

HOMOCYSTEINE AND LIPID PROFILE RESPONSE IN SEDENTARY YOUNG MEN TO SIX-WEEK HIGH-INTENSITY INTERVAL TRAINING

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Abstract

Objective: The purpose of this study, determine effect of six weeks high intensity interval training on homocysteine and Lipid Profile levels in sedentary young men. **Methods:** Eighteen inactive young men voluntarily participated in this study and randomly divided into two groups: Experimental (n: 9, age: 24.33±1.41, height: 176.22±4.91, weight: 72.27±6.59) and Control (n: 9, age: 23.27±2.01, height: 180.22±6.88, weight: 76.27±7.33) groups. Experimental group performed three high intensity interval training sessions per week for 6 weeks. Each session consisted of either four to six repeats of maximal sprint running within a 20 m area with 20–30 s recovery. Fasting blood samples were collected 24 hours before and 48 hours after exercise protocol. Data were analyzed by dependent t test. **Results:** Results showed that, although homocysteine levels in the experimental group reduced 18% compared with control group, but this change was not statistically significant ($P>0.05$). Although serum concentrations of triglycerides, low-density lipoprotein, total cholesterol and the ratio of total cholesterol to high-density lipoprotein decreased and high-density lipoprotein levels increased, but these changes were not statistically significant ($P>0.05$). **Conclusions:** According to the results of this study, high intensity interval training seems to be a time-dependent strategy with a relative improvement in homocysteine and Lipid Profile levels, could lead to the prevention of cardiovascular disease in sedentary individuals.

Key Words: homocysteine, Lipid Profile, High Intensity Interval Training, sedentary young men

Introduction

Over recent decades pattern of morbidity and mortality in the world has shifted from infectious diseases such as tuberculosis to non-communicable diseases such as cardiovascular diseases (CVD), cancer, chronic respiratory diseases and diabetes (Nordlieb, Wolda & Kloner, 2005). Several risk factors are associated with the development of cardiovascular disease, including poor diet, lack of sufficient physical activity, low aerobic fitness, obesity, high blood pressure and abnormal lipid profile (Gerber, Börjesson, Ljung, Lindwall & Jonsdottir, 2016). For many years' lipid profile has been regarded as the main indicator of cardiovascular diseases. Although the ration of HDL-C / LDL-C is the main indicator and risk factor for cardiovascular disease, studies have shown that some people with normal HDL-C and LDL-C are also suffering from cardiovascular disease (Ruiz, Castro-Pinero, Artero, Ortega, Sjostrom, Suni, Castillo & 2009). Thus, researchers have been looking for indicators to predict the risk of cardiovascular disease with more accuracy and sensitivity. In this regard, the America Heart Association (AHA) according to the extensive research has stated that cardiovascular diseases have inflammatory basis and general inflammation plays a pivotal role in the development of atherosclerosis (Ridker & Rifai, 2002). Some of the inflammatory indices that are considered as the predictors of cardiovascular diseases include: fibrinogen, haptoglobin, cytokines, serum amyloid A (SAA), high sensitivity C-reactive protein (hs-CRP), intracellular adhesion molecules (ICAM) and

vascular cell adhesion molecules (VCAM), and homocysteine HCY (Ruiz, Castro-Pinero, Artero, Ortega, Sjostrom, Suni & Castillo, 2009; Ridker & Rifai, 2002). Homocysteine is a non-protein amino acid, which is a homologue of the amino acid cysteine, and for having an extra methylene groups differs from cysteine. Homocysteine is synthesized from methionine (in the diet) by removal of C-terminal methyl group and can be recycled back into methionine or converted into the cysteine by help of vitamins B12 and B6 (Martí-Carvajal, Solà, Lathyris & Salanti, 2009; Martí-Carvajal, Solà & Lathyris, 2015). The elevated blood homocysteine to more than 16 mmol per liter is an unusual medical condition that is called hyperhomocysteinemia. Studies have demonstrated that the elevated level of homocysteine has a negative effect on cardiovascular system such as the oxidation of LDL, proliferation of smooth muscle cells, increased platelet adhesion and endothelial cell cytotoxicity, psychosis, Alzheimer's disease and fractures caused by osteoporosis (Blake & Ridker, 2001; Witkowska, 2005; Seshadri, Beiser & Selhub, 2002). It has been reported that 5 mmol per liter elevation in HCY can increase the risk of cardiovascular disease by 20 percent (McLean, Jacques & Selhub, 2004). Also, 25% drop in HCY (3 mmol per liter) is associated with 11% decrease in ischemia risk (Ueland, Refsum, Beresford & Vollset, 2000). Besides, per 1 micromol increase in HCY, the probability of disease increases by 6 to 7 percent (Clarke & et al., 2002). Several studies have shown that association between total

