

# DIURNAL VARIATION OF HAEMOSTATIC RESPONSE TO EXERCISE IN YOUNG SEDENTARY MALES

■ Accepted  
for publication  
05.08.2012

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**ABSTRACT:** The aim of this study was to evaluate diurnal variations in the haemostatic response to submaximal exercise performed by young, sedentary men. Fifteen healthy young sedentary males aged  $25.6 \pm 1.34$  (mean  $\pm$  SD) years performed two exercise sessions, morning and evening, at 70% of maximal oxygen consumption ( $VO_2\max$ ) on a cycle ergometer for 30 min. Platelet count (PC), activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen, tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) activity were measured as dependent variables. Exercise produced significant increases in PC and fibrinogen for both sessions ( $P \leq 0.05$ ), which returned to the resting values after recovery only in the evening session. APTT and PT shortened immediately after exercise, which remained after recovery for both sessions ( $P \leq 0.01$ ). Exercise presented significant increases in tPA activity ( $P \leq 0.001$ ), which returned to the baseline after recovery in both exercises. PAI-1 activity was significantly higher during the morning than evening ( $P \leq 0.05$ ), but no longer demonstrated exercise-related changes. It was found that exercise caused activation of both coagulation and fibrinolysis processes, partly related to the time of the day when the exercise was performed.

**KEY WORDS:** diurnal variation, coagulation, fibrinolysis, thrombus

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## INTRODUCTION

Haemostasis or hemostasis is a process which causes bleeding to stop, meaning to keep blood within a damaged blood vessel. Most of the time this includes blood changing from a liquid to a solid state. Intact blood vessels are central to moderating blood's tendency to clot. The endothelial cells of intact vessels prevent blood clotting with a heparin-like molecule and thrombomodulin and prevent platelet aggregation with nitric oxide and prostacyclin [2].

A number of studies have shown that acute physical exercise resulted in activation of the haemostatic system, which may trigger acute myocardial infarction [7,23,31]. Although evidence has shown that physical exercise alters platelet count (PC) [1,22], coagulation [18,23] and fibrinolysis, the variability of methods, exercise protocols and population (gender, age, healthy or patient, trained or sedentary) used in these investigations makes an assessment of the dimension and importance of these changes difficult [11,19,28]. In addition, the time of day as an important effective factor has been less discussed [1,28].

A wide range of physiological parameters such as body temperature and heart rate, haematological parameters such as haematocrit,

homeostasis and white blood cell and total cholesterol have a daily rhythm [2,16]. Circadian variation in resting haemostatic marker profiles have been reported previously, including platelet hyperactivity, hypercoagulability, hypofibrinolysis, increased blood viscosity and vascular spasm in the morning [2,24,25]. Also, cardiac related problems, which frequently occur during the morning [2], coincide with changes in haemostatic variables [25,26]. It may be hypothesized that exercise-induced haemostatic system activity is influenced differently at different times of the day [1]. However, although large studies have investigated diurnal variations in haemostatic profiles at rest, little is known concerning the diurnal variations in haemostatic response to submaximal exercise. Therefore, in this study first we investigated the effect of submaximal exercise on PC, coagulation and fibrinolytic responses. Then, we compared PC, coagulation and fibrinolytic changes during morning and evening exercise sessions.

## MATERIALS AND METHODS

**Subjects.** Fifteen healthy young sedentary males volunteered for this study after approval of the protocol by the Research Ethics Commit-

tee of the University of Mohaghegh Ardabili. These volunteers gave consent to participate after an explanation of the study and the measurements needed. The participants were non-smokers and they had no history or clinical signs of any disease. They were not taking any medication known to affect platelet, blood coagulation or fibrinolysis, e.g. aspirin, at least 2 weeks before exercise sessions.

