EFFECT OF CONTINUOUS AND INTERMITTENT EXERCISE TRAINING PROGRAMS ON PLATELET ACTIVATION AND FIBRINOLYTIC PROFILE OF SEDENTARY MALES

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ABSTRACT
The purpose of this study was to investigate the effectiveness of continuous and intermittent training program on platelet activation and fibrinolytic profiles in healthy sedentary males. 24 healthy sedentary males of 21.8±3.7 years participated in this study. They were divided into 3 groups, like, control group (n=9), continuous training group (n=7) and intermittent training group (n=8). In 12 week training period, the intensities for both the continuous group and intermittent group, for the first 4 weeks, had been kept low as a familiarization trial, but were increased considerably for the next 8 weeks. Platelet activation and fibrinolytic profiles were measured, at the beginning and at the end of training period, by detecting P-selectin and glycoprotein IIb/IIIa. Concentration of the tissue plasminogen activator (t-PA) and concentration of plasminogen activator inhibitor type-1 (PAI-1) antigen were measured as fibrinolytic profiles. VO₂ max of each person was evaluated before and after training, following graded exercise protocol till exhaustion. Both the intermittent and continuous type of training did not show any significant change in the gated percentage of CD62p and PAC-1 activation, t-PA and PAI-1 antigen, but improved VO₂ max significantly. In conclusion, our study found that 12 weeks of continuous and intermittent training had beneficial effect on VO₂ max, but failed to produce any significant variation in fibrinolytic profile and platelet activation variables. This might be due to the fact that a longer training period might be necessary to get the significant variation in coagulation variables rather than the cardio-respiratory variable like, VO₂ max.

Key Words: Continuous Training, Intermittent Training, Fibrinolytic Profile, T-PA Antigen, PAI-1 Antigen

INTRODUCTION
Coronary heart disease (CHD) is the leading cause of mortality worldwide with more than 4.5 million deaths occurring in the developing countries. Men are at higher risk than women (O’Connor et al., 2006). In Malaysia, CHD became the commonest cause of death since 1980 (Jeyamalar, 1991). Regarding cardiovascular disease admissions and death, since 1985 to 2004 in Malaysia, ischemic heart disease accounted for 25% to 33% of admissions and 27% to 35% of death (Zambahari, 2004). The mortality rate due to CHD was twice in males than their female counterparts (Khor et al., 1997). Recently, dysfunction of the haemostatic system in relation to atherosclerosis and CHD has become more recognized (El-Sayed et al., 2004; Abdullah et al., 2009). The cardio protective effect of exercise occurs by improving the haemostatic system, especially coagulation (Lockard et al., 2006) and fibrinolytic system (Stratton et al., 1991; De Geus et al., 1992; Szymanski and Pate, 1994; Wang, 2006). Previous investigations reported that exercise may reduce the CHD by reducing the coagulation potential (Lockard et al., 2006) and promoting the endogenous fibrinolysis activity (Meade et al., 1993; Fernhall et al., 1997; Wannamethee et al., 2002). Furthermore, exercise is found to improve fibrinolytic activity by increasing t-PA activity, reducing t-PA antigen and the PAI-1 antigen (Lowe et al., 1998; Szymanski et
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al., 2005; Lockard et al., 2006). However, changes in components of the homeostasis due to effects of exercise per se remain poorly understood and studies on effects of exercise on fibrinolytic system are few. The beneficial effects of exercise on physiological improvements are well known. However, there are many types of exercise training. Continuous exercise is defined as a long session with low intensity but without any break, whereas intermittent exercises are defined as short bouts of moderate intensity exercise with rest or low intensity activity in between (Hardman, 2001). Most people consider long and continuous workouts to be beneficial for maintaining cardiovascular fitness. However, exercising with intervals may be more effective (Lyndon et al., 2005). In addition, the National Initiative Health (NIH) and Human Health Service (HHS) suggested that shorter bouts of intermittent exercise of moderate intensity with at least 10 minutes duration accumulating 30 minutes per day, provided cardiovascular benefits (Lyndon et al., 2005). DeBusk et al., (1990) compared both intermittent and continuous types of exercise training comprising of same energy expenditure. They observed that, subjects who exercised in intermittent sessions experienced gain in physical fitness and improvement in blood lipid profile. Short bouts of intermittent exercise training lead to a significant improvement in cardiovascular fitness as compared to the longer continuous bout of similar duration (McFarlane et al., 2006). On the other hand, brisk walking training programme at 70-80% of predicted maximal heart rate for 30 min/day on 5 day/week showed increasing aerobic fitness, improved blood lipid profiles, enhancing the aspects of physiological well being of sedentary middle-aged adults (Murphy et al., 2002). Regular physical activity reduces the risk of cardiovascular disease and thromboembolism stroke (Haskell et al., 2007).