homocysteine level and atherosclerosis is even stronger than its association with cholesterol (Bots, Launer, Lindemans, Hoes, Hofman & Witteman, 1999). Atherosclerosis is known as a chronic inflammatory process. Several in-vitro studies have shown that homocysteine increases the production of several inflammatory cytokines (Sütken, Akalin, Ozdemir & Colak, 2014). However, the mechanism of homocysteine effect on blood circulation has not been fully understood. The self-oxidative feature of homocysteine in plasma enhances the production of reactive oxygen species that results in increased levels of LDL oxidation and damage to endothelial cells and also oxidative stress (Di Banfi, Stel & Cauci, 2009). There are numerous determinant factors for plasma levels of homocysteine that sport activity is one of these factors (Maroto-Sánchez, Lopez-Torres, Palacios & González-Gross, 2016). Some studies have shown an inverse correlation between blood levels of homocysteine and physical activity. Regular exercise reduces blood levels of homocysteine, therefore is beneficial for the prevention and treatment of cardiovascular disease (Hankey, Eikelboom, Ho & Bockxmeer, 2004). Furthermore, it has been reported that sport activities in sedentary people can improve traditional (lipid profile) and non-traditional (e.g., homocysteine) risk factors of cardiovascular diseases (Gaume, Mougin, Figard, Simon-Rigaud, N'Guyen & Callier, 2005). In another study the relationship between sport activity and blood concentrations of homocysteine has not been supported, although the type and intensity of exercise have been effective on homocysteine levels. According to the findings of these researches, endurance training as well as highly intensive training in some cases can increase and in other cases has no effect on homocysteine concentration (Gelecek, Teoman, Ozdirenc, Pinar, Akan & Bediz, 2007). One of the protocols that have recently attracted some attentions is high intense interval training (HIIT). This protocol includes interval training with high intensity and active rest intervals with very low intensity (Trapp, Chisholm, Freund & Boutcher, 2008). Although traditional high volume aerobic exercise training reduces the risk of cardiovascular and metabolic diseases, considerable amount of time should be allocated to such training (Trapp, Chisholm, Freund & Boutcher, 2008).

Therefore, HIIT is an efficient protocol in terms of time. Since there is scant evidence regarding the effect of HIIT on plasma levels of homocysteine and due to the controversial results about the effect of strength, aerobic and intensive training on plasma levels of homocysteine this study sought to answer the question whether the implementation of six-week HIIT has an impact on plasma homocysteine levels and lipid profile in sedentary young men?

Materials and methods

In this semi-experimental study, the study population consisted of sedentary male students in Tehran University Dormitory.

A total of 18 volunteers aged from 21 to 26 years were enrolled in the study. Initially, the objective and procedure of the study was explained to the subjects. Then information about physical activity and health status of the subjects was obtained using a questionnaire. When the written consents were obtained from the subjects they were randomly assigned into two control ($n = 9$) and experimental ($n = 9$) groups. All subjects' diet was based on the dining plan offered by Tehran University dormitory. Moreover, the subjects were requested to avoid tobacco, alcohol, caffeine supplements, dietary and medication supplements intake a week before the beginning of the six-week training program until the end of the study. None of the subjects had regular exercise such as high intensity interval training within the last six months. Two weeks before the start of training, initial assessment such as the determination of height, body weight, body fat and body mass index (BMI) was performed. The subjects' weight was measured using standard medical scales (SECA, Germany). To measure height, the subjects stood straight up with their back directly against the wall without shoes such that the heels, buttocks and shoulders touched the wall and they were asked to look straight-forward. Their height was measured using the stadiometer and recorded in centimeters. BMI was calculated by dividing weight (in kilograms) by height in meters square. To measure body fat percentage, Harpenden calipers and three-point method (chest, abdomen and thigh) were used. The subjects in the experimental group performed the training protocol over a distance of 20 meters marked by three cones, 3 sessions per week for 6 weeks as follows (Figure 1).

