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Effects of Exercise and Pineal Gland on Some of Coagulation Parameters in Olfactory Bulbectomy Rats

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Abstract
This interventional study was aimed to investigate the effects of physical exercises and pineal gland on thrombin time and coagulation time in olfactory bulbectomy rats. The experiment was performed on 120 white male wistar rats weighing 100-150 gr. Animals were divided into two groups; control group and experimental group (olfactory bulbectomy and epiphysectomy). In both group animals were divided into three subgroups: control (without physical exercises), short-time physical exercises (5 minutes) and long-time physical exercise (20 minutes). Blood samples were collected from rat tail tip in several stages as before and after intervention, to determine of coagulation time. Exercise plans included; swimming on water pool until 5 minute and 20 minute in experimental and control groups. Then, thrombin time was measured for each tissue after autopsy of animals. The results revealed that short- time physical exercise significantly decreased thrombin time on different tissues of rats compared baseline values in control group ($P<0.001$). In contrast, long- time physical exercise significantly increased thrombin time on different tissues in interventional groups ($P < 0.001$). The findings of this study indicated that in experimental groups, coagulation time increased after long-time physical exercise but decreased after short-time physical exercises. In conclusion, this study suggested that there is a functional relationship between the olfactory bulb and pineal gland via its regulation of circadian rhythms and exercise on changes of coagulation parameters in different tissues.
Keywords: Thrombin time; coagulation time; physical exercise; olfactory bulbectomy; epiphysectomy and rat.
Abbreviations: **CT**: coagulation time, **TT**: thrombin time, **OB**: olfactory bulbectomy **NOB**: without olfactory bulbectomy, **PEX**: physical exercise, **NPEX**: without physical exercise, **EP**: epiphysectomy, **NEP**: without epiphysectomy, **SCN**: suprachiasmatic nucleus, **vWF**: von Willebrand factor, **aPTT**: activated partial thromboplastin time, **tPA**: tissue plasminogen activator

INTRODUCTION

The pineal gland is an important neuroendocrine organ in the brain. It's effects are believed to be mediated solely by the secretion of melatonin, which follows a circadian rhythm (Bulbuller, 2005). It is, also called the pineal body, epiphysis cerebri, or the third eye, is a small endocrine gland in the vertebrate brain. It produces melatonin, a hormone that affects the modulation of wake/ sleep patterns and photoperiodic (seasonal) functions (Amawi and Salahat, 2013). Melatonin is a well-known hormone produced by pineal gland, synthesized from serotonin and famous for its various roles involved in oxidative stress, inflammation, and circadian rhythm (Hong et al, 2014).). The production of melatonin by the pineal gland is inhibited by light to the retina and permitted by darkness. It is onset each evening and the duration of melatonin secretion is directly proportional to the length of night (Amawi and salahat, 2015). It is well known that melatonin play an important role in the control of several physiological processes. A number of investigations have suggested that there is a functional relationship between the pineal gland, via its hormone melatonin and the coagulation system (Tunali et al, 2005). Investigating pineal role in regulating functional condition of blood coagulation can be beneficial to hemostasis. It is well known that physical activity induces modification in blood hemostasis and lead to an activation of blood coagulation and fibrinolysis (amawi and salahat, 2015; Mahmoodinezhad and et al, 2016)). Melatonin secretion can be influenced by acute exercise (Baltaci et al, 2006 ; Lopez BO et al, 2007) and exercise has been shown to affect plasma melatonin levels in humans and rodents. Plasma melatonin is increased during acute exercise sessions and can increase also after prolonged exercise (Sergiu et al, 2015. Increased platelet activation, reduced aggregation and platelet adhesion, increased parasympathetic control, and reduced sympathetic cardiac control have been reported after physical activity (Mahmoodinezhad and et al, 2016). Recently, a report showed that the chronobiological patterns should considere to analyze activity levels of coagulation factors (Pinotti et al, 2005). 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 (Chesebrom et al, 1995). 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 (Hilberg et al, 2005). Mamalian olfactory system regulates a wide range of multiple and integrative functions such as physiological regulation, emotional responses, reproductive functions and social behaviors (Geol et al,1998). Findings of Corthell showed that melatonin receptors are present in olfactory bulb and likely affect olfactory function. Additionally these data suggest that melatonin may be locally