#### *Study design*

Each participant made four visits to the Department of Physical Education and Sport Sciences Laboratory of the University of Mohaghegh Ardabili. During visit one, subjects were familiarized with the laboratory environment, test protocol, and blood sampling procedures. Then, the exercise tests were performed in three separate sessions with intervals of at least four days over a period of two weeks. The second visit included an incremental exercise test to measure maximal oxygen consumption ( $\dot{V}O_2\text{max}$ ) and maximal heart rate (HR<sub>max</sub>), and also the measurements of height, body mass, and body mass index (BMI). The third and fourth visits were the submaximal exercise sessions at 70% of their  $\dot{V}O_2\text{max}$ , one in the morning and one in the evening. The order of the sessions was randomized and counterbalanced. All morning testing was directed between 7:30 and 8:30 AM, and evening testing was directed between 5:30 and 6:30 PM. To minimize the effect of food ingestion on the blood parameters, in the morning session, fasting conditions (12 hours) were standardized for all subjects. In the evening session, volunteers arrived at the study centre at 12:00 PM to receive an adapted meal (~900 kcal; percentage of dietary energy: 15% protein, 30% lipid, and 55% carbohydrate) [7] and to rest for 5 h. Instructions before an evening session were to refrain from eating at least 5 hours before the testing session and to refrain from ingesting caffeine after 10 AM. Subjects were also instructed not to perform any training or other vigorous physical activities on the day prior to that when measurements were done. Standard environmental conditions (20–21°C ambient temperature, 55–65% relative humidity) were ensured during the exercises [1].

#### *Blood sampling*

Blood samples were drawn without stasis at rest, immediately after exercise and after 30 min of inactive recovery from an antecubital vein with subjects in the seated position. Pre-exercise and post-recovery samples were obtained after 30 minutes of sitting rest. Blood samples for determining haematocrit, haemoglobin and PC were collected into 5 mL vials containing 50  $\mu\text{L}$  of 15% K3-EDTA. Blood samples for determining blood coagulation and fibrinolytic variables were collected in 1.8 mL vials containing 3.8% sodium citrate. Within 30 min of phlebotomy, these blood samples were centrifuged for 20 min at 3,000 g and 4°C. All samples stored at –80°C until analyses.

#### *Maximal graded exercise test*

An incremental exercise protocol was performed on a cycle ergometer in order to determine  $\dot{V}O_2\text{max}$  and HR<sub>max</sub>. After a 5 min

warm-up period at a workload of 150 W, the workload was increased by 30W every 3 min until exhaustion. The  $\dot{V}O_2$  was measured at 10-second intervals using online computer-assisted circuit spirometry (Ganshorn Medizin Electronic GmbH Power Cube-Ergo, Germany), which had been calibrated before each subject according to the manufacturer's instructions.  $\dot{V}O_2\text{max}$  was recorded as the highest  $\dot{V}O_2$  value reached during the incremental test.  $\dot{V}O_2\text{max}$  was confirmed when three or more of the following criteria were met: (a) a plateau in  $\dot{V}O_2$  despite an increase in running speed; (b) a respiratory exchange ratio (RER) higher than 1.20; (c) a heart rate within 10 beats·min<sup>-1</sup> of its predicted maximum; and/or (d) visible subject exhaustion. The physiological data obtained from the maximal test were used for estimation of the relative workload corresponding to 70%  $\dot{V}O_2\text{max}$  for each subject.

#### *Submaximal exercise sessions*

The two submaximal sessions were performed on the cycle ergometer for 30 minutes. The exercise protocol consisted of a 5 min warm-up period in which workload was increased progressively corresponding to 70%  $\dot{V}O_2\text{max}$ , and a 25 min period at a constant workload corresponding to 70%  $\dot{V}O_2\text{max}$ .

#### *Haemodynamic variables*

After at least 30 min of sitting rest in a quiet, temperature-controlled room, resting heart rate and brachial blood pressures were measured with heart rate and blood pressure testing devices (Polar Electro 5610, Finland; Beurer, bm 58, Germany), in the morning with subjects in a fasted state (12 hours).

