
<td>69.7±50.63</td>
<td>2.12</td>
<td>0.05*</td>
<td>14</td>
<td>%↓2.83</td>
</tr>
<tr>
<td>VO2max (M/kg.min)</td>
<td>Experimental</td>
<td>5.77±30.77</td>
<td>2.08</td>
<td>0.056</td>
<td>14</td>
<td>%↑1.88</td>
</tr>
</tbody>
</table>

Table 2: Results of independent t – test for studying inter-group difference between values of ICAM-1 and VO2max

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>avg-standard deviation</th>
<th>t-value</th>
<th>P-value</th>
<th>Degree of freedom</th>
<th>Change percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL(mg/dc)</td>
<td>Control</td>
<td>81.25±16.10</td>
<td>-0.79</td>
<td>0.44</td>
<td>14</td>
<td>0.78%↑</td>
</tr>
<tr>
<td>HDL(mg/dc)</td>
<td>Experimental</td>
<td>74.80±16.41</td>
<td>44.37±6.02</td>
<td>0.48</td>
<td>0.63</td>
<td>14</td>
</tr>
<tr>
<td>TC(mg/dc)</td>
<td>Control</td>
<td>133.75±17.4</td>
<td>-0.73</td>
<td>0.47</td>
<td>14</td>
<td>1.43%↓</td>
</tr>
<tr>
<td>TG(mg/dc)</td>
<td>Experimental</td>
<td>78.25±26.42</td>
<td>68.50±24.17</td>
<td>-0.77</td>
<td>0.45</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 3: Results of independent t – test for studying inter-group difference of lipid profile
Adhesion molecules are proteins located on the cell surface involved in binding with other cells or with the extracellular matrix (ECM) in the process called cell adhesion (Carlson et al, 1988). Adhesion molecules soluble in blood flow can only be found in soluble form. A raise in their value is related to dyslipidemia, atherosclerosis (Blann et al, 1999), connective tissue disorders and some cancers (Hackman et al, 1996). Although there have been many studies on the effects of sport activities on CAMs, however more attention have been given to patients and their clinical situation. Nevertheless, considering the role of adhesion molecules in inflammation, a lot of attention has been given to them recently as important markers of inflammation, especially in obese individuals. Some evidence reveal that traditional indicators of cardiovascular diseases (lipid profile), do not seem to be a precise comprehensive indicator to predict those diseases(DabidiRoshan et al, 2005), and it is recommended that new inflammatory markers such as cellular adhesion molecules ICAM-1 possess higher sensitivity in predicting cardiovascular events (Larty, 2004). The results of current study showed that six weeks of HIIT leads to significance decrease in plasma ICAM-1 values in obese young female individuals. Sori et al. reported a significant decrease in values of sICAM-1 in obese individuals, after 16 weeks of aerobic exercise with the intensity between 60 to 65 percent of heart rate reserve three times a week and 45 to 60 minutes per session (Zabet et al, 2005). Jones Lee et.al. (2009) exposed 12 patients with malignant lung lesions to a 4-week exercise program with cycloergometer. In the first week, patients exercised with 60 % Vo2 Peak intensity for 20 minutes. The intensity and duration of the exercise were increased each week so that in the last week it was a 30 minute session with 65 % Vo2-peak intensity. After the program, a significant decrease was observed in the level of blood sICAM-1. Inaddition (Jones Lee et al, 2009), Ito et.al.(2002), presented the hypothesis of endothelial activity adjustment in significance decrease in cellular adhesion molecules (ICAM-1) levels, after studying significant effect of three months of aerobic activity with weight loss diet on the level of adhesion molecules (Ito et al, 2002). Sixt et al. (2009) and Puglisi et al (2008), reported level decrease of serum ICAM-1 in 50-70 year old male and female patients with coronary arteritis, respectively after supervised 4-week sport exercise ( six sets of 15-minute cycling at 80% of maximum heart rate five days a week) and after six weeks of walking (10 minutes a day in the first two weeks, 20 minute a day in the next two weeks, and 30 minutes a day in the last two weeks). These findings are in agreement with our results which recommend positive effect of sport activity on reduction of ICAM-1 value (Sixt et al 2009; Puglisi et al, 2008).