synthesized in the olfactory bulb and that affect olfactory bulb function (Corhtell et al, 2014). Montufar and et al, suggested in their studies that the main olfactory bulb represents a functional circadian pacemaker in many mammalians, during pre- visual stages of development the olfactory system plays a vital role in their survival (Montufar et al, 2012).Olfactory bulbs influence the effects of photic information on the circadian timing system (Geol et al,1997).Granados showed that olfactory bulb comprises a master circadian pacemaker, which enhances olfactory responsivity each night, drives rhythms and interacts with the suprachiasmatic nucleus (SCN) to coordinate other daily behaviors (Granados et al, 2006). On the other hand olfactory bulbs normally tend to increase the frequency of the animal's circadian oscillator (Pieper and et al, 1991). The effects of the olfactory stimuli on haemostasis in humans support the view point that simultaneous information and physico-chemical processes act together in parallel to play and important role in life activities of human organism. The blood coagulation time as on integral sign of human homeostasis is revealed to be influenced by olfactory stimuli through autonomic mechanisms (Sudakov et al, 1999). 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 (Chesebrom et al, 1995). In addition, the hemostatic 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 (Tikhomirova et al, 2007). 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(Hilberg et al, 2005). Also other 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 (Hilberg et al, 2005; Amawi, 2013; Rostami and et al, 2011). Physical activity exerts a considerable effect on coagulation system based on its type, duration and intensity (Ghaediyan et al, 2012). It has been demonstrated that exhaustive exercise alters blood coagulation and fibrinolysis (Hilberg et al, 2003) and it has been reported that exercise induced a significant increase in factor VIII activity with a significant shortening of activated partial thromboplastin time (EL-sayed et al, 2013).). Waha and coworker in their studies, demonstrated that increases t-PA levels in response to resistance exercise (Waha et al, 2015). It has been demonstrated that exhaustive exercise alters blood coagulation and fibrinolysis (Hilberg et al, 2003). It has been reported that exercise induced a significant increase in factor VIII activity with a significant shortening of activated partial thromboplastin time (EL-sayed et al, 2000). Hilberg et al demonstrated a decrease of the clotting factor and an increase of the fibrinolysis power in heath men (Hilberg et al, 2003). Improving the reaction of fibrinolytic and reducing activity of coagulation were reported in elderly males following the aerobic exercise (Amini and et al, 2011). In addition, blood haemostasis is a complex interaction among platelets, coagulation, and fibrinolysis. Acoording to the previous studies, the intensity of acute exercise is a critical factor affecting blood platelet function (Wang et al, 1997; EL- Sayed et al, 2005). Blood coagulation increases after the physical activity and remains increased for 1-24 hours (El- Sayed and et al, 2004). Peat and et al showed that partial thromboplastin activated time (aPTT) decreased immediately after exercise on both active and inactive people (Peat and et al., 2010). 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 (Nailin et al, 2007). 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 (Piccione et al, 2005). Few investigation exist on the relationship between the olfactory bulb, pineal gland, exercise and changes of coagulation time and thrombin time .Thus, the objective of this investigation was to examine effects of physical exercise and pineal gland on coagulation time and thrombin time in olfactory bulbectomy rats.

MATRIALS AND METHODS

Animal care and selection

120 white male Wistar rats 30 days old, weighing about 100-150 grams were used in these experiments. The animals were housed at an ambient temperature of 22 ± 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.

Experimental design and animal grouping

Animals were divided into two groups of 60 rats in each group. Control group (without olfactory bulbectomy and epiphysectomy) and experimental group (olfactory bulbectomy and epiphysectomy). In each group rats were divided into three subgroups: control (without physical exercise), short-time (5 minutes physical exercise) and long-time (with 20 minutes physical exercise). Blood samples were collected from rat tail tip in several stages as before olfactory bulbectomy, epiphysectomy, after, olfactory bulbectomy, epiphysectomy, after short-time and after long-time physical exercise from experimental group, to determine of coagulation time (Margolis, 1958).

