

## Chronic Effects of Aerobic Exercise Training on Serum Concentrations of Fibrinogen and Homocysteine in Males

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### Abstract

Coronary Artery Disease (CAD) is the number one killer of adults in the Iran. Fibrinogen is CAD risk factor that participation in both the atherogenic and thrombogenic processes. Also playing a role in thrombosis and atherogenesis is Homocysteine, as a risk factor for CAD.

Long-term exercise training and physical activity favorably modified several of the conventional CAD risk factors. No such association has been consistently shown between regular physical activity and exercise training with fibrinogen and homocysteine concentrations and the effects of physical activity and exercises training on fibrinogen and homocysteine were not clear. This study aimed to clarify whether long-term aerobic exercise training and physical activity reduced fibrinogen and homocysteine levels in men. This cross-sectional study involved 45 voluntary participants that divided into three groups of 15 each, as follows: active, sedentary, and CAD group. Fasting whole blood samples were collected from the left antecubital vein after 9–12 hours of fasting. The serum concentrations of fibrinogen were measured using the chrometric method. Enzyme-linked immune sorbent assay (ELISA) was used to measure the serum concentrations of homocysteine by Biomerio

fully automated analyzer. Between-group comparisons of estimated VO<sub>2</sub>max, homocysteine, and fibrinogen were performed using one-way analysis of variance (ANOVA) and the LSD Post Hoc test. Significant levels for all tests were set at  $p \leq 0.05$ . No significant between-group differences were found in the serum concentrations of homocysteine [F (2, 42) = 0.107,  $p = 0.898$ ] and the serum concentrations of fibrinogen [F (2, 42) = 0.468,  $p = 0.630$ ]. Significant differences of estimated VO<sub>2</sub>max were found between active and sedentary groups ( $p \leq 0.001$ ), and active and CAD groups ( $p \leq 0.001$ ). The results indicated that exercise training and physical activity does not have any desirable effects on the serum levels of homocysteine and fibrinogen. Hence, more studies are needed to clarify the effects of physical activity and exercise training on homocysteine and fibrinogen levels. More studies are required to clarify the optimal intensity, duration, and type of exercise to favorably modify these risk factors of CAD.

**Key Word:** Fibrinogen, Homocysteine, VO<sub>2</sub>max, CAD, Exercise.

### Introduction

Coronary Artery Disease (CAD) is the number one killer of adults in the Iran (Iranian Heart Association, 2002). Associated modifiable risk factors include

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hypertension, hypercholesterolemia, obesity, hyperglycemia, physical inactivity and smoking (American Association of Cardiovascular and Pulmonary Rehabilitation, 2004; Brubaker et al, 2002). Fibrinogen (Fib) has emerged as an independent risk factor of importance equal to or greater than of other previously described CAD risk factors (Brubaker et al, 2002). Fibrinogen is a large plasma glycoprotein, involved in blood clotting, and in the rheological characteristic of blood flow. It is CAD risk factor that manifests in coronary occlusive events through its participation in both the atherogenic and thrombogenic processes (Brubaker et al, 2002; Le Mura et al, 2004). Fibrinogen concentration is significantly and directly related to the incidence of coronary events (Ernst et al, 2003). Despite these recent advances in the understanding of Fib and its relationship to CAD, information is required on whether reduction of plasma Fib improves patient outcome and should therefore be incorporated into clinical practice (Le Mura et al, 2004). Also playing a role in thrombosis and atherogenesis is Homocysteine (Hcy), a sulfhydryl-containing amino acid formed by demethylation of methionine (Brubaker et al, 2002). Multiple studies have shown elevated Hcy concentrations in patients with CAD. Previous studies established the strength and independence of Hcy as a risk factor for CAD (Dimitrios et al, 2003; Franke et al, 1997; Sloma et al, 2003; Walus et al, 2003). A linear relationship between Hcy concentrations and CAD risk was reported by previous studies (Monica, 1997; Nygard et al, 1995). Few studies have been reported that analyze the relation between Hcy and Fib (Kassam et al, 2001; Monica, 1997; Nissen et al, 2002). It would appear to be logical to investigate the association between these two metabolic factors as Hcy has been shown to have a deleterious effect on the normal prothrombotic and anticoagulant activities of endothelial cells and Fib plays

