

**A STUDY ON AFFECT OF SEVERITY OF EXERCISE ON PLATELET FUNCTION**Srinivas Reddy Kilim<sup>1</sup>, P. V. V. Lakshmi<sup>2</sup>**HOW TO CITE THIS ARTICLE:**

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**ABSTRACT:** Platelets serve an important role in the Physiological process of hemostasis. Platelets also contribute to the formation of pathologic thrombus. Platelet count increases with exercise. Despite the increase in Platelet number with exercise, previous studies indicate that activation and aggregation may be influenced by numerous factors, predominantly by the intensity of exercise; this study was done to determine the effect of moderate and strenuous exercise on Platelet count, and Platelet activation. Platelet factor 4 and  $\beta$ -thromboglobulin ( $\beta$ -TG) are platelet-specific proteins stored in the  $\alpha$ -granules and secreted upon platelet activation. The degree of platelet activation can be assessed by measuring plasma and urinary Platelet factor 4 and  $\beta$ -thromboglobulin ( $\beta$ -TG). In this study 50 girls and 50 boys were randomly selected. Blood sample was used for measurement of resting hematological parameters (platelet count, RBC count and hemoglobin). The subjects were instructed to perform exercise with various intensities on bicycle ergometer. The post-exercise sample of blood was used for measurement of hematological Parameters (Platelet Count, RBC Count and Hemoglobin), and In vivo activation of platelets was measured by radioimmunoassay of platelet factor 4 (PF4) and beta thromboglobulin ( $\beta$ TG). Platelet count, increased from  $2.24 \pm 0.19$  to  $2.76 \pm 0.24 \times 10^3/\text{ml}$  ( $p < 0.01$ ) during Moderate exercise, and increased from  $2.24 \pm 0.19$  to  $2.98 \pm 0.27 \times 10^3/\text{ml}$ , ( $p < 0.01$ ) during Severe exercise. plasma PF4 increased from  $2.13 \pm 1.1$  to  $2.76 \pm 0.24 \text{ng/ml}$ , ( $p < 0.01$ ) and plasma  $\beta$  TG increased from  $11.46 \pm 5.3$  to  $12.85 \pm 5.8 \text{ng/ml}$  ( $p < 0.01$ ) during Moderate exercise, plasma PF4 increased from  $2.13 \pm 1.1$  to  $4.67 \pm 2.4 \text{ng/ml}$ , ( $p < 0.01$ ) and plasma  $\beta$  TG increased from  $11.46 \pm 5.3$  to  $16.53 \pm 7.5 \text{ng/ml}$  ( $p < 0.01$ ) during Severe exercise, in boys and similar results were found in girls. This study support that Moderate exercise leads to increase Platelet count without Platelet activation, and strenuous exercise cause increase in Platelet count with Platelet activation as indicated by increased plasma PF4 and  $\beta$ TG. Large numbers of studies have documented the beneficial effects of exercise on various aspects of physiology such as blood lipid profile, cardiovascular health, etc. strenuous aerobic exercise might counterbalance these advantageous modifications. Platelet activation may lead to promotion of thrombogenesis especially in sedentary and untrained individuals. Hence strenuous exercises in sedentary and untrained individuals have to be performed with caution.

**KEYWORDS:** Exercise, Platelet count, Platelet activation, Platelet factor 4,  $\beta$ -thromboglobulin.

**INTRODUCTION:** Circulating blood Platelets serve an important role in the Physiological process of hemostasis. The main function of platelets is to arrest bleeding by forming a haemostatic plug through their interaction with damaged vascular wall. Platelets also contribute to the formation of pathologic thrombus to occlude vessels, leading to diseases such as coronary artery disease or stroke. Physical exercise has been documented to result in alteration in many haemostatic parameters including platelet number, Size and Function.

Platelets must be activated to prevent bleeding by forming a haemostatic plug. Platelet activation in vivo is initiated by collagen and thrombin at a site of acute vascular injury, Circulating

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platelets must be able to sustain repeated contact with the normal vessel wall without premature activation to avoid harmful effect of pathologic thrombus.