Surgical procedure and epiphysectomy

Animals were deeply anaesthetized during all surgical procedures, with Ketamin 50 mg/kg BW and Xylazine 10 mg/kg BW, by intraperiton injection and submitted to the surgery, according to Hoffman and Reiter (1965). In brief, the anesthetized rats were placed in a stereotaxic apparatus for small animals and a sagittal opening was on the scalp. The skin and muscles were pushed aside in order to expose the lambda suture. By means of a circular drill a disc-shaped perforation was done around the lambda and the disc-shaped piece of bone was delicately removed. Thereafter, the pineal gland (which is located just below the posterior venous sinus confluence) was pulled out with fine forceps. After a brief period of haemostasis, the skull was closed by returning the disc-shaped bone and the scalp was sutured with cotton threads (Hoffman and Reiter, 1965).

Surgical procedure and olfactory bulbectomy

Animals were deeply anaesthetized during all surgical procedures, with Ketamin 50 mg/kg BW and Xylazine 10 mg/kg BW, by intraperitoneal injection and submitted to the surgery room, according to Leonard and Tuite (1981). In brief, the anesthetized rats were placed in a stereotaxic apparatus for small animals and skull covering the bulbs was exposed by skin incision and a burr hole was drilled, through which both olfactory bulbs were removed by suction with a hypodermic needle attached to a water pump. Finally, the burr hole was filled with bone wax in order to avoid further bleeding, after application of antibiotic powder (Neomaycin), the skin was closed with Histoacryl and animals were returned to their home cages (Leonard and Tuite, 1981).

Physical exercise procedure and swimming test

Animals 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 (Luki et al., 2001).Then rat were observed for 5 min and 20 min.

Determination of thrombin time in tissues

Ten days after olfactory bulbectomy and epiphysectomy in experimental and control groups, exercise programs included; swimming on water pool until five minute (short-time) and twenty minute (long-time) performed. Rats were killed and then, all of the animals were done autopsy. 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 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 (Flanders et al, 2003).

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 $p < 0.05$.

RESULTS

Thrombin time responses to short – time physical exercise (5 min) are presented in table one.

Table1. Effect of short-time physical exercise on TT (second) in different tissues in control group (NOB and NEP) in male rat

Group / Tissue	NPEX(N=10)		PEX(N=10)		P
	Mean	SD	Mean	SD	
Lung	20.0	0.0	17.1	0.5	<0.01
Brain	46.5	1.5	27.2	0.7	<0.01
Kidney	34.9	0.5	9.0	0.0	<0.001
Skeletal muscle	39.7	0.4	4.4	0.2	<0.001
Intestine	25.8	0.5	8.5	0.2	<0.01

According to number one table, thrombin time (TT) on different tissues significantly decreased in physical exercise (PEX) group as compared to without physical exercise (NPEX) group ($p < 0.001$). Our data clearly demonstrated that greatest decrease on TT was observed in skeletal muscles tissue (4.4 ± 0.2) and lower decrease on TT was seen in lung tissue (17 ± 0.5). Also, our data in table two showed that long–time physical exercise (20 min) significantly decreased TT on difference tissues in PEX group compared to NPEX group ($p<0.001$) and greater decrease was observed in brain tissue (9.8 ± 0.3) and lower decrease on TT was seen in intestine tissue (12.6 ± 0.4).

Table2. Effect of long-time physical exercise on TT (second) in different tissues in control group (NOB and NEP) in male rats.

Group / Tissue	NPEX(N=10)		PEX(N=10)		P
	Mean	SD	Mean	SD	
Lung	20.0	0.0	7.0	0.0	<0.01
Brain	46.5	1.5	9.8	0.3	<0.001
Kidney	34.9	0.5	7.4	0.5	<0.001
Skeletal muscle	39.7	0.4	7.9	0.4	<0.001
Intestine	25.8	0.5	12.6	0.4	<0.01

In addition, after olfactory bulbectomy and epiphysectomy as shown in table three, TT on different tissues significantly decreased especially on kidney tissue (18.4 ± 0.4) greater decrease, with short-time physical exercise as compared to NPEX rats ($p < 0.001$) and lower decrease was seen in skeletal muscle tissue (29.9 ± 0.5).