Previous studies exhibited that the short intermittent bouts of walking provided improvement in aerobic fitness, body composition, plasma lipoprotein and blood pressure compared to continuous or long session of physical training (Haskell et al., 2007). Several studies and investigations evaluated the effects of different types of exercise condition on the haemostatic variables (De Paz et al., 1992). However, studies on the effects of continuous and intermittent exercise training programs on haemostatic variables, especially on fibrinolytic markers are still lacking and remain controversial (El-Sayed et al., 2004) in literature. Hence, the present study was undertaken with a view to investigate the effectiveness of continuous and intermittent exercise training on fibrinolytic profile and platelet activation markers of sedentary individuals.

MATERIALS AND METHODS

Study Design

A randomized control trial was designed to compare the effects of continuous and intermittent exercise training program on platelet activation and fibrinolytic profile among healthy males. Subjects were divided into three groups (control, continuous and intermittent). Continuous and intermittent group followed their 12 weeks training programs. The control group did not perform any exercise and maintained their sedentary lifestyle during the 12 week intervention period. The complete protocol of the research study has been illustrated in Figure 1, in the form of a flow chart.

This study was approved by the Research and Ethics Committee (Human), Universiti Sains Malaysia, Health Campus, Kelantan. Written informed consent was obtained during the first screening visit.

The inclusion criteria were sedentary males who had not participated or trained regularly in any sport activities, had body mass index (BMI) in the range of 19-25kg/m², non-smoker, had normal blood pressure (< 140/90mmHg), had normal fasting total cholesterol level (<200mg/dl or 5.2mmol/L), normal fasting triglycerides level (<200mg/dl or 2.3mmol/L) and normal blood glucose (70-100mg/dl or 3.9-5.6 mmol/L).

The exclusion criteria included, on drug treatment (Aspirin, Non-steroidal anti inflammatory drug), high blood pressure (> 140/90mmHg), high blood glucose (> 5.6mmol/L), high level of lipid profile (> 5.2 mmol/L), BMI exceeding 25 kg/m², suffering from chronic disease such as diabetes mellitus,
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cardiovascular disease, chronic migraine and orthopedics problems that would interfere with exercise training.

Sample Size Calculation
The sample size was calculated using PS Power and Sample Size Calculation version 2.1.30 using 80% as power of the study with the 95% confidence interval keeping the t-PA antigen as the reference variable (Kulaputana et al., 2005). The sample size calculated for each group was 7. Hence, the calculated sample size was kept 8, considering 10% drop out.

Investigation Subjects
24 healthy sedentary males (19-34 yrs) who neither participated in any physical training programme nor in any regular sports were recruited for this study. The selected subjects were screened by previous medical history and blood biochemistry evaluation. The subjects were excluded if they had the chronic disease such as diabetes mellitus, cardiovascular disease, chronic migraine and orthopedics problems that would interfere with exercise training programme. All selected subjects agreed to participate willingly in the study. This study was approved by the Research and Ethics Committee (Human), Universiti Sains Malaysia, Health Campus, Kelantan. Written informed consent was obtained during the first screening visit.