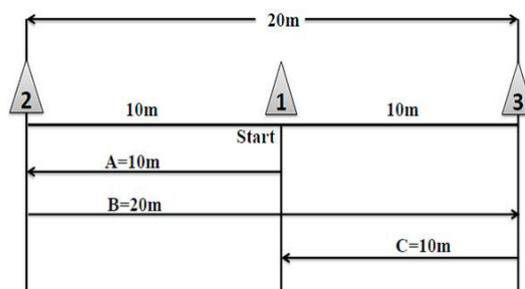


Figure 1. The schematic of HIIT protocol

With the start of the training protocol, the subjects with their maximum speed from the starting point (Cone number one) ran toward the second cone (route A), then returned and in the opposite direction (20 meters) ran toward the third cone with maximum speed (route B). Finally, they returned and ran toward the starting point (cone 1) with maximum speed (route C) to complete the distance of 40 meters. The subjects continued this trend with a maximum speed to complete 30 seconds time period of training protocol, and after 30 seconds of rest, they repeated this protocols. The training progressed by increasing the number of 30 seconds repetitions from 4 times per session in the first and second weeks to 5 times per session in the third and fourth weeks to 6 times per session in the fifth and sixth weeks.

Before the start of training protocol and at the end of each session proper warm-up and cool down movements was performed for five minutes (e.g., jogging, stretching and brief period of low-intensity exercise). Training protocol derived from 40 meters' sprint (sweep) testing, which is a valid test for assessing non-aerobic performance (Glaister, Hauck, Abraham, Merry, Beaver & Woods, 2009). During the six-week training protocol the subjects in the control group had no regular exercise. To determine the intensity of the training, the maximum heart rate (age - 220 = HRmax) were used and in all stages of HIIT the training intensity was over 90 percent of HRmax, which was calculated for each subject separately (heart rate monitor from Beurer (Germany) was attached to all subjects during 30 second maximum speed running to control the intensity of training according to their heart rate). In order to check the effect of training on plasma homocysteine and lipid profile, 24 hours before the first training session and 48 hours after the last session 5 cc of fasting blood was obtained from antecubital of all subjects in both groups (at 8:30 am). Blood samples were transferred immediately into tubes containing EDTA anticoagulant. All blood samples were centrifuged at 3000 rpm for 10 minutes at a temperature of 4 °C and the obtained plasma were kept at -80°C for further assessments. Serum homocysteine levels were measured by ELISA kit (axis-shield, UK) with sensitivity of 1 micromole per liter. Also, the values of lipid profile were measured using Pars test kit (Iran) by Noor Pathobiology laboratory in Tehran. The data collected was analyzed using SPSS 18 statistical software. The normal distribution of data was confirmed using the Kolmogorov-Smirnov test. To investigate the inter-group changes of data, independent t-test was used. P value less than 0.05 was considered statistically significant.

Results

Table 1. The average and standard deviation of anthropometric variables and VO2max subjects before and after intervention

Variables	Control Group		Experimental Group	
	Pre-test	Post-test	Pre-test	Post-test
Age (Year)	23.27 ± 2.01	-	24.33 ± 1.41	-
Stature (Cm)	180.22 ± 6.88	-	176.22 ± 4.91	-
Weight (Kg)	76.27 ± 7.23	77.30 ± 8.00	72.27 ± 6.59	72.11 ± 6.88
BMI (Kg/m ²)	23.54 ± 2.60	23.85 ± 3.24	23.32 ± 2.29	23.18 ± 2.44
WHR	0.84 ± 0.00	0.85 ± 0.02	0.84 ± 0.04	0.84 ± 0.05
Body Fat (%)	12.34 ± 3.19	13.02 ± 2.11	10.52 ± 2.12	*8.87 ± 2.22
VO ₂ max (ml.kg.min)	41.81 ± 2.51	41.2 ± 3.03	42.2 ± 2.85	*46.3 ± 2.01

*P<0.05

The subjects' general characteristics are presented in table 1. According to the results, there was not a significant difference in weight, BMI and ratio of waist to hips between the two groups after the training intervention.