#### *Blood analysis*

Haematocrit, haemoglobin and PC were measured using a blood cell counter (SYSMEX K 1000, Germany). Activated partial thromboplastin time (aPTT) and prothrombin time (PT) were determined using APTT-XL (Fisher Diagnostics, Middletown, USA); Thromboplastin D (Fisher Diagnostics, Middletown, USA). Fibrinogen was measured by the modified Clauss method (Mahsayaran, Iran). Plasma tissue plasminogen activator (tPA) activity (Zymutest tPA Activity, Hyphen Biomed, Neuville-Sur-Oise, France) and plasminogen activator inhibitor-1 (PAI-1) activity (Zymutest PAI-1 Activity, Hyphen Biomed, Neuville-Sur-Oise, France) were determined by enzyme-linked immunosorbent assays (ELISAs). Plasma tPA activity is expressed in international units (IU) and PAI-1 activity in arbitrary units (AU). One AU is defined as the amount of PAI-1 that inhibits 1 IU of human tPA per millilitre of plasma.

#### *Statistical analyses*

Results were expressed as means  $\pm$  SD. Morning and evening values were compared statistically using paired Student's t test. The statistical analysis of the responses to exercise was performed using two-way repeated-measures ANOVA to compare PC, coagulation and fibrinolytic parameters at rest, immediately after exercise and after

30 min of recovery during morning and evening tests. When the analysis of variance revealed a significant effect, Bonferroni's test was used for post hoc testing to determine the statistical significance, and also the partial eta squared test was used to determine the effect size (ES) of the independent variables (exercise and time of day) on the dependent variables. All statistical tests were performed at the 0.05 level of significance.

**RESULTS**

Baseline anthropometric, haemodynamic and cardio-respiratory fitness characteristics of the participants are presented in Table 1.

Percent changes in plasma volumes were estimated from the haematocrit and haemoglobin values [10]. The ANOVA indicated

**TABLE 1. PHYSICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE PARTICIPANTS**

n	15
Age (year)	25 ± 1.3
Height (cm)	176.4 ± 2.9
Body mass (kg)	71.8 ± 5.1
Body mass index (kg · m <sup>-2</sup> )	23 ± 1.5
Body fat (%)	12.8 ± 1.7
Heart rate (beats · min <sup>-1</sup> )	73.7 ± 3.7
Systolic BP (mmHg)	118 ± 4.8
Diastolic BP (mmHg)	79 ± 6.5
Mean arterial BP (mmHg)	92 ± 5
VO <sub>2</sub> max (ml · kg <sup>-1</sup> · min <sup>-1</sup> )	38.5 ± 4.3

Note: The values are mean ± SD; n, no. of subjects; BP, blood pressure; VO<sub>2</sub>max, maximal oxygen consumption.

**TABLE 2. PLATELET COUNT (PC) DURING EXERCISE AND RECOVERY**

	Resting	Post exercise	Recovery
PC			
a.m. (×10 <sup>9</sup> · l <sup>-1</sup> )	227 ± 19	304 ± 36 <sup>a,b</sup>	264 ± 27 <sup>a,b</sup>
p.m. (×10 <sup>9</sup> · l <sup>-1</sup> )	223 ± 16	284 ± 22a	237 ± 26

Note: The values are mean ± SD; the values with superscript letters (a, compared with baseline values; b, compared between two times of day in the same period) show significant differences at p ≤ 0.05; n = 15.

