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The Effect of Acute Training and Circadian Rhythm on Blood Hemostasis in Female Athletes

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Abstract

Background: Circadian rhythm and physical activity are factors that influence the homeostasis of blood. This study aimed to investigate the effect of exhaustive exercise in the morning and evening on the blood hemostasis in female athletes.

Methods: In the present quasi-experimental study, 30 female athletes aged 18-25 were selected by convenience sampling method and randomly divided into two groups (morning and afternoon exercises). The standard Bruce protocol test was used. In the present study, platelets, fibrinogen, and thromboplastin time were measured as indicators of blood coagulation before and after testing. Paired t-test and covariance analysis were used to analyze the measured indices and $P \leq 0.05$ was considered significant.

Results: An acute exhausting aerobic training session in both groups significantly increased platelet and fibrinogen levels, but a significant decrease was observed in thromboplastin time. Considering the training time, significant difference was observed in the blood thromboplastin time in the morning in comparison with the afternoon.

Conclusion: According to the results of this study, the circadian rhythm and acute exhausting aerobic training are effective factors on the blood coagulation and a training session in the morning compared with the evening training has a greater effect on the blood coagulation.

Keywords: Acute training, Thromboplastin, Fibrinogen, Platelet

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Introduction

Cardiovascular disease is one of the most common causes of death in the world.[1] One of the principal causes of this disease is thrombosis due to the increased activity of coagulation factors and the imbalance in hemostasis.[2] Sport and physical activity is directly related to the lower incidence of cardiovascular disease. Physical activity plays an important role in the automatic control of the cardiovascular system. Increased platelet activation, reduced aggregation and platelet adhesion, increased parasympathetic control, and reduced sympathetic cardiac control have been reported after physical activity. Physical activity affects the homeostatic system and prevents heart disease.[3] However, a number of studies have reported that acute physical exercise can increase the activity of clotting factors, an underlying factor in the formation of thrombosis. The formation of exercise-induced coronary thrombosis is not necessarily linked to the heart disease.[4] This shows the importance of studying the process of thrombosis. Blood coagulation increases after the physical activity and remains increased for 1-24 hours.[5] A study by Peat et al showed that partial thromboplastin activated time (aPTT) decreased immediately after exercise in both active and inactive people.[4] Menzel and Hilberg investigated the effect of one aerobic training session in young and middle-aged men, and reported a significant reduction in aPTT and no change in prothrombin time (PT).[6] Lekakis et al demonstrated that a single session of aerobic exercise in patients with high blood pressure was associated with an increase

in PT and a significant reduction in aPTT.[7] Hilberg et al demonstrated a decrease of the clotting factor and an increase of the fibrinolysis power in healthy men.[8] Improving the reaction of fibrinolytic and reducing activity of coagulation were reported in elderly males following the aerobic exercise.[9] In contrast, Nikokheslat et al studied the effects of resistance exercise on hemorheological and coagulation variables. Their results showed a significant decrease in the fibrinogen levels and no change in the PT and partial thromboplastin time (PTT).[10]

Based on the available evidence, many variables and physiological responses to exercise are under the influence of time and hourly changes of day and night over a 24-hour period.[11] Most of the time interactions of living organisms, including humans, follow circadian rhythms such as the cycle of rest and activity. Circadian rhythms are light and dark cycles. These rhythms also have 24-hour periods during consistent circumstances such as dark/dark cycles that begin through internal mechanisms which are called the circadian clock. Our circadian rhythms were circadian fluctuations in behavior and physiology that were essential to maintain homeostasis in organisms through molecular genetics mechanisms, produced as circadian hours. This rhythm pattern is produced in the core circadian oscillator located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Some evidence shows that the coordination of circadian physiological and cellular interactions affects the health of the organism.[12-14] Cardiovascular disease and thromboembolism reggae influences the circadian fluctuations, which may be closely associated with the biological clock. It seems that many rhythmic cycles, endothelial function, platelets, and the concentration and activity of several clotting proteins regulate the circadian fluctuations. Nonetheless, it is still unknown how homeostatic efficiency is influenced by the circadian cycle.[15]

Some studies confirm the existence of circadian rhythms in fibrinogen, PT, and PTT,[16,17] while some have stated that fibrinogen and platelets do not have such rhythms.[17,18] Some research has reported that the circadian rhythm is effective on the fibrinogen and plasminogen.[19,20] In a study by Kanabrocki et al, platelet and fibrinogen aggregation were reported in the afternoon and in the morning, respectively.[21] Yasuda et al stated that the thromboplastin time had a significant reduction in two of the four intervals studied.[22] It seems that platelets also have circadian rhythms. It has also been reported that fibrinogen follows a circadian rhythm similar to the blood pressure.[23]

The importance of blood hemostasis and the role of physical activity and circadian rhythm inspired the present study to investigate the effect of acute training and circadian rhythm on the blood hemostasis in female athletes.