Platelet count increases with exercise, due to release of fresh platelets from the spleen, bone marrow, or other reservoirs. Previous studies found an increase in Platelet count immediately after exercise. Despite the increase in Platelet number, most studies regarding behavior, mainly aggregation and secretion have been incomplete. Studies of platelet  $\alpha$ -granule release during exercise have been inconclusive. Platelet factor 4 and  $\beta$ -thromboglobulin ( $\beta$ -TG) are platelet-specific proteins stored in the  $\alpha$ -granules and secreted upon platelet activation. Platelet  $\alpha$ -granule contents in the plasma such as  $\beta$ -TG and platelet factor 4 can be measured using an ELISA or radioimmunoassay. Plasma levels of  $\beta$ -TG greatly exceed plasma levels of platelet factor 4, possibly due to more rapid binding of platelet factor 4 to endothelial cells and thus results in its removal from plasma. Therefore a higher ratio of  $\beta$ -TG to platelet factor 4 is always maintained in vivo.<sup>1</sup> The average dimension of a platelet is about 2 $\mu$ m. As non-activated platelets have an irregular shape, such measurements may not always be precise. The mean platelet volume (MPV) is therefore considered the most accurate measure of platelet size. Normal MPV ranges from 7 to 11fL. The degree of platelet activation also affects the measurement of MPV Treadmill exercise testing causes a rapid (within 30 min) activation of platelets, as indicated by a significant increase in MPV.

Earlier results of several studies indicate that plasma PF4 and BTG increase minimally, but significantly, in normal subject's during maximal exercise.<sup>2</sup> Moderate exercise leads to increase Platelet count without Platelet activation and aggregation. Platelet activation has been implicated in the pathogenesis of a number of diseases, which include atherosclerosis, coronary vascular disease, and cerebrovascular disease. Platelet activation may therefore be quantified by factors such as a change in shape and a tendency to aggregate, and also by measuring the blood and urine levels of relevant platelet metabolic products. For example, the alpha granule components, beta thromboglobulin and platelet factor 4.

The response of haemostatic system to exercise was dependent on the type of physical exercise, duration, intensity and degree of Training. Human lifestyle or physical activities have diverse effects on coagulation, fibrinolysis, and platelet reactivity. Habitual Physical exercise is associated with an overall decreased risk of acute heart disease. However strenuous exercise especially in sedentary & untrained can activate the haemostatic system leading to Thrombosis.

The purpose of this study was to determine the effect of moderate and strenuous exercise on Platelet count, and Platelet activation. Available evidence from recent studies support lifestyles that adopt strategies to engage in regular moderate exercise and relaxation, reduce platelet reactivity. The overall effects ought to translate into an improved cardiovascular or other beneficial clinical outcome in healthy individuals.

**MATERIAL AND METHODS:** In this study 50 girls and 50 boys were randomly selected. They gave their consent and understood the experimental procedure. To prevent confounding effects of smoking, all subjects were non-smokers. None of the subjects had taken aspirin, or other antiplatelet drug. None of the subjects were sedentary and no subject underwent physical training. The subjects completed the medical history form and a physical activity questionnaire. None of the subjects had history of any medical or surgical illness. They had no cardiovascular, Respiratory, Endocrine, renal or other disorders. Before the actual study subjects were familiarized with exercise on a bicycle ergometer (Aerofit) to eliminate the novel effect of new experience. After the subjects had arrived at

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the laboratory and rested for 30 minutes, blood sample was drawn from a forearm vein. Blood was added to Anti-coagulant. The Blood sample was used for measurement of resting hematological parameters (Platelet count, RBC count and hemoglobin) and baseline platelet factor 4 (PF4) and beta thromboglobulin ( $\beta$ TG) were measured by radio immunologic method. After an initial two minutes of unloaded pedaling on a bicycle ergometer the subjects were instructed to perform 3 minutes of work load exercise on bicycle ergometer (Aerofit). Immediately after the exercise session, another blood sample was drawn from the fore-arm vein and added with anti-coagulant. The post-exercise sample of blood was used for measurement of post-exercise hematological Parameters (Platelet Count, RBC Count and Hemoglobin) and platelet factor 4 (PF4) and beta thromboglobulin ( $\beta$ TG).