Determination of Maximal Oxygen Consumption
\( \dot{V}O_2 \) max of each individual was determined following a graded exercise protocol on an Excalibur Lode Cycle ergometer. The initial load was 50W and was increased at 16W for every 1 minute, till exhaustion. The pedaling frequency was kept between 60-70 rpm. The expired gas was analyzed on a portable computerized metabolic measurement system, Metamax 3B (Cortex Biophysik, Germany), pre-calibrated with known gas sample. The heart rate was recorded on a Polar transmitter (T61, polar Electroy, Finland). The \( \dot{V}O_2 \) max was recorded following the criteria described by American College of Sports Medicine (2006).

Determination of Sub-max Workloads
The data from Metasoft programme provided the information about the workload and oxygen consumption. From this information, the graph was plotted using the Microsoft Excel Professional 2003. From the graph the equation of \( Y= mx + c \) was provided. Then from equation, the training load for 40%, 45%, 60% and 75% \( \dot{V}O_2 \) max were estimated.

Training Program
The training groups participated in 12 weeks of training programme. Exercise was performed 3 times per week. The work load was continuously monitored using electronic cycle ergometer during each training session. Each training programme started by self warm-up for two minutes and ended with self cooling down. For continuous exercise, subjects pedaled the ergometer at 60/min at individual workload (at 60% of individual \( \dot{V}O_2 \) max) for 45 minute. In intermittent exercise, the subjects cycled at 45% of individual \( \dot{V}O_2 \) max for 10 min followed by 5 min rest and then at 75% of individual \( \dot{V}O_2 \) max for 10 min followed by 5 min rest. This repitition continued until 45 min (Figure 1). Familiarization to training was of a little lower intensity (Fig 1).

Blood Collection for Fibrinolytic Variables
The subjects reported in the laboratory after an overnight fast. They were allowed to rest for at least 20 minutes. Phlebotomy of the venous blood was drawn at antecubital vein with a BBRAUN venofix® 21-gauge butterfly needle, (Malaysia) and syringe 10 cc/ml TERUMO® SYRINGE, (Philippines). The first 2 ml of blood was discarded. The venous blood for fibrinolytic profile (about 8ml) was collected in three BD Vacutainer® NNC 0.109M trisodium citrate, UK container. All three container for fibrinolytic profile were prepared by centrifugation at 4300 rpm for 15 minute at 20°C and the plasma was separated and stored in aliquots and kept frozen in Thermo Electron Freezer at -80°C in until analyzed (Lippi et al., 2006).
Subject joins this study after signing the informed consent form approved by USM Ethical Committee (Human). Screening Health Status: Blood Pressure (BP); Electrocardiogram (ECG); Fasting blood total cholesterol; Fasting blood triglycerides; Fasting blood glucose.

Data entry, analysis, and interpretation using the SPSS Software

Figure 1: Flow chart of Experimental Protocol
Platelet Activation Measurement
The whole blood was stained within 10 minutes in the Felcon® 5 ml polystyrene round-bottoms 12 x 75 mm (BD, USA) and were placed in a rack before the start of laboratory session. For analysis purpose, two felcon tubes were used. The first tube was labeled as control and the other one was labeled as test. Then 20µl of PAC-1, CD62P and CD61 was pipetted into the test tubes. For the control tube, 2µl of Perdidine chlorophyll protein (PerCP), fluorescein isothiocyanate (FITC)-conjugated PE and 20µl CD61 were pipetted into control tube. 54µl of PBS buffer was added into control tubes. After that 5 µl of whole blood was added carefully to both tubes. Vortex was not done to avoid in vitro platelet activation. Control and test tubes were then incubated at the room temperature in dark for 15-20 minutes. After the incubation was completed, 1 ml of cold 2°-8° C 1% paraformaldehyde or cell fix was added to each tube and mix well. Fixing inhibit the PAC-1 from binding to paraformaldehyde. Stained and fixed samples were then kept in the dark at 2°-8° C for 30 minutes before they were analyzed with flow cytometer. To minimize in vitro artifacts, fixation and lysing procedures were omitted. Acquisition was immediately performed thereafter on a fluorescence-activated cell sorter (FACS) Calibur flow cytometer (B.D) with excitation by an argon laser at 488 nm. The cytometer was calibrated daily with standard B.D. Calibrate beads containing specific amounts of mean equivalent soluble fluorescein in molecules.