According to results from other studies, regular sport exercises prevent release of inflammatory mediators from adipose tissue, by reducing sympathetic stimulation and increasing anti-inflammatory cytokines, and as a result density of cellular adhesion molecules decreases (Tremblay et al 1994).

Another mechanism in reduction of ICAM-1 inflammatory markers may be anti-oxidation effect of aerobic sport activities, since oxygen free radicals lead to raising of inflammatory mediators and cellular molecules (Mogharnasi et al, 2011). On the other hand, according to studies revealing that anti-oxidation defense is reinforced as a result of aerobic exercise (Mogharnasi&Nasseh, 2011), sport activity may lead to decrease in ICAM-1 value through improving anti-oxidation system.

It is also determined that in an endurance sport activity, endocrine system, via increasing the secretion of epinephrine, norepinephrine, growth hormone (somatotrophin) and cortisol, can increase the fat oxidation (lipolysis) rate, hence fatty acid are used as fuel. Therefore, considering the loss of weight and body fat percentage in the current research, it is possible that HIIT sport activity has led to the loss of fat bulk through increasing lipolysis. Thus, according to studies that have shown that adipose tissue is an important place for secretion of inflammatory markers and cytokines (Zoppi et al, 2006), previous studies have shown that HIIT programs increase lipolysis capacity and mitochondrial enzyme activity (Babraj et al, 2009). Tremblay et al (1994) compared the effects of performing HIIT with those of moderate-intensity regular sport activities for 30 minutes and 5 times a week, on fat loss in young males and females. Their results showed that, the HIIT group experienced significantly more fat loss in comparison to moderate-intensity group (Tremblay et al, 1994). Yannakoulia et al. (2005) also reported that 12 weeks of aerobic activity had no effect on ICAM-1 level in obese girls. These studies observed ineffectiveness of sport activity on ICAM-1 inflammatory marker (Nassis et al, 2005), which is not in accordance with results of current study. Difference in intensity, duration, type of sport

<table>
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<th>ICAM-1 Post-test</th>
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<tr>
<td>LDL(mg/dc)</td>
<td>0.68</td>
<td>0.059</td>
</tr>
<tr>
<td>HDL(mg/dc)</td>
<td>-0.01</td>
<td>0.96</td>
</tr>
<tr>
<td>TC(mg/dc)</td>
<td>0.71</td>
<td>0.04*</td>
</tr>
<tr>
<td>TG(mg/dc)</td>
<td>0.13</td>
<td>0.74</td>
</tr>
<tr>
<td>Wt/kg.min ( VO2max)</td>
<td>-0.59</td>
<td>0.11</td>
</tr>
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**DISCUSSION**

Table 4. results of Pearson correlation coefficient between ICAM-1 and lipid profile after training intervention

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activity, presence or absence of diabetes and cardiovascular diseases, weight, age and sex of the subjects may be the possible reasons for this disagreement.

In this study, a meaningful increase of 12.05 percent in plasma volume in experimental group after six weeks of HIIT was observed. Regular endurance activity probably can have protective effects against cardiovascular diseases through several mechanisms. Sport activity directly effects cardiovascular system by increasing the volume of blood and plasma, decreasing blood viscosity, increasing stroke volume and VO2max. Plasma volume increase is one of the most changes that occur as a result of endurance exercises. If plasma volume increases, blood volume will increase as well, and thus more blood will enter the heart and stroke and cardiac output volumes increase, which leads to a raising in maximal oxygen consumption. Endurance exercise cause plasma volume to increase through two processes: 1) by augmenting secretion of anti-diuretic and aldosterone hormones it leads to retention of water in kidneys and thus increases plasma volume; 2) by increasing plasma proteins especial albumin, it raises blood osmotic pressure and thus more liquid remains in blood. As a results, by cooperation of these two processes, blood liquid i.e plasma is increased and blood viscosity is reduced (Dabidi-Roshan et al, 2005). Six-week HIIT program in this study may also lead to reduction of ICAM-1 via increasing consumed oxygen and plasma volume.

