A Comparative Study on Platelet Activation Markers Between Continuous and Intermittent Exercise Training Programs in Healthy Males

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Abstract

Background: Platelet plays an important role in the hemostatic system. The hyper-activation of platelet may lead to the development of thrombotic event in cardiovascular disease (CVD). Aerobic exercise is well known to reduce CVD risk and can reduce the platelet activation. The purpose of this study was to investigate the effect of the continuous and intermittent training programs on platelet activation.

Methods: There were 24 healthy sedentary males mean aged 21.75 ± 3.73 years old participating in this study. They were divided into three groups, which are control group (n = 9), continuous group (n = 7) and intermittent group (n = 8). Continuous training group cycled at 60% VO2 max for 45 minutes. For the intermittent training group, participants cycled at the 45% VO2 max and had a rest before the second session at 75% VO2 max, and the sessions were repeated twice. The exercise was performed non-stop until 45 minutes. Both exercise training groups performed the exercise three times per week for 12 weeks. Platelet activation was measured via detection of the P-selectin and glycoprotein IIb-IIIa using flow cytometry by indirect immunoflourescence.

Results: This study showed that, intermittent type of training showed a significant difference of the gated percentage of CD62p and PAC-1 activation. There was also a significant difference of PAC-1 after training between two groups.

Conclusions: As a conclusion, intermittent group pronounced more beneficial effects on platelet activation, whereas continuous group provided the beneficial changes on platelet activation.

Keywords: Platelet activation; PAC-1; CD62p; Exercise training

Introduction

Cardiovascular disease (CVD) is recognized as an important cause of morbidity and mortality [1, 2] particularly in men [3]. According to the report of Clinical Practice Guidelines Acute Myocardial Infarction 2001, in 1998 approximately 24.5% of deaths in government hospitals, in Malaysia, were due to cardiovascular problems. In Malaysia males had higher chronic heart disease (CHD) mortality rate than females [4] and Indians had a higher CHD mortality than Malays and Chinese [5].

Currently, exercise is considered as one of the preventive medicines for CHD. Higher levels of physical activity are associated with a reduced incidence of CHD. Physical inactivity is observed to be one of the risk factors for CHD [6]. Investigation on the effects of different types of exercise programs on platelet activation is one possible mean of assessing the beneficial effects in respect to cardiovascular protection. Studies by Wang et al (1994) indicate that moderate-intensity exercise training (60% VO2 max for 30 min per day, five times per week for 8 weeks) might reduce resting time but, in contrast, it can induce platelet aggregation and platelet activation. The aim of this study was to compare the platelet activation markers between continuous and intermittent exercises in healthy sedentary males. Besides that, it also can be used to identify the better training program that can be used as one of the ways to prevent CVD.

Materials and Methods

A total of 24 healthy sedentary males aged 19 - 34 with body mass index (BMI) in the range of 19 - 25 kg/m2 were recruited for this study. These sedentary males have not engaged in any active physical activity programs, except the routine daily living activities. The selected subjects were screened...
by medical history and blood biochemistry evaluation. They should have a normal blood pressure (< 140/90 mm Hg), normal fasting total cholesterol level (< 200 mg/dL or 5.2 mmol/L), normal fasting triglycerides level (< 200 mg/dL or 2.3 mmol/L), normal blood glucose (70 - 100 mg/dL or 3.9 - 5.6 mmol/L) and non-smokers. Subjects with high blood pressure (> 140/90 mm Hg), high blood glucose (> 5.6 mmol/L), high level of lipid profile (≥ 5.2 mmol/L), BMI exceeding 25 kg/m², ongoing drug treatment (aspirin, non-steroidal anti-inflammatory drug) and suffering from chronic disease such as diabetes mellitus, CVD, chronic migraine and orthopedics problems that would interfere with exercise training, were excluded from the study.

The subjects were divided into three groups (on the basis of similar VO₂ max), control group (n = 9), continuous training group (n = 7) and intermittent training group (n = 8). The intensities for both the continuous group and intermittent group had been kept low for the first 4 weeks as familiarization trial, but were increased considerably for the next 8 weeks. All of them were students and staff from the Hospital Universiti Sains Malaysia. All selected subjects agreed to participate willingly in the study. This study has been approved by the Research and Ethics Committee (Human), Universiti Sains Malaysia, Health Campus, Kelantan.

Blood collections throughout the study were collected at baseline level and immediately after the last bout of training programs. Before each blood collection session, all subjects were confirmed free from any illness and not on anti-inflammatory drugs medication for at least 10 days before phlebotomy. Blood taking procedure was done in the afternoon around 2:30 - 6:30 pm to avoid the diurnal variation.

Blood collection for platelet activation was designed to minimize artificial platelet activation and stasis during the phlebotomy [7]. It was performed after subjects rested for at least 15 min. First 2 mL of blood was discarded; 1 mL of blood was collected into 3.8% sodium citrate Greiner Bio-One tube [8] by venipuncture using a Venofix 21-gauge butterfly needle, Malaysia and BRAUN Discofix stopcock, Germany. It should be mixed vertically and slowly to prevent in vivo activation.

The expression of CD62 (P-selectin) and PAC-1 (conformational change of the GPIIb/IIIa complex) was measured by three colors flow cytometry by indirect immunofluorescence. The platelet population was detected by its light-scatter characteristics and expression of the monoclonal antibody CD61. Briefly, 5 μL of whole blood (3.2% citrate sample) was incubated with 10 μL of CD61 PerCP, 10 μL CD62p PE and 10 μL PAC-1 FITC with corresponding isotype control as a negative control. All samples were processed within 15 min of collection on an FACS Calibur flow cytometry (Becton-Dickinson, San Jose, CA).