Direct immunoflourescence staining assay used single or multicolor staining. In multicolor staining, one of antibody conjugate can be used to threshold data acquisition to analyze only those blood cells that bind an activation-independent, platelet-specific antibody such CD62 to recognized the platelets (Abrams et al., 1990; Abrams and Shattil, 1991). Then, used the another antibody which is conjugated to a different fluorochrome to simultaneously assess the binding of platelet –associated, activation–dependent antibodies for example CD62p or PAC-1. The combination of CD61, CD62p and PAC-1 reagents represents a three-color assay that reports the two aspects of platelet activation (Shattil et al., 1987).

Platelet Marker
CD61 monoclonal antibody is composed of mouse IgG1 heavy chains and kappa light chains. CD61 recognizes as Mr 110-kdalton (kDa) protein, also known as GP IIIa, the common β-subunit (intergrin β3 – chain) of the GPIIb/IIia complex and vitronectin receptor (VNR) (Modderman et al., 1989). The GP IIb/IIia complex and the VNR are intergrins that involved in cell adhesion (Parmentier et al, 1990). The CD 41 antigen and the CD 61 antigen forms the GP IIb/IIia complex, which acts as a receptors for fibrinogen, vWF, fibrinectin, vitronectin on activated platelet (Modderman et al., 1989). The CD61 antigen is found on all normal resting and activated platelet (Modderman et al., 1989). The CD61 antigen is also found on endothelia cells, megakaryocytes, and on some myeloid, erythroid, and T-lymphoid leukemic cell lines (Fitzgerald et al., 1987; Modderman et al., 1989).

Platelet Activation Marker
CD62p:
CD62P is composed of mouse IgG1 heavy chains and kappa light chains. The CD62p antigen, also known as platelet activation-dependent granule-external membrane (PADGEM) protein or granule membrane protein (GMP-140), is a 140 kilo Dalton (kd) single-chain polypeptide. The CD62p antigen is a member of the selectin family or adhesion molecules. It mediates the adhesion of activated platelets to neutrophils and monocytes in haemostasis. The CD62P antigen is an intergral membrane protein associated with α-granules of platelets, Weibel-Palade bodies of endothelial cells and megakaryocytes. The CD62P antigen is expressed on internal α-granule membrane of resting platelets. During the platelet activation and α-granule secretion, the α-granule membrane fuses with the external plasma membrane and the CD62P antigen which is expressed on the surface of the activated platelet.

PAC-1:
PAC-1 is pentameric IgM κ-immunoglobulin. PAC-1 is monoclonal antibody. PAC-1 recognizes an epitope on the glycoprotein IIb/IIia (GP IIb/IIia, α_{II}β_{3} ) complex of activated platelets at or near the
fibrinogen receptor/fibrinogen binding site. The GP IIb/IIIa complex is a member of a family of adhesive protein receptors found on a variety of cell types of integrin and cyto adhesion family. PAC-1 only binds to activated platelets and appears to be specific for this recognition site within GP IIb/IIIa. Approximately 45,000 to 50,000 GP IIb/IIIa receptors appear on the platelet surface upon activation. The binding of fibrinogen to the GP IIb/IIIa receptor is required for platelet aggregation. PAC-1 inhibits fibrinogen-mediated platelet aggregation.