The Platelets were counted using 1% Ammonium Oxalate. Hemoglobin was measured by Sahli's Hemoglobinometer. The data are presented as mean, and standard deviation. Student's t-test on the mean values was used to compare results in the two groups and the difference was considered significant if probability values were lower than 0.05. After 30 minutes another sample of blood was used for measurement of hematological Parameters (Platelet Count, RBC Count and Hemoglobin) and platelet factor 4 (PF4) and beta thromboglobulin ( $\beta$ TG).

Measurements of platelet reactivity either in vitro or in vivo (platelet secretary products, mainly beta thromboglobulin and platelet factor 4) are associated with considerable methodological difficulties and thus may account for the discrepancies of results reported in the literature.<sup>3</sup>

	Rest		Exercise				30 min			
	Boys	Girls	Boys		Girls		Boys		Girls	
			Mod- erate	Sev- ere	Mod- erate	Sev- ere	Mod- erate	Sev- ere	Mod- erate	Sev- ere
Platelet count x 10 <sup>3</sup> ml <sup>-1</sup>	2.24 ±0.19	2.10 ±0.12	2.76± 0.24	2.98± 0.27	2.63 ±0.23	2.87 ±0.26	2.45 ±0.21	2.55 ±0.22	2.25 ±0.16	2.35± 0.15
RBC count x 10 <sup>6</sup> ml <sup>-1</sup>	4.73 ±0.48	4.13 ±0.47	5.12 ±0.49	5.65 ± 0.45	4.65 ±0.49	4.89 ±0.46	4.95 ±0.49	5.12 ±0.38	4.32 ±0.49	4.54± 0.48
WBC count x 10 <sup>3</sup> $\mu$ l <sup>-1</sup>	6.37 ±1.9	5.22 ±1.6	7.45± 1.6	7.89± 1.8	6.35± 1.4	6.96± 1.6	6.95 ±1.9	7.12 ±1.5	5.43 ±1.8	5.86± 1.6
Hemoglobin g/dl	13.67 ±0.71	11.60 ±0.93	15.16 ±0.84	15.96 ±0.81	12.48 ±0.96	12.95 ±0.85	14.53 ±0.76	14.92 ±0.74	12.11 ±0.98	12.94 ±0.78
Plasma PF4 (ng/ml)	2.13 ±1.1	2.16 ±0.9	2.93 ±1.6	4.67 ±2.4	2.96 ±0.8	4.82 ±2.1	2.25 ±1.2	2.51 ±1.4	2.26 ±0.7	2.13± 1.1
Plasma $\beta$ TG (ng/ml)	11.46 ±5.3	10.26 ±4.2	12.85 ±5.8	16.53 ±7.5	11.32 ±4.4	17.81 ±5.8	11.86 ±5.7	12.78 ±3.8	10.65 ±4.5	11.46 ±5.3

**Table 1: Changes in Blood cell counts, Hb, Plasma Platelet Factor 4, and  $\beta$  Thromboglobulin in Moderate exercise and severe exercise**