Table3. Effect of short-time physical exercise on TT (second) in different tissues in experimental groups (EP and OB) in male rats.

Group / Tissue	NPEX(N=10)		PEX(N=10)		P
	Mean	SD	Mean	SD	
Lung	31.4	0.6	15.0	0.0	<0.01
Brain	60.0	1.3	20.0	0.3	<0.001
Kidney	54.3	0.9	18.4	0.4	<0.001
Skeletal muscle	45.2	0.5	29.9	0.5	<0.01
Intestine	40.0	0.0	20.6	0.3	<0.01

Also, results of this study revealed that in table four, long-time physical exercise significantly decreased TT on different tissues in OB and EP rats as compared to NPEX rats ($p < 0.001$), and greater decrease was observed in brain tissue (13.0 ± 0.0) and lower decrease was seen in lung tissue (25 ± 0.5) .

Table4. Effect of long-time physical exercise on TT (second) in different tissues in experimental groups (EP and OB) groups in male rats.

Group / Tissue	NPEX(N=10)		PEX(N=10)		P
	Mean	SD	Mean	SD	
Lung	31.4	0.6	25.4	0.5	<0.01
Brain	60.6	1.3	13.0	0.0	<0.001
Kidney	54.3	0.9	30.0	0.0	<0.01
Skeletal muscle	45.2	0.5	21.3	0.6	<0.01
Intestine	40.0	0.0	20.8	0.4	<0.01

Table5. Effects of short time and long- time physical exercise on CT (second) in experimental (OB and EP) rats.

Group / Exercise	NPEX(N=20)		PEX(N=20)		P
	Mean	SD	Mean	SD	
Short-time	296.9	8.57	212. 9	1.09	<0.01
Long-time	100.15	1.36	255.5	0.56	<0.001

According to data of table five, coagulation time (CT) significantly decreased in OB and EP rats after short-time physical exercise (212.9 ± 1.09) as compared to control group (296.9 ± 8.57) ($p<0.001$). In contrast, after long–time physical exercise, CT significantly increased in OB and EP rats (255.5 ± 0.56) compared to control group (100.15 ± 1.36) ($p < 0.001$).

DISCUSSION

The results obtained in the present investigation suggest that physical exercise significantly decreased TT and long-time physical exercise had a more decreasing effect on TT than short-time physical exercise, except kidney, skeletal muscle and intestine tissues (tables one and two). Exercises are known to have considerable effect on blood hemostasis (El- sayed et al, 2000; Wang et al, 1994). It is well known that physical exercise induces an activation of coagulation and fibrinolysis, but this reaction depends on the exercise type, duration and its intensity (Andrew et al, 1986; Dufau et al, 1984; EL-Sayed et al, 2004; Hillberg et al, 2003). The effect of muscular exercise on blood coagulation has been the subject of several investigations in both man and animals (Keenney and Laramie, 1962). 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 (El- Sayed et al, 2000). It seems that swimming caused activation of the clotting system by increasing fibrinolytic activity (Lins et al, 2003). It is well understood that physical activity evokes multiple

