Advances in Biology & Earth Sciences
Vol.2, No.1, 2017, pp.65-72

THE IMPACT OF SHORT-TIME EXERCISE ON SOME OF HEMOSTATIC FACTORS IN DIFFERENT TISSUES OF RATS

Hossein Rostami[1*], Ali Aliyev[1], Valida Madatova[1], Mahsa Rostami[2] Mehri Golchin[3]

[1]Department of Physiology, Faculty of Biology, Baku State University, Baku, Azerbaijan
[2]Department of Dentistry, Faculty of Dentistry, University of Ataturk, Erzurum, Turkey
[3]Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract. This study was designed to determine the effects of physical exercise on thrombin time and coagulation time in male rats. Male Wistar rats (n=40) weighing 90-100 gr were divided into two groups; control group (without exercise) and experimental group (exercise). In each group animals were divided into two subgroups: 30 days old rats and 90 days old rats . Blood samples were collected from rat tail tip in several stages as before and after physical exercise from experimental group and control group, to determine of coagulation time. Exercise programs included; swimming on water pool until five minute in experimental groups were performed. All of the rats were done autopsy. Thrombin time was measured for each tissue. Our data showed that in 30 days old rats, physical exercise significantly decreased thrombin time on all of tissues as compared baseline values in control group ($P<0.001$). In contrast, effect of exercise in 90 days old rats was not showed the same changes. In conclusion, these results suggest that there is a functional relationship between the exercise on changes of haemostatic parameters in different tissues.

Abbreviations: **CT**: coagulation time, **TT**: thrombin time, **EX**: exercise, **NEX**: without exercise

Keywords: *Thrombin time, coagulation time, short-time exercise, rats, circadian rhythm.*

Corresponding Author: *Hossein Rostami, Department of Physiology, Faculty of Biology, Baku State University, AZ1148, Z. Khalilov str., 23, Baku, Azerbaijan,*
e-mail: h_rostami_tab@yahoo.com

Manuscript received: *11 March 2017*

1. Introduction

It is shown that thrombin is the primary activator of platelets at the site of thrombus formation and a major driving force in thrombus growth [3] and it is well documented that short-term exercise increases fibrinolytic activity [32]. It is known that physical activity induces modification in blood hemostasis and lead to an activation of blood coagulation and fibrinolysis [1]. In addition, several studies have shown that strenuous exercise leads to a shortening of the activated partial thromboplastin time and results in an increase of thrombin generation markers [13]. In addition, the haemostatic system is involved not only in the maintenance of the liquid state of the blood, vascular wall resistance, and the arrest of bleeding from injured vessels, but also in the regulation of, hemodynamics and vascular permeability [33].

It has been demonstrated that exhaustive exercise alters blood coagulation and fibrinolysis [14]. It has been reported that exercise induced a significant

increase in factor VIII activity with a significant shortening of activated partial thromboplastin time [5]. In addition, blood haemostasis is a complex interaction among platelets, coagulation, and fibrinolysis. According to the previous studies. The intensity of acute exercise is a critical factor affecting blood platelet function [37]. Various studies identified increased platelet counts of 18-80% immediately after treatmill or bicycle exercising [6, 8]. An interesting possibility is that thrombin is involved in the platelet activation induced by strenuous exercise. Exercise also enhances blood coagulation and fibrinolysis , as evidenced by elevated plasma levels of prothrombin fragment, and tissue plasminogen activation [25]. The increase in clotting and fibrinolytic activity due to exercise has been widely documented in humans, both for maximal and near maximal effects, the increased fibrinolytic activity appears in counter balance the exercise-induced increase in coagulability [27]. Few investigation exist on the relationship between the exercise and changes of coagulation time and thrombin time. Thus, the objective of this investigation was to examine effects of physical exercise on changes of coagulation and thrombin time in male rats.