**TABLE 3. COAGULATION VARIABLES DURING EXERCISE AND RECOVERY**

	Resting	Post exercise	Recovery
PT			
a.m. (s)	11.2 ± 0.1	10.8 ± 0.1 <sup>a,b</sup>	10.9 ± 0.1 <sup>a,b</sup>
p.m. (s)	11.3 ± 0.1	10.9 ± 0.1 <sup>a</sup>	11 ± 0.1 <sup>a</sup>
APTT			
a.m. (s)	32 ± 1.5 <sup>b</sup>	28.2 ± 1.2 <sup>a,b</sup>	28.5 ± 1.4 <sup>a,b</sup>
p.m. (s)	33.4 ± 2.3	30.1 ± 1.7 <sup>a</sup>	30.1 ± 1.4 <sup>a</sup>
Fibrinogen			
a.m. (g · l <sup>-1</sup> )	2.4 ± 0.4	3 ± 0.4 <sup>a,b</sup>	2.8 ± 0.4 <sup>a,b</sup>
p.m. (g · l <sup>-1</sup> )	2.2 ± 0.2	2.6 ± 0.4 <sup>a</sup>	2.3 ± 0.3

Note: Values are mean ± SD; PT, Prothrombin time; APTT, activated partial thromboplastin time. The values with superscript letters (a, compared with baseline values; b, compared between two times of day in the same period) show significant differences at p ≤ 0.05; n = 15.

significant main effects of exercise (P ≤ 0.001, ES = 0.7) and time of day (P ≤ 0.036, ES = 0.22) for PC, which were corrected for plasma volume changes. Exercise produced significant increases in PC for both sessions. The increased PC returned to resting values after recovery only in the evening session. After exercise and after recovery, PC was larger in the morning than the evening session (Table 2).

The ANOVA found significant main effects of exercise (P ≤ 0.001, ES = 0.75) and time of day (P ≤ 0.016, ES = 0.28) for aPTT. Exercise produced significant shortening in aPTT, which remained after recovery in both sessions. Resting, after exercise and after recovery, aPTT was higher in the evening than the morning session (P ≤ 0.05). Exercise and time of day presented significant effects for PT (P ≤ 0.001, ES = 0.81; P ≤ 0.031 ES = 0.23, respectively). Exercise produced significant reductions in PT, which remained after recovery in both sessions. After exercise and after recovery, PT was lower in the morning than the evening session (P ≤ 0.05). Exercise and time of day exhibited significant main effects for plasma levels of fibrinogen (P ≤ 0.001, ES = 0.47; P ≤ 0.033, ES = 0.23, respectively). Exercise showed significant increases in fibrinogen for both sessions. The increased plasma fibrinogen levels returned to the resting values after recovery only in the evening session. Fibrinogen was not different at resting values at two different times of the day. Post-exercise and post-recovery fibrinogen values were significantly different (p ≤ 0.05), with evening fibrinogen values being lower (Table 3).

The ANOVA indicated significant main effects of exercise (P ≤ 0.001, ES = 0.86) and time of day (P ≤ 0.024, ES = 0.25) for tPA activity. Exercise presented significant increases in tPA activity for both sessions, which quickly returned to the resting values after recovery. Although tPA activity levels were greater during the evening than the morning session, this was significant after exercise only (P ≤ 0.05). The time of day showed significant main effects for PAI-1 activity (P ≤ 0.009, ES = 0.32). Resting, post-exercise and post-recovery PAI-1 activity was higher in the morning sessions compared with the evening sessions. PAI-1 activity did not change with exercise for either session (Table 4). There was no interaction effect of exercise and time of day for any parameter.

**TABLE 4. FIBRINOLYTIC VARIABLES DURING EXERCISE AND RECOVERY**

	Resting	Post exercise	Recovery
tPA			
a.m. (IU · ml <sup>-1</sup> )	3.6 ± 0.3	5.17 ± 0.3 <sup>a,b</sup>	3.8 ± 0.3
p.m. (IU · ml <sup>-1</sup> )	3.8 ± 0.4	5.54 ± 0.4 <sup>a</sup>	4.13 ± 0.4
PAI-1			
a.m. (AU · ml <sup>-1</sup> )	3.4 ± 0.4 <sup>b</sup>	3.6 ± 0.3 <sup>b</sup>	3.5 ± 0.3 <sup>b</sup>
p.m. (AU · ml <sup>-1</sup> )	3.1 ± 0.2	3.2 ± 0.3	3.1 ± 0.2

Note: The values are mean ± SD; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1, The values with superscript letters (a, compared with baseline values; b, compared between two times of day in the same period) show significant differences at p ≤ 0.05; n = 15.