effects on blood haemostasis and via reduced of inflammation and coagulation, which leads to reduce of mortality. Also, a recent study showed that anaerobic exercise accelerates blood coagulation and activates blood fibrinolytic activity (Nazar Ali and Hanachi, 2011). 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 content seems to be mediated by b-adrenergic receptors through a nitric oxide-dependent mechanism, the hemostastic system could be conditioned by endothelial function and be modified during the aging process (Jilma et al, 1997). Also, based on one marathon study, despite increased levels of B-thromboglobulin, platelet aggregation was found to be decreased after exercise (Rock et al, 1997) .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 (Rocker et al, 1986). Based on the results presented in this study, it was observed that TT significantly decreased on different tissues in OB and EP rats after short and long-time physical exercise in table three and four. Abdi and coworker demonstrated in their studies that glucose levels, PTT, T and TT decreased after olfactory bulb in rabbits, inaddition, physical activity reduce TT in different tissues (Abdi et al, 2015). Also, Amawi and et al showed in their experiments that thrombin time before physical activity was 31.1 ± 0.5 and after short- time exercise was 29 ± 1.0 and after long- time exercise was 16.5 (Amawi and et al, 2013). 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 (Kim and et al, 2015). Also Amawi showed that animals went through 5 minute physical activity, there was no statistically significant variation in blood CT of samples. However, there was a shortening in the blood CT for prolonged physical activity (Amawi and et al, 2013). Also, our data showed that CT significantly decreased in OB and EP rats after short- time physical exercise, but CT significantly increased after long-time physical activity in OB and EP rats (table five). Result of other research, showed that during exercise, clotting time decreased from 21.5 minutes to 9.9 minutes (Mc keever et al, 1990). It is reported that exogenously administered melatonin reduced the skin oxidant damage and normalized activated blood coagulation induced by thermal trauma (Tunali et al, 2005). It has been demonstrated that a dose-response relationship between the plasma concentration of melatonin and coagulation activity (Wirtz et al, 2008; Cardinali et al, 1993). Therefore the result of present study showed that role of olfactory bulb is functional on changes of thrombin time and coagulation time. Interestingly, our data showed that long-time physical exercise caused CT increased in OB and EP rats, but short-time physical exercise decreased it in OB and EP rats. Amawi and their cowerkwers showed that clotting time before physical activity were 51.17, after short- time exercise 81.2 and after long- time exercise 128.7 in inhibited pineal gland rats (Amawi et al, 2013). 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 (Ferguson et al, 1987). 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 (Andrew et al., 1986). 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 (Gunga et al, 2002). Posthuma and et al showed shortened clotting time (CT) and an increased maximum clot formation after long-term submaximal exercise (Posthuma and et al, 2015). The results of this study indicate that the pineal gland, olfactory bulb and physical activity play an important role on hemostasis system.

CONCLUSION

The findings of this study revealed that physical exercise could decrease thrombin time. Also, TT increased in OB and EP rats. Interestingly, our data showed that in OB and EP rats after long-time physical activity increased in coagulation time but after short-time physical activity decreased. These data clearly indicate that olfactory bulb and pineal gland (melatonin), similar to physical exercise play important role on hemostasis. However, further studies are needed to determine possible mechanisms of action, physical exercise, olfactory bulb and pineal gland on changes of others coagulation parameters.

REFERENCES

1. Abdi B, Aliyev A, Qaziyev A (2015). Investigation the effect of hypoxia and interception of olfactory nerve receptor on glucose, PT, T and TT of non- pregnant rabbit blood plasma. *Biological Forum.* 7(1): 879- 881.
2. Amawi KF, Salahat MA (2013). The impact of both physical exhaustion and disruption of circadianrhythm in blood coagulation factors in rats. *Advances in Life Science and Technology*. 7: 1- 6.
3. Amini A, Sobhani V, Mohammadi MT, Shirvani H (2016). Acute effects of aerobic , resistance and concurrent exercise and maximal shuttle run test(MSRT) on coagulation and fibrinolytic activity in non-athletic healthy young men. *J Sports Med Fitness*. 23 : 1- 6.
4. 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.