Methods

Sample Selection

Having filled out the personal and health information forms, the volunteers were assessed and 30 subjects were found to be appropriate, according to inclusion criteria. The inclusion criteria for the study were: athlete girls aged 18-25 years, presence in the city sports teams, regular weekly training programs, and a history of regular exercise of at least three years. The selected participants were randomly divided into two groups: morning training (20.87 ± 2.29 years old; 66.53 ± 3.83 kg), and afternoon training (21.13 ± 2.32 years old; 65.67 ± 4.99). Weekly regular training programs and at least three years history of regular exercise were also required factors.

Training Protocol

In this study, the subjects were asked to refrain from strenuous physical activity 24 hours before the intervention. Their sleep and nutritional status was controlled for 48 hours prior to the intervention as well. Blood samples were taken from the participants to determine blood factor levels in the morning and evening, before and after the test. Acute aerobic training was performed using the standard Bruce protocol test. Assessments and activities were conducted at 8:00-10:00 and 16:00-18:00 in the morning and evening groups, respectively. After the pretest, the research groups completed intense activity, following15 minutes of rest to determine posttest variables. Activities were carried out in the same day in both groups. In this study, to control the monthly menstrual cycle, the subjects were studied in a period of one week.

Measurement of Biochemical Indicators

To obtain the blood samples, a tourniquet was placed on the participants' arm and 2.0 mL of venous blood was drawn within less than one minute. The blood samples were then placed in plastic vials containing 20 µL of EDTA-K2 solution. The vials were transferred to the laboratory for analysis in less than 30 minutes. At the end of the study, blood samples were analyzed using ELFA method with bioMerieux laboratory kits (bioMérieux, France) and Vidas computer device.

Statistical Methods

In this study, paired *t* test was used to assess the changes within the groups, and analysis of covariance (ANCOVA) was used to compare the groups of research. All results were shown as mean ± SD and P values <0.05 were considered statistically significant.

Results

In this study, the effect of exhaustive exercise on the blood homeostasis of female athletes was investigated in the morning and afternoon. Table 1 shows the demographic and anthropometric indices of the groups in the pre-test. The results of intra-group changes in blood homeostasis parameters are stated in Table 2. Dependent *t* test results in Table 2 indicate the significant changes in levels of platelets, fibrinogen, and thromboplastin time after the aerobic exercise in the studied groups (exercises in the morning and evening).

The results of ANCOVA to compare the groups of exercises in the morning and evening (Table 3) showed a sig-

Table 1. Anthropometric indices in studied groups

	Age	Height	Weight
Morning activity	20.87 ± 2.29	176.33 ± 5.49	66.53 ± 3.83
Evening activity	21.13 ± 2.32	174.80 ± 4.12	65.67 ± 4.99

Table 2. Pretest and Posttest *T* Test Results

	Activity Time	Pretest	Posttest	t	*P*
Platelets (cell/ µL)	Morning	249.13 ± 63.38	271.20 ± 74.98	3.68	0.002**
	Evening	239.20 ± 57.30	261.47 ± 61.66	4.09	0.001**
Fibrinogen (mg/dL)	Morning	244 ± 19.13	257.33 ± 22.22	3.15	0.007**
	Evening	246.33 ± 13.32	252.60 ± 14.88	2.98	0.010**
Thromboplastin time (s)	Morning	30.49 ± 4.29	25.92 ± 2.93	5.65	0.000***
	Evening	31.60 ± 4.51	29.75 ± 4.11	3.54	0.003**

*P <0.05, ** P <0.01, *** P <0.001.

Table 3. Results of ANCOVA for Research Groups in Blood Variables (Platelets, Fibrinogen, Thromboplastin)

Variable	df	Mean Square	F	*P*	Eta Squared
Platelets	1	7.10	0.015	0.90	0.001
Fibrinogen	1	336.48	2.01	0.16	0.06
Thromboplastin	1	71.18	15.05	0.001**	0.35

*P<0.05, **P<0.01

nificant difference in the thromboplastin time ($P < 0.001$), but no significant difference was observed in the platelet and plasma fibrinogen ($P > 0.05$).