**Data Acquisition and Analysis**

Acquisition and analysis were performed on scatter gating or fluorescence gating. Scatter gating gating on forward scatter (FSC) and side scatter (SSC). Fluorescence gating was done on the activation-independent platelet marker, and then the light-scatter profile of the positive population independently analyzed. Venous blood typically demonstrates three subpopulation of particles. The majority of the particles consist of single intact platelets. A second population, typically representing 5% of all particles, exhibits greater light scatter than single platelets and represents platelets associated with large white blood cells (WBCs). A third population, representing 5% to 15% of the particles which is the light scatter is lower than single intact platelets, includes platelet-derived micro particles with an average diameter of 0.1 μm. 5. For fluorescence gating, excluded debris and background noise by setting the appropriate threshold.

**Flow Cytometry Technique**

The whole blood samples were analyzed by Becton Dickinson FASC Calibur flow cytometry. Flow cytometry is a rapid technique to measures the specific characteristics of large number of individual’s cells. Before performing the flow cytometry analysis, cells should labeled by fluorescent. Typically the cell is labeled with fluorescence conjugated monoclonal antibody (MoAb). In the flow cytometry chamber, the suspended cell pass through the flow chamber at 1,000 to 10,000 cells rate per minutes by application of the laser beam. After fluorescent activated the flouresphore at the excitation wavelength, a detector process the emitted fluorescence and the light scattering properties of each cell (Michelson, 1996).

Flow cytometry technique was used to characterize the alteration in the structure of the platelets due to platelet activation. Flow cytometry used with multiple fluorescent staining of platelets or in combination with monoclonal antibodies to measure circulating activated platelets. Flow cytometric detects activated platelets by determining the changes in shape of the activated platelet, detection of specific antigens on the membrane of activated platelet (P-selectin, GP IIb/IIIa) or platelet surface bound proteins (such as fibrinogen) or the detection of the expressed procoagulant surface. The activated platelet exhibits alterations in the density of the surface glycoprotein, ligands or expression of new epitopes on the platelet surface. These alterations provide the possible means to detect and quantify the platelet activation. It is because, when the platelets are activated, they will expressed the surface GP IIb/IIIa complex and change their conformation which is generate the new epitope to be detected by monoclonal antibodies. The platelet population was identified on its forward and side scatter distribution and 20,000 events were analyzed for with mean fluorescence with CELL Quest software.

The instrument uses the principle of a 5W argon laser and operated at 200mW power at a wavelength of 488nm. The fluorescence was detected using with 530/30 band pass filter, and PE fluorescence was detected with a 585/42 filter. The instrument was calibrated for fluorescence and light scatter daily using 2µm Calibrite beads (Becton Dickinson) (Shattil et al, 1987).

**Fibrinolytic Tests**

ELISA method is a stands for Enzyme-Linked Immunoabsorbent assay. ELISA is a biochemical technique used mainly in immunology to detect the presence of an antibody or antigen in a sample. The enzyme chosen is one that is capable of catalyzing the reaction to generate a colored product from colorless substrate. The amount of antibody bound to antigen is then proportional to the amount of colored end product that can be visualized in the quantitative test or measured in a spectrophotometer by optical density scanning (Roderick and Helbert, 2002).
These assays used to detect specific antibodies in test samples. Antigen attached to the solid phase, is reacted with the test sample, potentially contains antibody. The reactants incubated and the surface washed. Subsequently, an enzyme-labeled anti-immunoglobulin added incubation and the surface washed again. Retention of enzyme on the surface again washed. Retention of enzyme on surface detected upon the addition of the appropriate substrate and indicates the presence of antibody being sought. The amount of the enzyme detected is directly proportional to the concentration of antibody in sample (Roderick and Helbert, 2002).

Sandwich ELISA alternately referred to double antibody EIA. In sandwich EIA procedures, antibody immobilized on the solid phase. Subsequently, the test sample, potentially containing the bi- or polyvalent antigen added and the reaction is incubated and washed to separate non-reactants. Enzyme-labeled antibody is than added to the surface, incubated and wash repeated. The amount of antibody bound to antigen present on the surface after the final wash is directly proportional to the amount of antigen in the sample (Roderick and Helbert, 2002).