5. Baltaci AK, Cumraligil B, Kilic M, Kaya O, (2006). Effect of acute swimming exercise on lactate levels and its relation with zinc in pinealectomized rats. *Cell. Biochem. Func*. 19:25-29.
6. Bulbuller N, Dogru O, yekeler H, Cetinkaya Z, Ilhan N, Kirkil C (2005). Effect of melatonin on wound healing in normal and pinealectomized rats. *Journal of Surgical Research*. 123: 3-7.
7. Cardinali DP ,Delzar MM,Vacas MI(1993). The effect of melatonin human platelets. *Acta Physiol Pharmacol*. 43:1-13.
8. Chesebrom JH, Toschi V, Leffino M , Gallo R (1995). Evolving concepts in the pathogenesis and treatment of arterial thrombosis. *J. Med*. 62:275-286.
9. Corthell JT, Olcese J, Trombly OQ (2014). Melatonin in the mammalian olfactory bulb. *Neuroscience*. 7 (261):74-84.
10. Dufau XB, Order U, Hollman W (1984). Can physical exercise induce an effective fibrinolysis? *Thromb Res*. 36: 37- 43.
11. El-sayed TY, Hassan FM(2013). Assessment of platelets count and coagulation parameters among Sudanese patients with liver cirrhosis. *Rawal Med J*. 38(8):215-218.
12. El-Sayed M,Omar A , Lin X (2000). Post -exercise Alcohol ingestion perturbs blood haemostasis during recovery. *Thrombosis Research*. 99:523-530.
13. El-Sayed MS, EL-Sayed ZE, Ahmadizad S (2004). Exercise and training effects on blood haemostasis in health and disease. *An Update Sports Med*. 34(3): 181- 200.
15. El-Sayed MS, Ali N, EL-sayed AZ (2005). Aggregation and activation of blood platelets in exercise training. *Sports Med*. 35:11-12.
15. Ferguson EW, Bernier IL, Banta GR, Yu-Yahiro J, Schoomaker EB (1987). Effect of exercise and conditioning on clotting and fibrinolytic activity in men. *J Appl Physio.* 62:1416-21.
16. Flanders MM, Crist R, Rodgers GM (2003). Comparison of five thrombin time reagents. *Clin. Chem*, 49:169-172.
17. Ghaediyan S, marefati H, Nabipur F, naghizadeh MM(2012). The effect of a moderate aerobic exercise on the blood coagulation markers in young non- athletic females. *J Jahrom University Med Sci*. 10(2): 54- 60.
18. Goel N, Lee T R,Pieper D R (1998). Removal of olfactory bulbs delay photic reentrainment of circadian activity rhythms and modifies the reproductive axis in male octodon degus. *Brain Research*.792:229-236.
19. Goel N,Lee TM (1997).Olfactory bulbectomy impeds social but not photic reentrainment of circadian rhythms in female octodon degus. *J.Biol.Rhythms*. 12:362-370.
20. Granados-Fuentes D,Tseng A, Herzog ED (2006). A circadian clock in the olfactory bulb control olfactory responsivity. *The Journal of Neuroscience*. 26(47):12219-12225.
21. Gunga HC, 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.
22. Hilberg T, Glaser D, Prasa D (2005) .Pure eccentric exercise doesn't activate blood coagulation. *Eur J Appl Physiol*, 94:718-721.
23. Hilberg T, Prasa D,Sturzebecher J(2003a). Blood coagulation and fibrinolysis after extreme short-term exercise. *Thrombosis Research*, 109:271-277.
24. Hillberg T, Glaser D, Reckhart C, Prasa D, Sturzebechr J,Gabriel HH (2003b). Blood coagulation and fibrinolysis after long- duration treatmill exercise controlled by individual anaerobic threshold, *Eur J App Physiol*. 90: 639- 642.
25. Hoffman, RA, Reiter RJ(1965). Rapid pinealectomy in hamsters and other small rodents. *Anat. Rec*. 153:19-22.
26. Hong Y, kim H, Lee Y, Lee S, Kim K, Jin Y, Lee SR, Chang KT, Hong Y(2014). Salutary effects of melatonin combined with treadmill exercise on cartilage damage. *J Pineal Res*. 57(1): 53- 66.
27. Jilma B, Dirnberger E, Eichler HG (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.
28. Keeney CE, Laramie DW (1962). Effect of exercise on blood coagulation. *Circ. Res*, 10: 691-695.Kim JH, Wu T, Roberge R, Coca A (2015). Hyperthermia exaggerates exercise induced aggregation of blood platelet. *Extreme Physiology and Medicine*. 4(1): 1- 2.
29. Leonard BE, Tuite M (1981). Anatomical, physiological and behavioral aspects of olfactory bulbectomy in the rat. *Int. Rev. Neurobiol*. 22:251-286.
30. Lins M,Speidel T,Bastian A (2003). Swimming and hemostasis during rehablitation in patient with coronary heart disease. *Thrombosis Research*. 108:191-194.
31. Lopez BO, Urquijo MDC (2007). Influence of physical training on the Syrian hamster's melatonin rhythm. *Neuroscience Letters*. 428: 68- 71.
32. Luki I, Dalvi A, Mayorga A J (2001). Sensitivity to the effects of pharmacologically selective