a key role in coagulation, platelet aggregation, and fibrinolysis, all which have a role in thrombosis and hypercoagulation (Monica, 1997). Usually, lifestyle changes are preferable to medication in primary or secondary prevention of chronic diseases such as CAD. Physical activity and exercise training are commonly recommended lifestyle intervention for individuals at risk for, or diagnosed with CAD. While it is generally accepted that regular physical activity and exercise training reduces the risk of CAD, the physiologic effects are only partially understood (Dishman et al, 2004; Le Mura et al, 2004; Mora et al, 2006; Nieman, 2003). Long-term exercise training and physical activity favorably modified several of the conventional CAD risk factors including blood lipids, obesity, blood pressure, and glucose intolerance, however, the magnitude of change in each of these factors by themselves is moderate (American Association of Cardiovascular and Pulmonary Rehabilitation, 2004; Dishman et al, 2004; Le Mura et al, 2004; Nieman, 2003). The inverse association between physical activity and CAD remains after controlling for the previously listed variables (Blair et al., 1996). Regular physical activity and exercise training have been shown to reduce plasma Fib in healthy subjects, as well as those with CAD (Jae et al, 2008; Le Mura et al, 2004). No such association has been consistently shown between regular physical activity and exercise training and Hcy concentrations (Monica, 1997; Nygard et al, 1995). No statistically significant correlation were found between Hcy concentrations and physical activity levels and the effects of physical activity and exercises training on Hcy were not clear (Franke et al, 1997; Monica, 1997; Nygard et al, 1995; Nissen et al, 2002; Mc Kenzie, 2003; Sloma et al, 2003). Others studies found an inverse correlation between mean Hcy concentrations and the amount of exercises and physical activity rating (Kassam et al, 2001; Foody et al,

2002; Ernest et al, 2003; Walus et al, 2003; Dimitrios et al, 2003; Antonopoulos et al, 2003; Tamvakos et al, 2003; Hayden et al, 2004; Tello-Montoliu et al, 2006; Jae et al, 2008). The most pronounced differences were found between the physically inactive and active subjects ( $12.8 \mu\text{mol.l}^{-1}$  vs.  $11.2 \mu\text{mol.l}^{-1}$ , respectively; or  $9.7 \pm 0.43$  vs.  $8.3 \pm 0.66 \mu\text{mol.l}^{-1}$ , respectively). Moderate physical activity and active exercises were associated with almost identical mean Hcy concentrations. Heavy physical activity and exercise training conferred a further reduction in mean Hcy concentrations. With increasing activity levels, a reduction in skewness of Hcy distribution was observed. This suggests that exercise training, especially heavy physical activity, exerts its most favorable effect in subjects with hyperhomocysteinemia. Therefore, there are few data on the association between Hcy concentrations and physical activity and the results of exercise training affects on Hcy are unclear and scattered.

The purpose of this study is to investigate the association between maximum oxygen consumption ( $\text{VO}_2\text{max}$ ) as a physical activity rating index, Hcy, and Fib concentrations in active, sedentary, and with CAD males and to determine if regular physical activity and aerobic exercise training are associated with altered plasma Hcy and Fib concentrations in this same population. This study aimed to clarify whether long-term aerobic exercise training and physical activity reduced Hcy and Fib levels, which are the conventional risk factors for CAD, in men.

## Methods

This descriptive, retrospective study compared the concentrations of Hcy and Fib in the study participants.

## Participants

This cross-sectional study involved 45 voluntary participants was based on National Health Interview Survey and

Physical Activity Rating (PA-R) Questionnaire. The participants signed an informed consent and were divided into three groups of 15 each, as follows: active, sedentary, and CAD group. The subjects in the active, sedentary, and CAD groups were randomly selected from 19 men who participated in morning exercises training of keshvari aerobic exercise club (Tehran), 18 employees of Islamic Azad University (Tehran), and 17 CAD outpatients from Amir Alam Hospital (Tehran), respectively. Physical activity level was determined using the American College of Sports Medicine (ACSM) standard in the PA-R questionnaires (Le Mura et al., 2004; Nieman, 2003). All men in the active and sedentary groups had no symptoms of cardiovascular diseases, diabetes, or hypertension; based on health/risk factor questionnaire. They had not received any special medications or supplements and did not follow any specific diet, based on health/risk factor questionnaire.