**Tissue Plasminogen Activator Antigen (t-PA antigen)**
Tissue plasminogen activator (t-PA) constitutes an important agent in fibrinolytic pathway and the levels of t-PA are thought to have minor effect on fibrinolytic potential (Ranby and Brandstrom, 1988). The physiological role of t-PA is to activate plasminogen to plasmin, which is turn degrades fibrin to soluble degradation products. The t-PA antigen (TECHNOZYM®, Austria) ELISA kit uses the double antibody principle to measure t-PA antigen concentration. Normal range for t-PA antigen is between 2-8 ng/ml.

**Plasminogen Activator Inhibitors type-1 (PAI-1)**
The PAI-1 antigen (TECHNOZYM®, Austria) ELISA kit testing is based on a double antibody principle. Plasminogen activator inhibitor type 1 (PAI-1) is the primary inhibitor of tissue plasminogen activator (t-PA), a key enzyme in fibrinolysis. An increased plasma level of PAI-1 antigen is associated with impaired fibrinolytic function. Elevated levels of PAI-1 have been observed in thrombolytic disease, acute myocardial infarction, deep vein thrombosis, as well as normal pregnancy and sepsis (Declerck et al., 1988; Ranby and Brandstrom, 1988). Normal range for PAI-1 antigen is between 7-43 ng/ml.

**Statistical Analysis**
Statistical analysis was performed using SPSS software program version 12.00. Two-way repeated measure ANOVA were employed to determine effects of exercise training of different types of training program between and within groups, followed by paired ‘t’ test.

**RESULTS AND DISCUSSION**
Pre and post-training physical variables, including the relative $\dot{V}O_2$ max are shown in Table 1. In our study, the relative $\dot{V}O_2$ max of both the continuous and intermittent training groups improved significantly, Continuous and intermittent type of training improved the $\dot{V}O_2$ max of sedentary persons was observed in previous studies (Hardman, 2001; Murphy et al., 2002). Cardio respiratory endurance is defined as ability to sustain prolonged, rhythmic exercise. It is related with the highest rate of oxygen consumption obtainable by the active muscle during maximal or exhaustive exercise (Wilmore et al., 2008). Both the exercise training program increased the $\dot{V}O_2$ max reflected an increase in cardio-respiratory system efficiency. Previous researchers also observed that both the continuous and intermittent exercise training program were able to improve the $\dot{V}O_2$ max (Debusk et al., 1990; Jakicic et al., 1999; Schmidt et al., 2001; Hardman, 2001). But in our study, we observed that the degree of improvement in $\dot{V}O_2$ max was higher after intermittent type of training as compared to the continuous type.

Mean t-PA antigen and PAI-1 antigen did not show any significant differences either in the control group or in the experimental groups.
Table 1: Summary mean± SD of pre and post training anthropometric profiles and cardiorespiratory fitness in groups. (*Significant difference within group (pre and post), *p < 0.005)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=9)</th>
<th>Continuous (n=7)</th>
<th>Intermittent (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-training</td>
<td>58.68±12.95</td>
<td>59.16±9.38</td>
<td>59.59±7.95</td>
</tr>
<tr>
<td>Post training</td>
<td>59.58±12.71</td>
<td>50.00±9.52</td>
<td>59.71±8.35</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-training</td>
<td>20.21±3.45</td>
<td>19.91±3.45</td>
<td>21.04±2.38</td>
</tr>
<tr>
<td>Post training</td>
<td>20.70±3.49</td>
<td>19.99±3.42</td>
<td>20.95±2.30</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-training</td>
<td>10.69±6.17</td>
<td>10.38±4.39</td>
<td>10.84±4.00</td>
</tr>
<tr>
<td>Post training</td>
<td>10.60±5.76</td>
<td>10.26±4.76</td>
<td>9.56±4.12</td>
</tr>
<tr>
<td>VO₂ max (ml/kg/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-training</td>
<td>33.07±4.87</td>
<td>33.37±3.78</td>
<td>36.37±5.41</td>
</tr>
<tr>
<td>Post training</td>
<td>33.40±5.38</td>
<td>39.61±6.42*</td>
<td>40.75±4.92*</td>
</tr>
</tbody>
</table>