However, the body fat percentage in the experimental group was significantly reduced and a significant increase was observed in VO2max in the experimental group (Table 1). As presented in Table 2, the results of independent t-test show that the implementation of six-week HIIT, insignificantly changed the amounts of homocysteine in the experimental group compared to the control group (P= 0.22, 18% decrease). Nevertheless, performing HIIT for six weeks had no significant effect on the values of total cholesterol (P=0.12, 3.64% decrease), triglycerides (P=0.4, 8.14 % decrease), HDL-C (P =0.55, 1.41% increase), LDL-C (P=0.12, 3.62% decrease), VLDL (P =0.41, 8.12 % decrease) and the ratio of TC to HDL-C (P =0.14, 4.76% decrease).

Table 2. Changes homocysteine and lipid profile parameters before and after the intervention in the control and experimental groups

Variables	Group	Pre-test	Post-test	T	P-value
Homocysteine (µmol/l)	C	29.98 ± 14.17	32.5 ± 11.62	2.43	0.22
	E	31.65 ± 24.9	25.73 ± 11.32		
Total cholesterol (mg/dl)	C	137.33 ± 16.80	138.55 ± 17.08	- 1.68	0.12
	E	140.11 ± 18.63	135.00 ± 12.66		
Triglyceride (mg/dl)	C	79.77 ± 39.22	81.22 ± 35.74	0.81	0.40
	E	76.33 ± 21.22	70.11 ± 28.19		
HDL-C (mg/dl)	C	39.00 ± 3.77	38.77 ± 2.72	0.60	0.55
	E	39.44 ± 4.53	40.00 ± 4.47		
LDL-C (mg/dl)	C	84.42 ± 13.06	85.22 ± 12.46	- 1.69	0.12
	E	85.97 ± 13.67	82.85 ± 11.13		
VLDL (mg/dl)	C	16.80 ± 7.38	17.01 ± 6.04	0.84	0.41
	E	15.26 ± 4.24	14.02 ± 5.63		
TC/HDL-C	C	3.57 ± 0.77	3.60 ± 0.63	- 1.54	0.14
	E	3.57 ± 0.53	3.40 ± 0.47		

C = control group, E = experimental group

Discussion and conclusion

The results of this study showed that the implementation of six-week HIIT is associated with partial improvement in lipid profile and plasma levels of homocysteine (18% decrease). The change in the values of these factors is not statistically significant. It has been demonstrated that weight loss in obese people decreases all risk factors of coronary artery disease associated with type 2 diabetes mellitus and improves hyperglycemia (Hu, Tuomilehto, Silventoinen, Barengo & Jousilahti, 2004). The available data suggest that reducing weight accompanied by diet therapy or sport activity improves lipid profile and can lead to increased HDL-C and decreased insulin resistance (Meires & Christie, 2011). However, in patients with varying degrees of physical activity, lipoprotein values based on the type and mode of exercise is different. Previous studies have shown that the distance of running (Mileage) per week is