$\text{VO}_2\text{max}$  as a PA-R index was estimated on the basis of non-exercise prediction equations for  $\text{VO}_2\text{max}$  developed by researchers at the University of Houston using age, physical activity status, sex and BMI (Nieman, 2003). Body mass index ( $\text{BMI} = \text{Wt. [kg]} / \text{Ht. [m}^2\text{]})$  was determined by obtaining each subject's height and weight using a calibrated medical Seca Bella scale (Germany). Participants were instructed to fast, consume no alcohol, or engage in physical activity for 12 h prior to blood sampling. Fasting whole blood samples were collected from the left antecubital vein at 7–8 AM after 9–12 hours of fasting by a certified phlebotomist and aspirated into one 4.5 ml evacuated tube containing sodium citrate. Tube were mixed to avoid coagulation, chilled, and centrifuged at 2000 g for 20 min within one hour after sampling. The plasma fraction from tube was each transferred to a plastic vial and frozen at  $-20^\circ \text{C}$ . Hcy and Fib concentrations were measured within 4

weeks after sample collection in ZAND medical laboratory, Tehran, Iran; by Biomerio fully automated analyzer. The serum concentrations of Fib were measured using the chromometric method. Enzyme-linked immune sorbent assay (ELISA) was used to measure the serum concentrations of Hcy.

### Statistical analysis

The normality of distribution and homogeneity of variances were calculated using Kolmogorov–Smirnov and Levine tests, respectively. Between-group comparisons of estimated VO<sub>2</sub>max, Hcy, and Fib were performed using one-way analysis of variance (ANOVA) and the LSD Post Hoc test. Data was analyzed using SPSS (Version 17, Statistical Analysis software). Significant levels for all tests were set at  $p \leq 0.05$ .

### Results

The descriptive characteristics of the subjects and variables are presented in Table 1. No significant between-group differences were found in the serum concentrations of Hcy [ $F(2, 42) = 0.107$ ,  $p = 0.898$ ] and the serum concentrations of Fib [ $F(2, 42) = 0.468$ ,  $p = 0.630$ ]. Serum concentrations of Fib in active group ( $287.86 \pm 51.56$ ) was not significantly lower than sedentary ( $299.80 \pm 49.21$ ) and CAD ( $307.20 \pm 63.80$ ) groups.

Significant between-group differences were found in the estimated VO<sub>2</sub>max [ $F(2, 42) = 26.545^*$ ,  $p \leq 0.001$ ]. Significant differences of estimated VO<sub>2</sub>max were found between active and sedentary groups ( $p \leq 0.001$ ), and active and CAD groups ( $p \leq 0.001$ ). Estimated VO<sub>2</sub>max in active group ( $39.04 \pm 2.56$ ) was significantly higher than sedentary ( $32.64 \pm 3.05$ ) and CAD ( $30.37 \pm 4.27$ ) groups. No significant difference of estimated VO<sub>2</sub>max was found between sedentary and CAD groups ( $p = 0.073$ ).

Table 1. Descriptive characteristics of the subjects and study variables (mean  $\pm$  SD).

Variables	Active (n=15)	Sedentary (n=15)	CAD (n=15)
Age (yrs)	47.86 $\pm$ 5.33*	43.53 $\pm$ 4.34*	48.13 $\pm$ 5.85*
BMI (kg·m <sup>-2</sup> )	27.96 $\pm$ 2.26	26.26 $\pm$ 2.96	26.44 $\pm$ 2.34
PA-R	5.73 $\pm$ 0.59**	0.80 $\pm$ 0.41**	1.00 $\pm$ 0.37**
VO <sub>2</sub> max (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	39.04 $\pm$ 2.56***	32.64 $\pm$ 3.05***	30.37 $\pm$ 4.27***
Hcy ( $\mu$ mol.l <sup>-1</sup> )	11.73 $\pm$ 2.62	12.40 $\pm$ 3.86	11.96 $\pm$ 5.11
Fib (mg.dl <sup>-1</sup> )	287.86 $\pm$ 51.56	299.80 $\pm$ 49.21	307.20 $\pm$ 63.80

\*Active and sedentary groups ( $p = 0.028$ ), sedentary and CAD groups ( $p = 0.020$ )

\*\*Active and sedentary groups ( $p \leq 0.001$ ), active and CAD groups ( $p \leq 0.001$ )

\*\*\*Active and sedentary groups ( $p \leq 0.001$ ), active and CAD groups ( $p \leq 0.001$ )

Note: CAD, Coronary Artery Disease; BMI, Body Mass Index;  
PA-R, Physical Activity Rating; Hcy, Homocysteine; Fib, Fibrinogen.