## DISCUSSION

This study evaluated the haemostatic responses to submaximal exercise performed by young, sedentary males and also compared the responses in the morning and evening. One main finding of the present study was that both exercises stimulate both coagulation and fibrinolysis. During recovery, fibrinolytic activity returned quickly to the baseline values, while the coagulation activity was maintained. The other main finding of this study was that PC and coagulation activity were higher during morning than evening exercise. But, the net activity of fibrinolysis was greater in the evening sessions compared with morning sessions. Although similar changes of the haemostatic system have been reported following exercise [1,28], this is the first study to investigate changes of both coagulation and fibrinolysis activity, simultaneously, at rest and following exercise at different times of the day.

There is now a large and impressive literature showing that regular physical activity reduces coronary and cardiovascular morbidity by improving an individual's haemostatic profile both at rest and during exertion [27,30]. However, there is a paradox, as acute physical exertion caused the prothrombotic state and therefore it has been associated with an increased risk of cardiovascular events [23,30,31]. Of course, some studies reported that exercise alters coagulation and fibrinolytic parameters in a normal range [19]. On the other hand, chronobiology science has started to evaluate the effects of time on a wide range of biological parameters [16,25] and also evaluated the changes caused by exercise on parameters at different times of day [1,25,28]. Especially from the haematological point of view, diurnal rhythm changes of haemodynamics such as heart rate and blood pressure [2,16,25] and haemostatic variables such as tPA and PAI-1 have been well documented [20,28]. A large number of these studies demonstrated that the best time of day for safety is in the afternoon and evening [16,28]. One study even suggested that people ought to avoid doing exercise early in the morning [16].

In this study, we observed essentially the same number of platelets from baseline samples. A significant increase in platelet numbers was observed after both exercises, which was independent from plasma volume changes. This post-exercise increase is reported to be due to a fresh release of platelets from the spleen, bone marrow and lungs [1,11]. The temperature-induced vasodilatation can be considered as a stronger induction of young platelet release from its stores [1]. Although mechanisms explaining the higher post-exercise and post-recovery levels of PC in the morning than evening exercises are not known, circadian rhythm may have an important role. Platelet aggregation is dependent on the PC. Therefore, a standardized PC is an important parameter to take into account, especially because blood PC increases with exercise [7]. Platelets play a central role in maintaining haemostasis and must be present in adequate numbers and have normal function.

The increase in coagulation activity by a rise in fibrinogen accompanied by shortening of aPTT and PT after exercise and persist-

ing during the recovery period is not so easily explained. Several mechanisms could play a role in the changes that we observed in the activation of the intrinsic and extrinsic pathways during both exercises. aPTT is a screening assay used to detect deficiencies in the intrinsic pathway of coagulation with specific sensitivity to factors VIII, IX, XI and XII [29]. The shortening of aPTT was largely determined by the increase in factor VIIIa, which is strongly related to von Willebrand factor and adhesive protein that is released from endothelial cells during exercise [18,31]. Also, the diminished liver blood flow during exercise could prolong the clearance of blood clotting factors [7]. In addition to the observed changes in coagulation, the enhanced shear stress and shear-induced platelet aggregation, known to occur during exercise, might potentiate the risk for the development of thrombosis [30]. The observed aPTT results were in agreement with those of Peat *et al.* [23].

Less well documented are the PT changes during exercise, and few investigators have observed a detectable change in PT. Our findings are in agreement with those of Van Den Burg *et al.* [31], who noted a small but significant shortening in PT. The PT is used to detect deficiencies in the extrinsic and common pathway of coagulation. Specifically, the PT is shortened if there is an increase in factor II, V, VII, X and/or fibrinogen [29]. The shortened PT may also be related to the increased body temperature [4].