the best predictor of HDL-C levels among runners (Stefanick, Mackey & Sheehan, 1998). In a study by Rotkis et al. (1982), 90 runners with the average running distance of 13, 27, and 58 miles per week were compared with 19 non-runners. The average HDL-C in runners was as follows: in 28 runners 47 mg/dl, 30 runners 53 mg/dl and in 32 runners was 60 mg/dl. The average HDL-C in 19 non-runners was 34 mg/dl (Rotkis, Cote, Coly & Wilmore, 1982). Anaerobic trained athletes have lower levels of HDL-C and lower ratio of HDL-C to total cholesterol compared with endurance athletes such as runners (Petridou, Lazaridou & Mougios, 2005). A study by Nikkila showed that serum lipid and lipoprotein in anaerobic sprinters whose training programs mostly included short-term efforts, did not increase. Besides, there was not any significant difference in total cholesterol or triglycerides among skaters (speed skating), weightlifters, and sedentary men (Nikkilä, Taskinen, Rehunen & Harkonen, 1978). By comparing the results of studies by Rotkis and Nikkila with the results of present study it can be concluded that because HIIT was the training protocol in this study and as the mileage compared to endurance training was very short, therefore the values of lipid profile in sedentary young men did not change significantly. Nevertheless, the minor changes that observed were positive and from clinical perspective it seems that spending even a little time on HIIT can relatively improve the lipid profile in sedentary men. Although in people with high aerobic fitness compared to people with low aerobic fitness, total cholesterol levels is lower, it has not been conclusively proven that the practice of sport activity can reduce total cholesterol level. Shoup and Durstine have shown that strength training does not increase the conversion of HDL-C to HDL-2 and HDL-3 subunits (Shoup & Durstine, 1991). This also applies for LDL-C. Although it has been shown that strength training reduces LDL-C level, there is still insufficient information about the biochemical mechanism of this change. However, the initial VO₂max values are associated with significant changes in serum lipids and lipoproteins. The subjects with higher VO₂max values show fewer changes in lipids and lipoproteins after training compared to those with lower initial VO₂max. Therefore, it is possible that the change in blood lipid and lipoprotein to be higher in people who do regular intense aerobic exercise resulting in reduced risk of cardiovascular disease (Durstine, Grandjean, Davis, Ferguson, Alderson & DuBose, 2001). given that in this study VO₂max values increased by 8 %, it can be said that one of the possible reasons for relative improvement in lipid profile is due to an increased aerobic fitness following the six weeks' implementation of HIIT. Since the subjects in this study consisted of sedentary and healthy young men and as the values of the lipid profile of the people in pre-test step was in the normal range, therefore such small changes resulting from the implementation of HIIT is clinically important. The findings of this study showed that the implementation of six-week HIIT has no significant effect on plasma levels of

homocysteine in sedentary men. Since there are several controversial reports about the effects of sport and physical activity on plasma levels of homocysteine, in the following some of these researches that are mostly related to aerobic and few numbers are related to strengthen (endurance) training will be discussed. The studies by Mora et al. (2006), Nygard et al. (1995), and Danker et al. (2002) showed the significant effect of physical activity on homocysteine concentration (Mora, Lee, Buring & Ridker, 2006; Danker, Cheitrit & Sela, 2002; Nygard, Vollset & Refsum, 1995). The study by De Berr et al. (2001), Rousseau et al. (2005) and Chrysohoou et al. (2004), reported insignificant relationship between physical activity with the concentration of total homocysteine, which is consistent with the results of the present study (Chrysohoou, Panagiotakos, Pitsavos, Zeimbekis, Zampelas & Papademetrio, 2004; De Bree, Verschuren, Henk & Kromhout, 2001; Rousseau, Robin, Roussel, Ducros & Margaritis, 2005). The discrepancy between the results of these studies is probably due to the difference in training protocol, intensity of training, gender, age, level of readiness (fitness), health status of the subjects as well as the difference in laboratory test analyses and the time of blood collection. Also, several studies have reported that intensive sports activities compared with prolonged exercise, which was mostly ineffective, can slightly increase the plasma homocysteine levels. Moreover, reduced homocysteine levels are mostly observed in people with high homocysteine levels or lack of physical activity (Boreham, Kennedy, Murphy, Tully, Wallace & Young, 2005). In this regard, Acura et al. (2006), conducted an aerobic training on 30 people for 20 weeks and came to the conclusion that the value of homocysteine in people with homocysteine concentrations greater than 15 micromoles per liter can decrease with regular aerobic exercise (Okura, Rankinen, Gagnon, Lussier-Cacan, Davignon & Leon, 2006). In contrast, Wright et al. (1998), in a study on 20 healthy and active men aged 24 to 39 years showed that running on a treadmill with 70 percent of maximum heart rate for 30 minutes has no effect on the homocysteine concentrations in healthy men (Wright, Francis & Cornwell, 1998). The results of this study are in line with the results of the present study and it seems that plasma homocysteine levels are not affected by the intensity of training. Although the results of this study showed no statistically significant changes in plasma levels of homocysteine, the small changes indicate 18 % decrease in homocysteine levels. Also, it has been reported that 25% decrease in HCY (3 mmol per liter) is associated with 11 % decrease in cardiovascular disease (Ueland, Refsum, Beresford & Vollset, 2000). Therefore, these results demonstrate that HIIT can reduce the amount of in homocysteine by 18 % in healthy sedentary individuals, thereby can prevent the incidence of cardiovascular disease. In addition, endurance exercises may lead to significant increases in homocysteine levels and the most important determining factor is probably the duration of exercise (Herrmann, Schorr, Obeid,