2. Materials and methods

2.1.Animal care and selection

Male Wistar rats (n=40), weighing about 90-100 gr were used in these experiments. The animals were housed at an ambient temperature of 25 ± 2º under a 12 h/12 h light-dark cycle and acclimated to these conditions for 10 days before use in experiments. All rats had free access to standard feed and water. Animals were used under ethical approval of department.

2.2. Experimental design and animal grouping

Animals were divided into two groups of 20 rats in each group. Control group (without exercise) and experimental group (exercise). In each group rats were divided into two subgroups of ten: 30 days old rats, and 90 days old rats. Blood samples were collected from rat tail tip in several stage as before physical exercise and after physical exercise from experimental group, to determine of coagulation time [21].

2.3. Physical exercise procedure and swimming test

Rats were put in the centre of a plastic pool (80 * 60* 30 cm) with vertical walls, filled with 20 cm of water at 25 degree centigrade. This large pool dimension is more suitable for mice testing [20]. Then rat were observed for 5 minute.

2.4. Determination of thrombin time in tissues

In experimental groups, exercise programs included; swimming on water pool until five minute. Rats were killed and then, all of the animals were done autopsy. Blood, liver, spleen, heart muscle, lung, brain, kidney, skeletal muscle and intestine tissues were isolated. After isolation of mentioned tissues, were weight on calibrated and accurate scale 500 mg from each tissue was detached and was

crushed on special mortar. Detached tissue mixed with five milliliter physiological serum solution (NaCl). After complete crushed of each tissue, samples of prepared solution tissues and blood were poured on natrium oxalated test tube. Then, this test tubes centrifuged with a 1500 round for twenty minute. Thrombin time was measured for each tissue after prepared plasma from mentioned tissue, and documented [10].

2.5. Statistical analysis

All results were expressed as the mean ± SD, with the range in parentheses. An independent Student' s t- test was used to analyze all the parameters (Statistical software, Stat Soft). ANOVA was used to compare means of more than two independent groups. Statistical significance was attained at $p < 0.05$.

3. Results

The changes of thrombin time responses to physical exercise in 30 day old rats, are presented in table one. Our results showed that thrombin time (TT) on different tissues significantly decreased in experimental (EX) group as compared to control group, without physical exercise (NEX) ($p < 0.001$). Our data clearly demonstrated that greatest decrease on TT was observed in skeletal muscle tissue (from 39.7 ± 0.35 to 4.4 ± 0.21) and minimum decrease on TT was observed in spleen tissue (from 21.1 ± 0.9 to 19.5 ± 0.41). Also, in 90 day old rats, our data in table two showed that exercise significantly decreased TT on difference tissues in experimental group as compared to control group ($p < 0.001$) and greater decrease was observed in blood tissue (from 42.3 ± 2.17 to 14.01 ± 0.13), minimum decrease on TT was observed in kidney tissues (from 17.02 ± 0.23 to 13.9 ± 0.48) and conversely increased TT on the other tissues e.g. lung, brain, skeletal muscle and intestine in experimental group as compared to control group ($p < 0.001$). In addition, as shown in table three, TT on different tissues significantly decreased especially on brain tissue in 90 day old rats (23.01 ± 0.31) without physical exercise as compared to 30day old rats (46.5 ± 1.5) ($p < 0.001$), and minimum decrease on TT was observed in spleen tissue in 90 day old rats (18.7 ± 1.2) without physical exercise as compared to 30 day old rats (21.1 ± 0.9). On the contrary, TT on blood and heart muscle tissues significantly increased in 90 day old rats as compared to 30 day old rats. Also, our results in table four showed that exercise significantly increased TT on lung, kidney, skeletal muscle and intestine tissues in 90 day old rats as compared to 30 day old rats ($p < 0.001$), and greater increase was observed in skeletal muscle tissue 4.4 ± 0.21 in 30 day old rats and 22.8 ± 0.62 in 90 day old rats. In contrast, exercise significantly decreased TT on blood, liver, heart muscle, spleen and brain tissues in 90 day old rats as compared to 30 day old rats ($p < 0.001$), and greater decrease was observed in spleen tissue. According to data of table five, coagulation time (CT) significantly increased in 30 day old rats and 90 day old rats after short-time physical exercise ($p<0.001$). In addition, our data showed that, maximum increase was in 90 day old rats as compared to 30 day old rats. Interestingly, decreasing of CT in 90 day old rats as compared to 30 day old rats in control group, was notable ($p < 0.01$).