Table 2: Mean and SD of pre and post training fibrinolytic profile in different study groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=9)</th>
<th>Continuous (n=7)</th>
<th>Intermittent (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t-PA Antigen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>Pre-training</td>
<td>2.25±0.31</td>
<td>3.00±0.54</td>
</tr>
<tr>
<td></td>
<td>Post training</td>
<td>2.37±0.38</td>
<td>2.57±0.90</td>
</tr>
<tr>
<td>PAI-1 Antigen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>Pre-training</td>
<td>9.68±7.86</td>
<td>8.59±4.20</td>
</tr>
<tr>
<td></td>
<td>Post training</td>
<td>9.61±7.54</td>
<td>6.94±3.33</td>
</tr>
</tbody>
</table>

Among healthy individuals, the rise of t-PA antigen had shown a sign of future cardiovascular risk (Ridker et al., 2004). Relative enhancement of t-PA antigen is a signal of fibrinolysis impairment among the susceptible individuals. Our present study showed a tendency or reduction in both the t-PA antigen and PAI-1 antigen in continuous training group, though not significantly. In fact, exercise training is supposed to increase the fibrinolytic potential via decreasing the t-PA antigen and PAI-1 antigen level (Lee and Lip, 2003). Hence, Hittel et al., (2003), found that the activation of fibrinolytic gene mRNAs and proteins as mention physical training. However, we just expected the decline of the t-PA/PAI-1 complexes in fibrinolytic system. Decline of the t-PA/PAI-1 complexes indicate that there is increase of t-PA activity or active t-PA, which reflected an improvement of the fibrinolytic system. Van Guilder et al., (2005) did not find any significant difference in the t-PA release in people having normal body weight.
De Paz et al., (1992) showed that there was no significant differences of t-PA antigen in both trained and untrained group, but there was a significant difference in the t-PA activity in trained group. De Gues et al., (1992), also did not find any significance reduction of PAI-1 activity as a result of eight weeks training. Stratton et al., (1991) also failed to find any significant changes of the fibrinolytic profile after six weeks of training of young adults. It can also be highlighted here that a prolonged continuous/intermittent training of more than the 12 weeks might be beneficial to improve the fibrinolytic profile of sedentary men. In contrast, Womack et al., (2003) observed that t-PA antigen decreased in chronic exercise training. Similar studies on young sedentary males highlighted significant differences between training group and control group after 12 weeks of training programs on PAI-1 antigen, PAI-1 activity and t-PA antigen (van den Burg et al., 1997). Six months of aerobic training program also revealed the improvement on the endogenous fibrinolytic profiles among overweight sedentary healthy men and women (Kulaputana et al., 2005). The improvements observed were related to the reduction of central abdominal obesity. Van Guilders et al., (2005) found that the t-PA antigen released was related to the body mass, fat percentage and BMI. The exact explanation is still unclear whether or not the regular exercise might improve the endothelial function. However, most of the thrombogenic profiles such as t-PA and PAI-1 antigen were conducted on the overweight or obese people. Accordingly, the improvements were observed related to the BMI and waist-hip ratio and reduction of anthropometric profile.

Mean of gated percentage of CD 62p in continuous and intermittent group were found to decrease from 1.16±1.17 to 0.83±0.35 (p>0.05) and from 1.30±0.98 to 0.55±0.34 (p>0.05) respectively after continuous and intermittent type of training, respectively. However, these declines were not found to be statistically significant (Table 3).

