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*Original Contribution*

# COMBINED EFFECT OF PHYSICAL ACTIVITY AND EPIPHYSECTOMY ON BLOOD COAGULATION IN RATS

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**ABSTRACT**
Purpose: Many investigations indicate the direct and indirect effects of pineal gland on coagulation system particularly on thrombin time. It is well known that exhaustive exercise alters blood coagulation and fibrinolysis, but the assessment of the dimension and importance of these changes is difficult. This study was designed to determine the effect of physical activity interaction epiphysectomy with on coagulation system in rats.Methods: Sixty healthy male rats 30 days old were selected. They were divided into experimental and control groups. Epiphysectomy was performed on experimental animals. Coagulation time and thrombin time were measured on each subgroup (control, short-time physical activity and long-time physical activity). Results: There was a significant difference between experimental and control groups before and after epiphysectomy ($P<0.001$). Physical activity decreased thrombin time on different tissues of rats. Conclusions: Our results suggested effect of physical activity on thrombin time and coagulation time and also the role of pineal gland on thrombin time in rats.

**Key word:** Physical activity; Pineal gland; Thrombin time; Coagulation time; Rat

## INTRODUCTION

The pineal gland (epiphysis) synthesizes and secretes melatonin, a structurally simple hormone that communicates information about environmental lighting to various parts of body. Ultimately, melatonin has the ability to entrain biological rhythms and has important effects on physiological function on many animals. Numbers of investigations indicate the direct and indirect effects of melatonin on coagulation system (1-3). Pinotti, et al in their studies concluded that the chronobiological patterns should consider analyzing activity levels of coagulation factors (4). According to the results of another study, exogenously administered melatonin reduces skin oxidant damage and normalizes the activated blood coagulation induced by thermal trauma (3). It is known that physical activity induces modification in blood hemostasis and leads to an activation of blood coagulation and fibrinolysis (5). Physical stress is associated with the activation of blood cell (6). 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 (7).

It is well known that exhaustive exercise alters blood coagulation and fibrinolysis (8). But the assessment of the dimension and importance of these changes is difficult. Long-term physical exercise is known to promote changes in the coagulatory and fibrinolytic activities of the blood. In contrast, changes in the procoagulatory components of the hemostasis remain rather poorly understood and studies on the effects of exercise on the anticoagulatory system are few (9). Few studies exist on the relationship between the clotting times and exercise. The increase in clotting and fibrinolytic activity due to exercise has been widely documented in human and experimental animals (10).In this study we considered effect of physical activity

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*Running Head: physical activity and pineal gland on thrombin time*

interaction with pineal gland on thrombin time in rats.

## MATRIALS AND METHODS

In this experimental study sixty healthy male Wistar rats 30 days old were used, ranging 150-190 g body weight. All animals were supervised in the animal care facility for at least 15 days before any studies. Animals were used under ethical approval of department, kept under controlled conditions of temperature (22-26 $^oC$), suitable humidity (50-60%), and 12-hour light-dark period. The animals were housed on adjacent metabolism cages, providing free access to drinking water, and a standard chow was daily provided within 20 minutes after the photophase started.

They were divided into two groups; control group without surgical intervention (epiphysectomy) and experimental group with surgical intervention. In each group subjects divided into three subgroups; control (without physical activity), short-time (5 minutes daily) and long-time (with 20 minutes daily) physical activity by running on treadmill for 5 days. Epiphysectomy surgery was performed by Aulov method (11) on experimental subjects. Animals anesthetized by Ketamine (75 mg/kg) and Xylazine (10 mg/kg) by intraperitoneal injection. Then, rats were slaughtered and all of the animals were done autopsy. Blood, liver, spleen and heart muscle tissues were isolated. Thrombin time was measured for each tissue. After liberating the plasma from the whole blood and tissue by centrifugation, bovine Thrombin was added to the sample of plasma. The clot was formed and was detected optically. The time between the addition of the thrombin and the clot formation was recorded as the thrombin time. Collected data were analyzed by SPSS software statistical significance was calculated using the independent student's T test. ANOVA was used to compare means of more than two independent groups. The level of significance in all cases was set at a two-tailed $P<0.05$.

## RESULTS

There was a significant difference in thrombin time on all of different tissues according to epiphysectomy. Most difference was observed on liver and least one was found on heart tissue **(Table 1)**. . Analysis of data also showed significant differences between subgroups according to physical activity. Both short-time and long-time physical activity decreased thrombin time on different tissues of animals but long-time is more effective than short-time **(Table 2)**. Data analysis showed significant differences in results between intact and epiphysectomized animals after short-time activity that most difference was found on spleen **(Table 3)**. Analysis of results also indicated significant differences after long-term physical activity according to epiphysectomy status so that most increasing effect was found on blood **(Table 4)**.

