Correlation of VO$_2$max with Serum Concentrations of Fibrinogen and Homocysteine

Ardeshir Zafari$^1$*, Hojatollah Nikbakht$^2$, Ali Mohammad Amirtash$^2$, Manocher Gharooni$^3$

$^1$Department of Physical Education and Sports Sciences, Zanjan Branch, Islamic Azad University, Zanjan, IRAN
$^2$Department of Physical Education and Sports Sciences, Tehran Research and Sciences Branch, Islamic Azad University, Tehran, IRAN.
$^3$Department of Cardiology, Amir Alam Hospital, Tehran University of Medical Sciences, Tehran, IRAN.

ABSTRACT
Coronary Artery Disease (CAD) is the number one killer of adults in the Iran. Fibrinogen is a risk factor for CAD, 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 correlations of physical activity and exercises training with fibrinogen and homocysteine were not clear. This study aimed to clarify correlations of physical activity with 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 chronometric method. Enzyme-linked immune sorbent assay was used to measure the serum concentrations of homocysteine by Biomerio fully automated analyzer. Pearson correlation equations were used assess the relationship between the estimated VO$_2$max with Hcy and Fib levels. The significance of the Pearson correlation coefficients was determined using the Fisher T test. Correlation coefficients were compared using the Fisher Z test. Significant levels for all tests were set at $p \leq 0.05$. Mean differences of Hcy ($p=0.898$) and Fib ($p=0.630$) between groups were not significant. The correlation coefficients between VO$_2$max with serum concentrations of Fib and Hcy were not significant in active, sedentary, CAD and total groups. Therefore, the levels of Hcy and Fib did not alter by regular aerobic physical activity. 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 Hcy and Fib.

Key Word: Fibrinogen, Homocysteine, VO$_2$max, CAD, Exercise.

INTRODUCTION
Coronary Artery Disease (CAD) is the number one killer of adults in the Iran [11]. Associated modifiable risk factors include hypertension, hypercholesterolemia, obesity, hyperglycemia, physical inactivity and smoking [1, 4]. Fibrinogen (Fib) has emerged as an independent risk factor of importance equal to or greater than of other previously described CAD risk factors [4]. 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 [4, 14]. Fibrinogen concentration is significantly and directly related to the incidence of coronary events [7]. 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 [14].

Also playing a role in thrombosis and atherogenesis is Homocysteine (Hcy), a sulfhydryl-containing amino acid formed by demethylation of methionine [4]. 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 [5, 9, 21, and 24]. A linear relationship between Hcy concentrations and CAD risk was reported by previous studies [17, 20]. Few studies have been reported that analyze the relation between Hcy and Fib [13, 16, 19]. 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 prothrombolytic 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 hyper coagulation [16].

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 [6, 14, 17, 18]. Long-term exercise training and physical activity activity 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 [1, 6, 14, 18]. The inverse association between physical activity and CAD remains after controlling for the previously listed variables [3]. Regular physical activity and exercise training have been shown to reduce plasma Fib in healthy subjects, as well as those with CAD [12, 14]. No such association has been consistently shown between regular physical activity and exercise training and Hcy concentrations [16, 20]. 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 [9, 15, 16, 19-21]. Others studies found an inverse correlation between mean Hcy concentrations and the amount of exercises and physical activity rating [2, 5, 7, 8, 10, 12, 13, 23, and 24]. 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 (VO\textsubscript{2max}) as a physical activity rating index with 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 correlations between physical activity with Hcy and Fib levels, which are the conventional risk factors for CAD, in men.

MATERIALS AND METHODS

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 [14, 18]. 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.

VO\textsubscript{2max} as a PA-R index was estimated on the basis of non-exercise prediction equations for VO\textsubscript{2max} developed by researchers at the University of Houston using age, physical activity status, sex and BMI [18]. Body mass index (BMI = Wt. [kg]/Ht. [m\textsuperscript{2}]) was determined by obtaining each subject’s height and weight using a calibrated medical Seca Bella-840 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°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 chronometric method. Enzyme-linked immune sorbent assay (ELISA) was used to measure the serum concentrations of Hcy.

Pearson correlation equations were used assess the relationship between the estimated VO₂max with Hcy and Fib levels. The significance of the Pearson correlation coefficients was determined using the Fisher T test. Correlation coefficients were compared using the Fisher Z test. Significant levels for all tests were set at p≤ 0.05.

RESULTS AND DISCUSSION

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]. Significant between-group differences were found in the estimated VO₂max [F (2, 42) = 26.545*, p ≤ 0.001]. Significant differences of estimated VO₂max were found between active and sedentary groups (p ≤ 0.001), and active and CAD groups (p ≤ 0.001).

The correlation coefficients between VO₂max with serum concentrations of Hcy were presented in Tables 2. The correlation coefficients between VO₂max with serum Hcy concentrations in active group (r = -0.251, p = 0.367), sedentary group (r = -0.367, p = 0.178), CAD group (r = -0.141, p = 0.617), and total (r = -0.038, p = 0.805) were not significant. No significant between-group differences were found in the correlation coefficients of VO₂max with serum Hcy concentrations.

The correlation coefficients between VO₂max with serum concentrations of Fib were presented in Tables 3. The correlation coefficients between VO₂max with serum Fib concentrations in active group (r = -0.358, p = 0.190), sedentary group (r = -0.139, p = 0.622), CAD group (r = -0.214, p = 0.443), and total (r = -0.147, p = 0.336) were not significant. No significant between-group differences were found in the correlation coefficients of VO₂max with serum Fib concentrations.

Estimated VO₂max values were expected to differ between the active, sedentary, and CAD groups. Therefore, in this study, VO₂max 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.
groups (p = 0.02). Using the age coefficient in the formula to estimate VO\(_{\text{max}}\) (-0.381), it was found that the maximum difference in estimated VO\(_{\text{max}}\) attributable to the age difference between the active and sedentary groups (4.33 years) was 1.65 ml·kg\(^{-1}\)·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 VO\(_{\text{max}}\) 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. The correlation coefficients between VO\(_{\text{max}}\) with serum concentrations of Fib and Hcy were not significant in active, sedentary, CAD and total groups. In this study, the levels of Hcy and Fib did not differ between the study groups. Therefore, the levels of Hcy and Fib did not alter by regular aerobic physical activity. 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 did not correlate with Fib and Hcy concentrations and did 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) showed that physical activity correlated with Fib and Hcy concentrations and 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 are negatively associated with plasma Fib in males and 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\(_{12}\), 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 decrement of body mass. 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 required to reduce these risk factors are unknown. More studies are required to clarify the optimal intensity, duration, and type of exercise to favorably modify these risk factors of CAD.

CONCLUSION

The results of this study indicated that regular physical activity does not have any desirable effects on the serum levels of Hcy and Fib, and the correlations between physical activity rating and VO\(_{\text{max}}\) with Fib and Hcy are not significant. Hence, more studies are needed to clarify the effects of physical activity and exercise training on Hcy and Fib levels. More studies aimed are needed to clarify correlations between physical activity and exercise training with Hcy and Fib levels, which are the conventional risk factors for CAD.

REFERENCES