4. Discussion

The results obtained in the present investigation suggest that physical exercise significantly decreased TT on 30 day old rats and exercise had a more decreasing effect on skeletal muscle tissue (Table 1).

Table 1. Effect of exercise on thrombin time (second) in different tissues in 30 days old male rats.

Group / Tissue	NEX		EX		P
	Mean	SD	Mean	SD	
Blood	29.5	1.23	21.3	0.59	< 0.01
Liver	41.1	1.6	16.2	0.52	< 0.001
Heart Muscle	20.2	0.53	17.01	0.14	< 0.01
Spleen	21.1	0.9	19.5	0.41	< 0.05
Lung	20.1	0.42	17.1	0.5	< 0.05
Brain	46.5	1.5	27.2	0.68	< 0.01
Kidney	34.9	0.46	9.01	0.14	< 0.001
Skeletal Muscle	39.7	0.35	4.4	0.21	< 0.001
Intestine	25.8	0.49	8.5	0.23	< 0.001

Table 2. Effect of exercise on thrombin time (second) in different tissues in 90 days old male rats.

Group / Tissue	NEX		EX		P
	Mean	SD	Mean	SD	
Blood	42.3	2.17	14.01	0.13	< 0.001
Liver	18.6	1.28	8.7	0.23	< 0.001
Heart Muscle	30.3	1.8	10.8	0.3	< 0.001
Spleen	18.7	1.2	5.02	0.12	< 0.001
Lung	9.9	0.77	21.2	0.39	< 0.001
Brain	23.01	0.31	24.8	0.7	< 0.05
Kidney	17.02	0.23	13.9	0.48	< 0.05
Skeletal Muscle	18.6	1.05	22.8	0.62	< 0.05
Intestine	17.5	0.4	18.1	0.69	< 0.05

Exercise are known to have considerable effect on blood haemostasis [5, 36]. It is well known that physical exercise induces an activation of coagulation and fibrinolysis, but this reaction depends on the exercise type, duration and it's intensity [2, 4, 7, 15, 31]. The effect of muscular exercise on blood coagulation has been the subject of several investigations in both man and animals [17]. It is known that physical activity induces modification in blood hemostasis. Exercise induced a significant increase in factor VIII activity with a significant shortening of activated partial thromboplastin time [5]. It seems that swimming caused activation of the clotting system by increasing fibrinolytic activity [19]. It is well understood that physical activity evokes multiple effects on blood haemostasis and via reduced of inflammation and coagulation , which leads to reduced of mortality. Also, a recent study showed that anaerobic exercise accelerates blood coagulation and activates blood fibrinolytic activity [26, 35]. In this regard, it is known that as the stimulus responsible for exercise-induced increase in plasma

von Willebrand factor (vWF) and coagulation factor VIII (FIII) content seems to be mediated by b-adrenergic receptors through a nitric oxide-dependent mechanism, the haemostastic system could be conditioned by endothelial function and be modified during the aging process [16, 24]. Also, based on one marathon study, despite increased levels of B-thromboglobulin, platelet aggregation was found to be decreased after exercise [29]. Platelet activation during exercise may be related to shear stress causing endothelial damage, increase in plasma thrombin generation, catecholamines and mobilization of more active platelets from the reticuloendothelial system [30]. Based on the results presented in this study, it was observed that TT significantly decreased on liver, spleen, lung, brain, kidney, skeletal muscle and intestine tissues in 90 days old rats as compared to 30 days old rats in control group. Despite this, it was demonstrated TT significantly increased on lung, kidney, skeletal muscle and intestine tissues in 90 days old rats as compared to 30 days old rats in experimental group (Table 3, Table 4).