Statistical results from independent t-test in this study showed that performing HIIT for six weeks has changed lipid profile, but none of these changes were statistically significant. Results of this study can be compared with the work of Ferrier et al. (2001) where they reported no significant effect on LDL-C, HDL-C and TG in ISH patients (Ferrier et al, 2001). In addition, Zoppini et al. (2006) after a six month program of moderate-intensity endurance activity twice a week in obese subjects with diabetes reported a meaningful reduction of plasmatic density of ICAM-1 and P-Selectin and no change in lipidic levels (Zoppini et al, 2006). Kraus et al. (2002) found that beneficial effects of exercise on several types of lipoproteins are highly correlated to high intensity of the exercise. Higher amount and intensity of exercise led to better improvement compared with lower amount and intensity of exercise and control group. Furthermore (Kraus et al, 2002), in their review, Kessler et al (2012) reported that at least 8 weeks of aerobic or anaerobic interval training is necessary to improve HDL-C, and in those studies that uses less than eight weeks of training, no changes in lipid profile were reported (Kessler et al, 2012). Okura et al (2003) observed that reduction of LDL-C and maximum consumed oxygen is more observable in high-intensity sport activity than low-intensity activity (Okura et al, 2003). According to the previous studies it can be concluded that to reduce lipid profile significantly, in addition to exercise intensity its duration must be increased as well. In this study, six weeks of HIIT led to improvement in lipid profile, but that improvement was not statistically significant. Whyte et al (2010) studied the effects of two weeks of sprint interval training on lipid profiles and health related factors in sedentary obese men. Their results showed that although SIT led to improvement in some of variables related to cardiovascular diseases, however those changes were not significant. These authors recommended short duration of trainings as the reason of no changes in lipid profile. Nowadays, positive relation between plasmatic density of vascular inflammatory factor and obesity, especially central obesity and blood triglyceride, and its negative relation with density of plasma HDL-C is determined. Regarding to the relationship of SICAM-1 with fat distribution and lipid profile (Whyte et al, 2010), Ito et al. (2002) reported a significant correlation between plasma SICAM-1 and obesity, especially central obesity, in overweight women (Ito et al, 2002). In another study, lack of positive relation of triglyceride and also negative relation of HDL-C with SICAM-1 was reported (Marsh &Coombes, 2005). While in another study the absence of a meaningful relationship between adhesion molecules and lipidic levels of serum in myocardial Anemia was emphesised (Morisaki et al, 1997). Effects of regular sport activity on endothelial function may occur following the increase of plasma HDL-C level. HDL-C leads to release of prostacyclin (PGI-2) from the wall of arteries or smooth muscular cells which in turn inhibits platelet formation and leads to reduction of adhesion molecule in both body and lab environment (Lerch et al, 1998). Nevertheless, due to lack of a significant relation between plasma ICAM-1 and HDL-C, this mechanism probably has a secondary role in significant reduction of plasma ICAM-1 level and subsequent mechanisms have an effect as well. Indeed, two reasons for the absence of a meaningful correlation of lipid profile, especially HDL-C are reported which are as follows. First, in healthy individuals HDL-C in not high enough (53mg/dL) to play its role in reduction of plasma ICAM-1 and second, relation between plasma ICAM-1 and ICAM-1 attached to membrane, is not high enough to lead to a correlation between HDL-C and ICAM-1 (Morisaki et al, 1997).

CONCLUSION

According to the results of this study and comparing them to existing information, it seems that in terms of time effectiveness performing HIIT is an effective factor in preventing and treating risk factors of chronical diseases including obesity and cardiovascular diseases and also improving lipid profile of obese young women.
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