Statistical analyses of the data were performed using SPSS software programs version 12. Paired t-test also was employed to determine the effects of pre- and post-training on platelet activation markers with the assumption of normality, random sampling and equal variance were met. All data were presented as mean ± SD. Statistical significance was set at P ≤ 0.05.

Results

The results showed that the means of gated percentage of CD62p in continuous and intermittent groups were found decreasing from 0.92 ± 0.97 to 0.70 ± 0.34 (P > 0.05) and from 0.86 ± 0.81 to 0.31 ± 0.20 (P < 0.05) respectively after training, while the mean of gated percentage of PAC-1 in continuous and intermittent groups were found from 1.35 ± 1.21 to 2.04 ± 1.72 (P > 0.05) and from 1.53 ± 1.09 to 0.99 ± 0.56 (P > 0.05) respectively before and after training. From the above data observed, CD62p level in intermittent group showed a significant difference. Comparison between PAC-1 level in continuous (post-training) and intermittent groups (post-training) also show a significant difference (P < 0.05).

Discussion

Regular physical activity was found to reduce the risk of CVD and thromboembolism stroke [9]. Previous study evaluated that, short intermittent bouts of walking provide improvements in aerobic fitness, body composition, plasma lipoprotein and blood pressure, compared to continuous or long session of physical training [9]. Several studies and investigations were conducted to evaluate the effects of different types of regular exercise or exercise conditioning on the hemostatic variables. However, the studies on the effect of short bout and long bout exercise training programs on hemostatic variables are still lacking.

There were few studies showing contradictory, some of the studies reported that regular exercise provided no changes [10] and some of them claimed that there is an improvement effect of regular exercise [11, 12]. Study by Ribeiro et al [13] showed that, factors such as age, gender, body composition, training status and methods of analysis might influence the hemostatic parameter to make the actual effect of exercise become contradictory. According to the Macchi et al [14], exercise will induce the platelet activation via significantly increased of P-selectin expression from the alpha granules and Weibel-Plade. Normally, exercise induces change in platelet activation state as a result of endogenous release of ADP and epinephrine [12]. Exercise is also noted to enhance the platelet activity which is accelerating the hemostatic plug which could turn to thrombosis.

Our study also discovered that the platelet activation referred to the gated percentage of CD62p decreased in both exercise groups, either continuous or intermittent group, immediately after compared to resting value. It may indicate
that regular exercise leads to less activation of the platelet during exercise. Our finding was similar with Smith [15], who also supported that the platelet became less activated in trained people compared to untrained peoples when they performed exercise. The resting platelet of physically active men was found to be less adhesive compared to sedentary people [16]. According to El-Sayed et al [10] the physical conditioning caused less aggregation events probably due to increase of epoprostenol, which is the potent, platelet aggregation inhibitor and arise of nitric oxide, which has the potent anti-platelet effect.

The study by Di Massimo et al [17] on sedentary healthy subjects who were trained at moderate intensity found that the total antioxidant stress increased and it might be the cause of decreased platelet responses to the ADP and collagen. The activation of the platelet during exercise may be due to release of plasma catecholamine. Sedentary people tend to experience more activated platelet due to greater release of catecholamine during exercise compared to physically active people [12]. Accordingly the exercise training or regular exercise possibly will reduce the response to catecholamine during exercise and lead to less activation of the platelets. This condition may reduce the risks of prothrombotic events.

The study also showed that the PAC-1 is slightly decreased in intermittent group. PAC-1 is a monoclonal antibo- body which appears to be specific for the fibrinogen binding site on platelet GPIIa/IIIb. Biologically, if there is decreased CD62p-selectin activation, it is supposed to decrease the gated percentage of PAC-1 which notices less activation of GPIIb/IIIa.

Recently the effects of exercise on the platelet activation pronounced have produced the conflicting results and the exact effect on platelet activation is still undetermined. Moreover, the different studies are performed in different intensity and types of training programs, and the other platelet activation markers (platelet factor 4, thromboxane and β-thromboglobulin). As a fact, regular physical activity is generally a part of a healthy lifestyle that is known to reduce the risk of CVD. Training-related adaptation expected to improve the platelet activation marker could be responsible for reduced risk of CHD.

Regarding to our study, there were beneficial effects of exercise training in improving the platelet activation. It may be due to improvements of several factors including the physiological improvement in hemostatic equilibrium. According to the Kestin et al [17] and Lockard et al [18] improvements in the platelet may possibly mediate the reduction of cardiovascular risk of exercise training. However, there are other platelet activation markers that should be monitored to evaluate the actual changes in hemostatic system due to chronic training programs.

For the further studies, there are a few recommendations that could be taken into consideration such as a larger sample size for more input. Besides that, the experimental and control group must be fully monitored to make sure that they are not involved in other sports or physical activity, and they are well defined sedentary peoples. Duration of the intervention programs is also suggested to increase up to 6 months frequently in every week. The intensity and training design also should be evaluated for more details. It is also suggested that more parameters and markers should be included to confirm the changes in platelet activation.

**Conclusion**

For the conclusion, platelet activation marker shows improvement in a continuous group where gated percentage CD62p and PAC-1 were decreased after the training programs. Nevertheless, the changes are also not significantly different. As a conclusion, intermittent exercise provides the favorable effects on platelet activation, whereas the continuous exercise gives more beneficial effect on the platelet activation.

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**Author Contributions**

NH, RH and AKG were making conception and design, analysis and interpretation of the data. NH was drafting of the article and making critical revision of the article. NH, RH and AKG gave the final approval of the article.

**Disclosure**

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**Conflict of Interest**

No conflict of interest.

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