No significant changes were also observed in mean of gated percentage of PAC-1 in continuous and intermittent training (Table 3). The platelet percentage were gated at more than 80% and CD62 quadrant were set without –ve control tube.

**Table 3: Summary of mean ± SD for pre and post training of platelet activation parameters in different study groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Control (n=9)</th>
<th>Continuous (n=7)</th>
<th>Intermittent (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet (CD62p) (Gated %)</td>
<td>Pre-training</td>
<td>1.07 ± 0.79</td>
<td>1.16 ± 0.82</td>
<td>1.30±0.98</td>
</tr>
<tr>
<td></td>
<td>Post training</td>
<td>1.18 ± 0.78</td>
<td>0.83 ± 0.35</td>
<td>0.55±0.34</td>
</tr>
<tr>
<td>Platelet (PAC-1) (Gated %)</td>
<td>Pre-training</td>
<td>0.98 ± 0.64</td>
<td>1.13 ± 0.75</td>
<td>0.89±0.60</td>
</tr>
<tr>
<td></td>
<td>Post training</td>
<td>1.12 ± 0.84</td>
<td>1.13 ± 0.80</td>
<td>1.13±1.47</td>
</tr>
</tbody>
</table>

According to Macchi et al., (2002), exercise induces platelet activation via significant enhancement of P-selectin expression from the alpha granules and Weibel-Plade bodies. Normally, exercise-induced changes in platelet activation occur as a result of endogenous release of ADP and epinephrine (Kestin et
Exercise also noted to enhance the platelet activity that is accelerating the haemostatic plug, which could turn to thrombosis. In this study, the platelet activation observed based on the gated percentage of CD62p and PAC-1. Percentage of gated CD62p, showed a tendency to decline in both the exercise groups. This indicated that regular exercise lead to less activated platelets. Smith (2003) also supported that the platelet become 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 (Wang et al., 1994). This might be due to increase of epoprosstenol, which is a potent platelet aggregation inhibitor and arise of nitric oxide having a potent anti-platelet effect (El-Sayed et al., 2004).

The activation of the platelet during exercise also may due to the release of plasma catecholamine. Sedentary people tend to have more activated platelet due to greater release of catecholamine during exercise compared to physically active people (Kestin et al., 1993). 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.

Nevertheless, our study found that the PAC-1 was slightly increased in the intermittent group. PAC-1 is monoclonal antibody, which appears to be specific for the fibrinogen-binding site on platelet GP IIb/IIIa. Biologically, if there are decreased of CD62p-selectin activation, there are suppose to decrease the gated percentage of PAC-1, which is notice less activation of GP IIb/IIIa. We assumed that this pattern occurred possibly due to the non-specific binding because we did not use the RGDS. RGDS is alpha chain tetrapeptide, Arg-Gly-Asp-Ser, which can be able, inhibit fibrinogen binding to ADP-stimulated platelet to prevent the aggregation (Bennett et al., 1988). RGDS have inhibitory effect on the peptides, which is specify that they interact with GP IIb/IIIa in a mutually exclusive manner. Mutually exclusive binding suggests that either the alpha or gamma chain peptides bind to identical or overlapping sites on the GP IIb/IIIa complex or that one peptide induces a change in the complex. Recently the effects of exercise on the platelet activation have produced conflicting results and the exact effect on platelet activation still undetermined. Moreover, different studies performed different intensity and types of training programs, and others platelet activation marker (platelet factor 4, thromboxane and β-thromboglobulin).

Regular physical activity is a part of healthy lifestyle that is known to reduce risk of cardiovascular disease. Training-related adaptation is expected to improve the coagulation and fibrinolytic profile. Our study found a beneficial effect of continuous as well as intermittent type of training in improving the VO2 max, but failed to produce any significant variation in fibrinolytic profile and platelet activation variables. This might be due to the fact that a longer training period might be necessary to get the significant variation in coagulation variables. Further study on this aspect is necessary.

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