Scharhag, Urhausen & Kindermann, 2003). In this regard, Herrmann et al. (2003) showed that endurance exercises cause a significant increase in plasma homocysteine values. The discrepancy between reported results stems from the difference in the intensity and duration of physical activity (Herrmann, Schorr, Obeid, Scharhag, Urhausen & Kindermann, 2003). In addition, Gelecek et al. (2007) showed that physical activity based on the intensity, volume, length and type of training can have an additive effect on homocysteine concentrations (Gelecek, Teoman, Ozdirenc, Pinar, Akan & Bediz, 2007). Saboorisarein et al. (2012), performed Bruce test until exhaustion step and stated that lack of sufficient time is an effective factor in significant increase of homocysteine levels

(Saboorisarein, Yazdanpoor & Jahromi, 2012). Considering the results of aforementioned studies and with regard to the results of the present study, which showed no significant change in plasma homocysteine levels after 6-week HIIT, it seems that the duration of exercise or training is the most effective factor on plasma homocysteine levels.

Since HIIT training model was used in this study, probably due to the limited training time in this method, significant change in plasma homocysteine levels was not achievable. Taken together, it seems implementation of HIIT for six weeks as a time-dependent strategy by relative improvement of homocysteine and lipid profile can prevent the onset of cardiovascular disease in sedentary men.

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HOMOCYSTEINE I LIPID PROFIL ODGOVORA U SEDENTARNIH MLADIH NA ŠESTO-TJEDNI INTERVALNI TRENING VISOKOG INTENZITETA

Sažetak

Cilj: Svrha ove studije je utvrditi učinak šest tjedana intenzivnog treninga na razinu homocisteina i razine lipidnih profila kod mladih sjedećih osoba. Metode: Osamnaest neaktivnih mladih ljudi dragovoljno je sudjelovalo u ovoj studiji i slučajno su podijeljeni u dvije skupine: Eksperimentalnu (n: 9, uzrast: 24,33 ± 1,41, visina: 176,22 ± 4,91, težina: 72,27 ± 6,59) i Kontrolnu (n: 9, uzrast: 23,27 ± 2,01, visina: 180,22 ± 6,88, težina: 76,27 ± 7,33) skupinu. Eksperimentalna skupina provodila je tri treninga s intervalima visokog intenziteta tjedno tijekom 6 tjedana. Svaka sesija sastojala se od četiri do šest ponavljanja maksimalnog sprinta koji se izvodio unutar područja od 20 m s oporavkom od 20-30 sek. Uzorci krvi prikupljeni su 24 sata prije i 48 sati nakon proteklog vježbanja. Podaci su analizirani ovisnim t testom. Rezultati: Rezultati su pokazali da iako je razina homocisteina u eksperimentalnoj skupini manja za 18% u usporedbi s kontrolnom skupinom, ova promjena nije statistički značajna (P > 0,05). Iako su povišene koncentracije triglicerida u serumu, lipoproteina male gustoće, ukupnog kolesterola i omjera ukupnog kolesterola do lipoproteina visoke gustoće, povećane su razine lipoproteina visoke gustoće, no te promjene nisu bile statistički značajne (P > 0,05). Zaključci: Prema rezultatima ove studije, intervalni trening visokog intenziteta čini se da je strategija koja ovisi o vremenu, a moguće i da relativno poboljšava razinu homocisteina i lipida, što može dovesti do prevencije kardiovaskularnih bolesti kod osoba sjedećih.

Ključne riječi: homocistein, lipidni profil, intenzivni intervalni trening, sjedeći mladi ljudi

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