The increased blood coagulation activity may also be related to the observed increased concentration of circulating blood coagulation factor fibrinogen [7]. Plasma fibrinogen, the circulating precursor of fibrin, is a major independent risk factor for atherosclerotic cardiovascular diseases [14]. Previous studies reported that plasma fibrinogen levels increased after exercise [7,22]. The present findings confirmed these earlier observations. Fibrinogen is an acute phase reactant protein. Elevated fibrinogen is an indication that an acute phase response is occurring that may lead to increased levels of factor VIII, von Willebrand factor and PAI-1. Increased fibrinogen levels are triggered by catecholamine release as well as by local ischaemic effects that occur during exercise, which may accelerate the pre-existing prothrombotic potential of the atherosclerotic vessel wall [21]. Also, fibrinogen genotypes may interact with physical activity in determining the variations in fibrinogen levels [6].

The observed coagulation diurnal rhythm at rest (only aPTT), immediately after exercise and after recovery may be related to the circadian variations of haemostatic variables [2,25] as well as blood fluidity [16]. Also, it is revealed that vascular endothelial function has a 24-h variation [26]. The vascular endothelium has a direct role in the haemostatic system. In addition, several studies have suggested that postprandial and fasting lipoproteins are associated with plasma levels or activation state of coagulation factors, and particularly of FVII, which plays a key role in the initiation of the clotting cascade [17,24].

The responsible system for contending against increased coagulation is the fibrinolytic system. The fibrinolytic system plays an important role in regulating the formation and removal of thrombi.



Fibrinolysis is initiated by the release of tPA from vascular endothelial cells. tPA, in the presence of fibrin, converts plasminogen to plasmin, which in turn lyses fibrin in the thrombus. The activity of tPA in circulating blood is regulated by the secretion of tPA by the vascular endothelium, clearance of tPA by the liver and the inhibition of tPA by PAI-1 [12]. Decreased fibrinolytic activity has been associated with an increased risk of arterial thrombosis [20]. Increased tPA activity after exercise [19,28], returning quickly to baseline values during recovery, have been reported previously [13]. The processes responsible for the increase in fibrinolytic potential during exercise may comprise an increase in the release of tPA from vascular endothelium as well as a reduction in the clearance by the liver due to reductions in liver blood flow [28,31]. Also, various stimuli such as catecholamines [3] and shear stress have been demonstrated as mechanisms responsible for increase in the release of tPA [15]. In addition, Björkman et al. [3] demonstrated that stimulation of cardiac sympathetic nerves induces a marked coronary release of tPA, and part of this response may be mediated through stimulation of  $\beta$ -adrenergic receptors. However, the regulation of synthesis and secretion is far from clear.

The mechanisms responsible for the greater increase in fibrinolytic activity during the evening exercise have not yet been completely resolved, although it is most likely due to the underlying di-

urnal variations at rest [28]. PAI-1 is hypothesized to be the major regulator of diurnal variations. Fibrinolytic activity in blood follows a circadian rhythm. Peak levels of PAI-1 activity occur in the morning, with the lowest levels occurring in the evening [20]. It was reported that PAI-1 production is regulated by the molecular clock, and this relationship is in turn modulated by metabolic factors such as glucose [9]. Also, the renin-angiotensin-aldosterone system appears to regulate PAI-1 production, at least in part, via effects on the peripheral clock transcriptional machinery [5]. The higher tPA activity observed during evening exercise may in part be explained by the lower PAI-1 activity, resulting in fewer tPA-PAI-1 complexes and thus more active tPA [8].

## CONCLUSIONS

Exercise resulted in activation of both coagulation and fibrinolysis processes. But during recovery, fibrinolytic activity decreased quickly to the baseline values, while the coagulation activity was maintained. In addition, the time of day plays an important role in exercise-induced haemostatic changes. PC and coagulation activity were higher during morning than evening exercise. But, the net activity of fibrinolysis was greater in the evening sessions compared with morning sessions.

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