Table3. Thrombin time (second) changes in different tissues of control group (NEX) by age in male rats.

Group / Tissue	30 Day		90 Day		P
	Mean	SD	Mean	SD	
Blood	29.5	1.23	42.3	2.17	< 0.01
Liver	41.1	1.6	18.6	1.28	< 0.01
Heart Muscle	20.2	0.53	30.3	1.8	< 0.01
Spleen	21.1	0.9	18.7	1.2	< 0.05
Lung	20.1	0.42	9.9	0.77	< 0.01
Brain	46.5	1.5	23.01	0.31	< 0.01
Kidney	34.9	0.46	17.02	0.23	< 0.01
Skeletal Muscle	39.7	0.35	18.6	1.05	< 0.01
Intestine	25.8	0.49	17.5	0.4	< 0.05

Table 4. Thrombin time (second) changes in different tissues of experimental group (EX) by age in male rats.

Group / Tissue	30 Day		90 Day		P
	Mean	SD	Mean	SD	
Blood	21.3	0.59	14.01	0.13	< 0.05
Liver	16.2	0.52	8.7	0.23	< 0.01
Heart Muscle	17.01	0.14	10.8	0.3	< 0.05
Spleen	19.5	0.41	5.02	0.12	< 0.01
Lung	17.1	0.5	21.2	0.39	< 0.05
Brain	27.2	0.68	24.8	0.7	< 0.05
Kidney	9.01	0.14	13.9	0.48	< 0.05
Skeletal Muscle	4.4	0.21	22.8	0.62	< 0.001
Intestine	8.5	0.23	18.1	0.69	< 0.001

It is well known, significant increase in TAT concentration in association with strenuous exercise, has been reported [12, 28]. On the other hand, some authors have proposed that coagulation is upregulated with increasing age due to higer

plasma levels of factor VIII, prothrombin fragment 1+2 , thrombin – antithrombin complex, fibrinogen and shortened activativated partial thromboplastin time, in sum numerous coagulation parameters were significantly increased with age [23]. Also, our data showed that in table five, CT increased in experimental group on 90 day old rats and 30 day old rats ($p < 0.01$). Result of a research, showed that during exercise, clotting time decreased from 21.5 minutes to 9.9 minutes [22]. It is reported that exogenously administered melatonin reduced the skin oxidant damage and normalized activated blood coagulation induced by thermal trauma [34]. Therefore the result of present study shows the role of exercise on changes of thrombin time and coagulation time. Interestingly, our data showed that physical exercise caused CT increased in 90 days old rats as compared to 30 days old rats in experimental and control groups. Kim showed that moderate exercise in the heat significantly elevated platelet aggregation as indicated by decreased CT whereas CT was not altered in non-hyperthermia exercise condition [18]. One of the possible explanation for this is that physical exercise effects may be more effective than circadian rhythms effect on coagulation time change. Researchers have shown that exhaustive exercise alters blood coagulation and fibrinolysis [9]. In this regard, investigators have found that an increase in the components of the factor VIII complex and a shortening of whole blood clot lysis time after exercise [2]. In addition, after strenuous short-term exercise in male subjects varying fitness was observed signs of an increased blood coagulation and fibrinolysis by measuring global tests, factor VII, tPA, and fibrin split products such as D-dimer and fibrin monomers [11].

Table 5. Changes of coagulation time (second) on control group (NEX) and experimental group (EX) by age in male rats.

Group	**NEX (N=10)**		**EX (N=10)**		**P**
	30 day	90 day	30 day	90 Day	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
CT	138.5 ± 1.99	57.3 ± 3.35	296.9 ± 8.57	269.1 ± 1.4	< 0.05

5. Conclusion

Conclusively, the results of this study indicate that short- time physical exercise could decrease thrombin time. Interestingly, our data showed that after short- timephysical activity CT increased. These data clearly indicate that physical exercise play important role on haemostasis. However, further studies are needed to determine possible mechanisms of action, physical exercise, on changes of coagulation and thrombin system.