antidepressants in different strains of mice. *Psychopharmacology*. 155: 315-22.
33. Mahmoodinezad S, Shakerian S, Ghalavand A, Motamedi P, Delaramnasab M(2016). The effect of acute training and circadian rhythm on blood hemostasis in female athelets. *Int J Basic Sci Med*. 1(1): 8-12.
34. Margolis J (1958). The kaolin clotting time. A rapid one-stage method for diagnosis of coagulation defects. *J. Path*, 11: 406-409.
35. Mc keever KH, Hinchcliff KW, Kociba GJ, Reed SM (1990). Changes in coagulation and fibrinolysis in horses during exercise. *Am J Vet Res*. 51 (9): 1335- 9.
36. Montufar- Chaveznava R, Hernandez- Campos O, Hudson R, Caldelas I (2012). Differential maturation of the molecular clockwork in the olfactory bulb and suprachiasmatic nucleus of the rabbit. *Neuroscience.* 5(207) : 198-207.
37. Mucignat-Caretta C,Bondi M,Caretta A (2006) . Time course of alteration olfactory bulbectomy in mice. *Physiology & Behavior*. 89: 637-643.
38. 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.
39. 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.
40. Peat E, Dawson M, Mckenzie A, Hillis WS(2010). The effects of acute dynamic exercise on hemostasis in first class Scottish football referees. *BR J Sports Med*. 44(8): 573- 578.
41. 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.
42. Pieper DR, Lobocki CA (1991). Olfactory bulbectomy lengthens circadian period of locomotor activity in golden hamsters. *Am J Physiol*. 261(4): 973- 8.
43. Pinotti M, bertolucci C,Portaluppi F,Colognesi I(2005). Daily and circadian rhythms of tissue factor pathway Inhibitor and Factor VII activity. *Arteriosclerosis Thrombosis and Vascular Biology*. 25:646.
44. Posthuma JJ, Vander Meijden PEJ, Cate HT, Spronk HMH(2015). Short and long term exercise induced alteration in hemostasis: A review of the literature. *Blood Reviews*. 29: 171-178.
45. Rostami H, Aliyev A, madatova M, Arasteh A (2011). Effects of physical activity on some of hemostatic parameters changes in epiphysectomized rats. *A J P P*. 5(19): 2156- 2160.
46. Rock G, Tittley P, Pipe A (1997). Coagulation factor changes following endurance exercise. *Clin J. Sport Med*. 7:94-99.
47. Rocker L, Drygas WK, Heyduk B (1986). Blood platelet activation and increase in thrombin activity following a marathon race. *Eur J Appl Physiol*. 55:374-380.
48. Sergiu D, Cristian P, Monika P(2015). Melatonin and exercise. *Palestrica of the Third Millennium Civilization & Sport*. 16(1): 69- 73.
49. Sudakov KV, Uryvaev YVV, Petrov GA (1999). Odogenic change of blood coagulation in humans. *Integative Physiological and Behavioral Science*. 33(3): 150-154.
50. Tikhomirova SV, Vikulov AD, Baranov AA, Osetrov IA (2007). Plasma coagulation Hemostasis in physically active subjects during adaptation to physical exercise. *Human Physiology*. 33(6) :736-74.
51. 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..
52. Waha JE, Goswami N, Schlagenhauf A, Martin KBL, Reibnegger G, Roller RE, Hinghofer SH, Cvirn G (2015). Effects of exercise and nutrition on the coagulation system during bedrest immobilization. *Medicine.* 94(38): 1-6.
54. Wang JS, Jen CJ,lee H, Chen HI (1997). Effect of short-term exercise on femal platelet function during different phases of the menstrual cycle. *Arteriosclero.Thromb.Vase.Biol*. 17: 1682-1686.
55. Wang JS,Jen CJ,Kung HC, Lin LJ, Hsiue TR, chen HI,(1994). Different effect of strenuous exercise and moderate exercise on platelet function in men. *Circulation*. 90(6): 2877-2885.
56.Wirtz PH, Spillmann M, Bartschic EV, Vonkanel R (2008). Oral melatonin reduces blood coagulation activity: a placebo controlled study in healthy young men . *J Pineal Res*. 44: 127-133.