Discussion

The results of the present study showed that a single session of exhausting exercise significantly increased the blood platelets in the female athletes in both morning and evening groups. The results of some studies[24-26] are consistent. Aldemir and Kiliç in their study showed that an aerobic exercise at 70% maximal oxygen consumption led to a significant increase in the number of platelets.[24] Aldemir and Kiliç showed that the number of platelets increased because of sport activities. This increase can be related to the physical activity and the release of new platelets from the spleen's vascular bed, bone marrow, and other body platelets reservoirs.[24] Studies have also shown that the secretion of epinephrine can cause strong contraction of the spleen (the source of one third of the body's stored platelets). Since during activity, especially strenuous activity, epinephrine levels increase, this could explain why platelets increase immediately after exercise. Some studies have even shown that waking up in the morning is a form of very light exercise that can cause an increase in the platelets. Furthermore, when the platelets significantly increase along with a significant change in the mean platelet volume (MPV), the results can possibly be attributed to the platelets activity and the initiation of blood coagulation in them.[26]

Moreover, in this study, a significant increase in the blood fibrinogen level in female athletes' blood samples was seen following an acute exhausting aerobic training session by both groups. These findings are in agreement with the reports by Ahmadizad et al and Prisco et al.[27,28] In contrast, the findings of some researchers (e.g., Rankinen et al[29]) were not similar to our results. These differences in fibrinogen may be due to the differences in the type and severity of the protocol.

Fibrinogen is a type of inflammatory protein that is increased in response to the inflammatory conditions. An increase in the plasma fibrinogen level can be caused by increased blood viscosity and a shift of plasma water or its production in the liver.[30] Since the intensity of the activity in this study was exhausting exercise, increased fibrinogen could have been caused by high-intensity workouts due to the body's inflammatory response. Some researchers claim that if the changes in the plasma volume be accounted for, a decrease in the fibrinogen levels can be observed after the moderate to intense exercise.[29] Because of the intensity of training in our study, plasma volume is probably decreased due to the perspiration, the transfer of plasma water into the interstitial fluid, and an increase in the blood pressure; therefore, the increase of fibrinogen is likely related to the decrease of blood plasma volume, which in turns leads to an increase in the blood concentration and fibrinogen.

The results of ANCOVA showed that there was not a significant difference in the rate of change of blood platelets (8.86% in the morning and 9.32% in the evening) and fibrinogen (5.46% in the morning and 2.55% in the evening) after intervention in an acute exhausting aerobic training session. These results are consistent with findings of Mohammad Najad Panah Kandi et al,[31] and in contrast with findings of other researchers such as Aldemir and Kiliç.[24] The effect of physical activity and its timing is a complex interaction that can occur in coagulation and platelet indices.[24] However, detailed studies on humans concerning this issue do not exist. The results of a study by Aldemir and Kiliç showed that the amount of platelets in both activity times (morning and afternoon) on the bike ergometer increased, however the increase was only statistically significant in the morning. The

MPV reduction in both activity times was not significant, however more reduction was observed after evening activity.[24] Ahmadizad and El-Sayed stated that the time of day had no significant effect on the fibrinogen and average blood pressure response to acute exercise endurance.[32] Considering the aforementioned points, platelets and fibrinogen indices seem to be hardly influenced by the circadian rhythm.

Study of the effect of the acute exhausting aerobic training in the morning and evening showed a significant difference between the thromboplastin time in the morning and in the evening. These findings are compatible with the results of many researchers, such as Menzel and Hilberg and Lekakis et al.[6,7]

In this study, a significant reduction was observed in the thromboplastin time in two exercise times (morning and evening) which was similar to the findings of Peat et al[4] and Lekakis et al.[7] The reduction of thromboplastin time was significantly higher in the morning than in the evening. Thromboplastin time is an indicator of coagulation that is much slower than PT and its mechanism begins with the damage of blood vessels and contact with collagen. Von Willebrand factor is a high molecular weight plasma protein which connects the platelets to the damaged endothelium. Therefore, the activity of endothelial cells caused by stress probably causes an increase in the Von Willebrand factor, which is also secondary to the increased levels of factor VIII, and ultimately decreases thromboplastin time.[6,7] Thromboplastin time reduction is likely to increase the risk of inappropriate blood clotting, such as thromboembolism. Given that the normal value of thromboplastin time is 25 to 39 seconds, exercising in the morning and evening is not considered a risk factor for athletes.

Conclusion

Overall, the findings of our study showed that an acute exhausting aerobic training session in the morning and evening significantly increased the platelet and fibrinogen levels and reduced the thromboplastin time. Further, our results indicated that the time of exercise in the morning and evening did not have a significant effect on the levels of fibrinogen and platelets; however it was effective on the thromboplastin time. The reduction in the amount of thromboplastin time was normal, although it was more apparent in the morning exercises. According to the findings, exercises in the morning have a greater effect on the blood coagulation time.[20,33]

Ethical Approval

In this study ethical considerations including informed consent in writing of the subjects was observed. Also the exercise protocol was performed in the presence of a medical assistant in the event of an accident or potential injury.

Competing Interests

Authors declare that they have no competing interests.

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