References

1. Amawi K.F., Salahat M.A., (2013) The impact of both physical exhaustion and disruption of circadian rhythm in blood coagulation factors in rats, *Advances in Life Science and Technology*, 7, 1- 6.
2. Andrew M., Carter C., Brodovich H., Heigenhauser G., (1986) Increases in factor VIII complex and fibrinolytic activity are dependent on exercise intensity, *J. Appl. Physiol*., 60, 1917-1922.
3. Chesebrom J.H , Toschi V., Leffino M., Gallo R., (1995) Evolving concepts in the pathogenesis and treatment of arterial thrombosis, *J. Med.*, 62, 275-286.
4. Dufau X.B., Order U., Hollman W., (1984) Can physical exercise induce an effective fibrinolysis? *Thromb Res*., 36, 37- 43.
5. El-Sayed M., Omar A., Lin X., (2000) Post-exercise Alcohol ingestion perturbs blood haemostasis during recovery, *Thrombosis Research*, 99, 523-530.
6. El-Sayed M.S., Ali N., EL-Sayed A.Z., (2005), Aggregation and activation of blood platelets in exercise training, *Sports Med*., 35, 11-12.
7. El-Sayed M.S., EL-Sayed Z.E., Ahmadizad S., (2004) Exercise and training effects on blood haemostasis in health and disease, *An Update Sports Med*., 34(3), 181- 200.
8. El-Sayed T.Y., Hassan F.M., (2013) Assessment of platelets count and coagulation parameters among Sudanese patients with liver cirrhosis, *Rawal Med J*., 38(8), 215-218.
9. Ferguson E.W., Bernier I.L., Banta G.R., Yu-Yahiro J., Schoomaker E.B., (1987) Effect of exercise and conditioning on clotting and fibrinolytic activity in men, *J. Appl. Physiol.*, 62, 1416-1421.
10. Flanders M.M., Crist R., Rodgers G.M., (2003) Comparison of five thrombin time reagents, *Clin. Chem.*, 49, 169-172.
11. Gunga H.C., Kirsch K., Beneke R., Boning D., Hopfenmuller W., Leithauser R., (2002) Markers of coagulation, fibrinolysis and angiogenesis after strenuous short-term exercise (wingate-test) in male subjects of varying fitness levels, *Int. J. Sports Med.*, 23, 495-499.
12. Herren T., Bartsch P., Haeberli A., Straub P.W., (1992) Increased thrombin-antithrombin III complexes after 1 h of physical exercise, *J. Appl. Physiol.*,73, 2499-2504.
13. Hilberg T., Glaser D., Prasa D., (2005) Pure eccentric exercise doesn't activate blood coagulation, *Eur J Appl Physiol*., 9, 718-721.
14. Hilberg T., Prasa D.,Sturzebecher J., (2003a) Blood coagulation and fibrinolysis after extreme short-term exercise, *Thrombosis Research*, 109, 271-277.
15. Hillberg T., Glaser D., Reckhart C., Prasa D., Sturzebechr J., Gabriel H.H., (2003b) Blood coagulation and fibrinolysis after long- duration treatmill exercise controlled by individual anaerobic threshold, *Eur J App Physiol*., 90, 639- 642.
16. Jilma B., Dirnberger E., Eichler H.G., (1997) Partial blockade of nitric oxide synthase blunts the exercise-induced increase of von Willebrand factor antigen and of factor VII in man, *Thromb Haemost*., 78, 1268-1271.
17. Keeney C.E., Laramie D.W., (1962) Effect of exercise on blood coagulation, *Circ. Res*., 10, 691-695.
18. Kim J.H., Wu T., Roberge R., Coca A., (2015) Hyperthermia exaggerates exercise induced aggregation of blood platelet, *Extreme Physiology and Medicine*, 4(1), 1- 2.
19. Lins M., Speidel T., ,Bastian A., (2003) Swimming and hemostasis during rehablitation in patient with coronary heart disease, *Thrombosis Research*, 108, 191-194.