***Table 1***. *Comparison of thrombin time (second) in different tissues according to epiphysectomy status.*

| Group / Tissue | Control (Intact) (N=10) | | Experimental (Epiphysectomized) (N=10) | | P |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| Blood | 29.50 | 3.88 | 23.50 | 5.40 | <0.01 |
| Liver | 41.00 | 4.90 | 18.10 | 6.80 | <0.001 |
| Heart | 20.00 | 2.10 | 16.50 | 2.47 | <0.01 |
| Spleen | 21.10 | 3.00 | 14.80 | 3.85 | <0.001 |
| Total | 27.90 | 5.50 | 18.20 | 2.620 | <0.001 |

***Table 2**. Thrombin time in different tissues according to physical activity.*

| Group / Tissue | Without physical activity (N=10) | | Short-time physical activity (N=10) | | Long-time physical activity (N=10) | | P |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | |
| Blood | 29.50 | 3.88 | 21.30 | 1.86 | 17.60 | 2.43 | <0.001 |
| Liver | 41.00 | 4.90 | 16.20 | 1.63 | 7.40 | 1.53 | <0.001 |
| Heart muscle | 20.00 | 2.15 | 17.00 | 1.85 | 12.30 | 1.86 | <0.01 |
| Spleen | 21.10 | 3.00 | 19.50 | 1.29 | 6.00 | 1.20 | <0.05 |
| Total | 27.90 | 5.50 | 18.50 | 1.90 | 10.65 | 4.15 | <0.001 |

***Table3.** Thrombin time in different tissues after short-time physical activity according to epiphysectomy status.*

| Group / Tissue | Intact (N=10) | | Epiphysectomized (N=10) | | P |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| Blood | 21.30 | 1.86 | 30.20 | 1.05 | <0.001 |
| Liver | 16.20 | 1.63 | 20.70 | 1.10 | <0.01 |
| Heart muscle | 17.00 | 1.85 | 20.00 | 2.30 | <0.05 |
| Spleen | 19.50 | 1.29 | 29.90 | 1.29 | <0.001 |
| Total | 18.50 | 1.90 | 25.20 | 4.85 | <0.01 |

**DISCUSSION**

Evidences implicate melatonin which is synthesized and released in the pineal gland in a broad range of effects with a significant regulatory influence over many of the body's physiological functions (12, 13).

Our study results showed decreasing effect of pineal gland on thrombin time in rats.

The findings provide preliminary support for a protective effect of melatonin in reducing the atherothrombotic risk (14). Wirtz and et al showed a dose-response relationship between the plasma concentration of melatonin and coagulation activity (15). It was showed exogenously administered melatonin normalizes the activated blood coagulation (2). Numerous studies of melatonin, by now widely acknowledged as a circadian rhythm-affecting neurohormone, is capable of promoting platelet production by megakaryocytes, of acting on the latter's ion channels by way of the outward currents, and of performing a physiological anti-aggregation function thus lengthening platelet life span (1).

***Table 4.*** *Thrombin time in different tissues after long-time physical activity according to epiphysectomy status.*

| Group / Tissue | Intact (N=10) | | Epiphysectomized (N=10) | | P |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| Blood | 17.60 | 2.43 | 75.30 | 2.43 | <0.001 |
| Liver | 7.40 | 1.53 | 20.00 | 3.20 | <0.001 |
| Heart muscle | 12.30 | 1.86 | 38.00 | 2.21 | <0.001 |
| Spleen | 6.00 | 1.20 | 59.90 | 2.64 | <0.001 |
| Total | 10.65 | 4.15 | 48.30 | 19.30 | <0.001 |

Results of our study also showed decreasing effect of short-time physical activity on thrombin time in different tissues. Hilberg and et al showed that maximal short-time exercise does not lead to a relevant activation of blood coagulation in healthy young subjects. It is only slightly altered within the normal rang. In this study immediately after exercise, a shortening of a PTT was seen (8). There are conflicting results about effect of exercise on prothrombin time and thrombin time. Most of them showed no demonstrable effect on prothrombin time, although some have shown a significant shortening of thrombin time (16). Swimming caused activation of the clotting system by increasing fibrinolytic activity (17).

Our results also showed long-time physical activity has more decreasing effect on thrombin time than short-time physical activity. The effect of muscular exercise on blood coagulation has been the subject of several investigations in both man and laboratory animals and relative results have indicated that coagulation is accelerated immediately after muscular exercise. Several studies have shown effect of strenuous exercise on coagulation (7). According to results of Riberiro and et al exhaustive exercise in adults decreases activated partial thromboplastin time (18). Furthermore, it is well known that exhaustive exercise alters blood coagulation and fibrinolysis (19). However, Hilberg and et al showed that maximal exercises with duration up to ninety second did not lead a relevant activation of blood coagulation (8).

The findings of the present study suggested the role of pineal gland on thrombin time in male rats. We also concluded that physical activity decreases thrombin time in different tissues of rats.

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