## Discussion and Conclusion

Estimated  $\text{VO}_2\text{max}$  values were expected to differ between the active, sedentary, and CAD groups. Therefore, in this study,  $\text{VO}_2\text{max}$  was estimated using a non-exercise-based formula derived by the University of Houston researchers that uses age, physical activity, BMI, sex, and a constant coefficient. In this study, the sex coefficient was fixed since all the participants were male and BMI did not significantly differ between the study groups (see Table 1). The age difference was significant between the active and sedentary groups ( $p = 0.028$ ) and between the active and CAD groups ( $p = 0.02$ ). Using the age coefficient in the formula to estimate  $\text{VO}_2\text{max}$  (-0.381), it was found that the maximum difference in estimated  $\text{VO}_2\text{max}$  attributable to the age difference between the active and sedentary groups (4.33 years) was  $1.65 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , which is negligible. The difference in the mean PA-Rs of the three groups was significant (see Table 1). Despite the significant age difference between the groups, the differences in estimated  $\text{VO}_2\text{max}$  between the active and sedentary groups and between the active and CAD groups were attributable to differences in the PA-Rs of the groups. Mean differences of Hcy ( $p=0.898$ ) and Fib ( $p=0.630$ ) between groups were not significant. In this study, the levels of Hcy and Fib did not differ between the study groups. These results and those reported by Franke et al (1997), Monica (1997), Nygard et al (1995), Nissen et al (2002), Mc Kenzie (2003), and Sloma et al (2003) suggest that regular physical activity and exercise training does not reduce the serum levels of these risk factors. Although the results of Kassam et al (2001), Foody et al (2002), Ernest et al (2003), Walus et al (2003), Dimitrios et al (2003), Antonopoulos et al (2003), Tamvakos et al (2003), Hayden et al (2004) Tello-Montoliu et al (2006), and Jae et al (2008) show that regular physical activity may reduce serum levels of these risk factors.

The concentration of plasma Fib is regulated by both genetic and environmental influences. Factors that are positively associated with plasma Fib in males include age, smoking, stress, obesity (especially abdominal obesity), LDL cholesterol, hypertension, and diabetes. Those with a negative association include estrogen replacement and HDL cholesterol. Previous finding indicated that the same variables which modulate the risk of CAD such as physical activity and exercise training and maintaining proper weight may also lower Fib concentrations. Plasma Hcy increases with age and is higher in men and smokers. The effects of high concentrations of Hcy on risk of CAD are not mediated by known risk factors. It seems likely that a genetic component is involved in at least some cases. In particular, several metabolic defects involved in the metabolism of Hcy can lead to elevations in its concentration. In addition, vitamin B<sub>6</sub>, B<sub>12</sub>, and folate are involved as cofactors in this metabolic process, and inadequate amount of these vitamins, either through a deficiency in intake or through other conditions, can also lead to high concentrations of Hcy. Also, the levels of Hcy are affected by gender and body mass. The results of this study indicated that exercise training and physical activity does not have any desirable effects on the serum levels of Hcy and Fib. In this study, factors such as gender, age, body mass index, LDL cholesterol, HDL cholesterol, hypertension, and diabetes were controlled by questionnaire. However, optimal control of factors such as stress, diet, smoking, body fat, and heredity was impossible. Furthermore, the optimal intensity, duration, and type of physical activity and exercise training required to reduce these risk factors are unknown. Hence, more studies are needed to clarify the effects of physical activity and exercise training on Hcy and Fib levels. More studies are required to clarify the optimal intensity, duration, and type of exercise to

favorably modify these risk factors of CAD.

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