20. Luki I., Dalvi A., Mayorga A.J., (2001) Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice, *Psychopharmacology*, 155, 315-322.
21. Margolis J., (1958) The kaolin clotting time. A rapid one-stage method for diagnosis of coagulation defects, *J. Path*., 11, 406-409.
22. Mc Keever K.H., Hinchcliff K.W., Kociba G.J., Reed S.M., (1990) Changes in coagulation and fibrinolysis in horses during exercise, *Am. J. Vet. Res.,* 51 (9), 1335-1339.
23. Menzel K., Hilberg T., (2009) Coagulation and fibrinolysis are in balance after moderate exercise in middle- aged participants, *Clinical and Applied Thrombosis Hemostasis*, 15(3), 348- 355.
24. Menzel K., Hilberg T., (2011) Blood coagulation and fibrinolysis in healthy untrained subjects: effects of different exercise intensities controlled by individual anaerobic threshold, *European Journal of Applied Physiology*, 11(2), 253- 260.
25. Nailin Li , Shu He , Margareta B. , Paul H., (2007) Platelet activity, coagulation and fibrinolysis during exercise in Healthy males, *Arterioscler. Thromb.Vasc. Biol* ., 27, 407-413.
26. Nazar Ali P., Hanachi P., (2011) To investigate the fibrinogen and some of coagulation factors in anaerobic exercise training women, *World Applied Sci. J*., 12, 72-75.
27. Piccione G., Fazio F., Giudice E., (2005) Exercise -induced changes in the clotting times and fibrinolytic activity during official 1600 and 2000 meters trot races in standard bred horses, *Acta. Vet. Brno*, 74, 509-514.
28. Prisco D., Paniccia R., Guarnaccia V., Olivo G., Taddei T., Boddi M., Gensisi G.F., (1993) Thrombin generation after physical exercise, *Thromb Res*., 69, 159-1 64.
29. Rock G., Tittley P., Pipe A., (1997), Coagulation factor changes following endurance exercise, *Clin J. Sport Med*., 7, 94-99.
30. Rocker L., Drygas W.K., Heyduk B., (1986) Blood platelet activation and increase in thrombin activity following a marathon race, *Eur. J. Appl. Physio*l., 55, 374-380.
31. Smith J.E., (2003) Effects of strenuous exercise on haemostasis, *Br. J. Sports Med*., 37, 433- 435.
32. Szymanski L.M., Pate R.R., (1994) Fibrinolytic responses to moderate intensity exercise, Comparison of physically active and inactive men, *Arteriosclerosis Thrombosis and Vascular Biology*, 14, 1746- 1750.
33. Tikhomirova S.V., Vikulov A.D., Baranov A.A., Osetrov I.A., (2007) Plasma coagulation Hemostasis iphysically active subjects during adaptation to physical exercise, *Human Physiology*, 33(6), 736-774.
34. Tunali T., Sener G., Yarat A., Emekli N., (2005) Melatonin reduces oxidative damage to skin and normalizes blood coagulation in a rat model of thermal injury, *Life Sci*.,76, 1259-1265.
35. Waha J.E., Goswami N., Schlagenhauf A., Martin K.B.L., Reibnegger G., Roller R.E., Hinghofer S.H., Cvirn G., (2015) Effects of exercise and nutrition on the coagulation system during bedrest immobilization, *Medicine*, 94(38), 1-6.
36. Wang J.S., Jen C.J., Kung H.C., Lin L.J., Hsiue T.R., Chen H.I., (1994) Different effect of strenuous exercise and moderate exercise on platelet function in men, *Circulation*, 90(6), 2877-2885.
37. Wang J.S., Jen C.J., Lee H., Chen H.I., (1997) Effect of short-term exercise on femal platelet function during different phases of the menstrual cycle, *Arteriosclero.Thromb.Vase.Biol*., 17, 1682-1686.