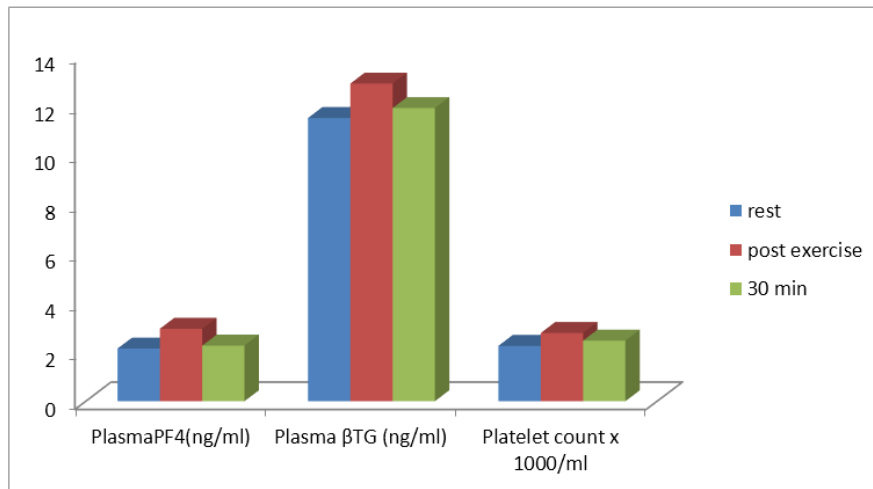
The analysis of data in boys showed an increased Platelet count, from 2.24±0.19 to 2.76±0.24 x10<sup>3</sup>/ml (p <0.01) during Moderate exercise, and from 2.24±0.19 to 2.98±0.27x10<sup>3</sup>/ml, (p <0.01) during Severe exercise.

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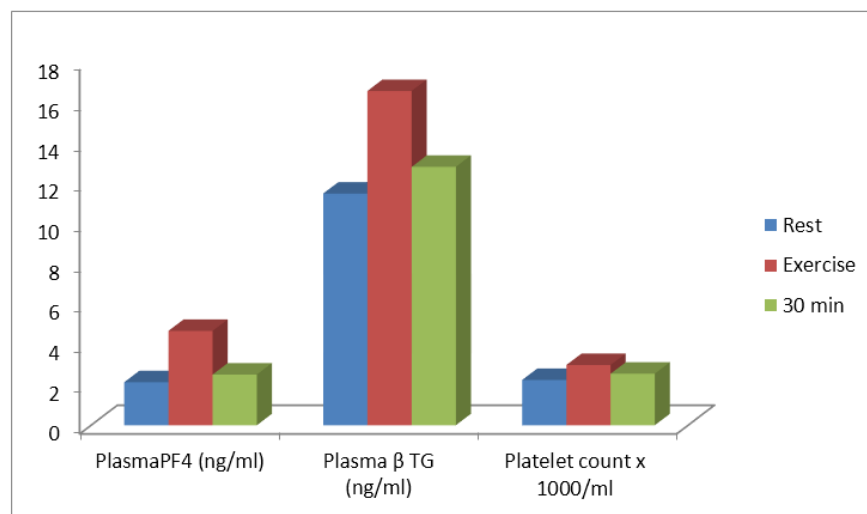
In girls Platelet count increased, from  $2.10 \pm 0.12$  to  $2.63 \pm 0.23 \times 10^3/\text{ml}$  ( $p < 0.01$ ) during Moderate exercise, and from  $2.10 \pm 0.12$  to  $2.87 \pm 0.26 \times 10^3/\text{ml}$ , ( $p < 0.01$ ) during Severe exercise.

In boys during Moderate exercise, plasma PF4 increased from  $2.13 \pm 1.1$  to  $2.76 \pm 0.24 \text{ ng/ml}$ , ( $p < 0.01$ ) and plasma  $\beta$  TG increased from  $11.46 \pm 5.3$  to  $12.85 \pm 5.8 \text{ ng/ml}$  ( $p < 0.01$ ), In boys during Severe exercise, plasma PF4 increased from  $2.13 \pm 1.1$  to  $4.67 \pm 2.4 \text{ ng/ml}$ , ( $p < 0.01$ ) and plasma  $\beta$  TG increased from  $11.46 \pm 5.3$  to  $16.53 \pm 7.5 \text{ ng/ml}$  ( $p < 0.01$ ),

In girls during Moderate exercise, plasma PF4 increased from  $2.16 \pm 0.9$  at rest to  $2.96 \pm 0.8 \text{ ng/ml}$ , ( $p < 0.01$ ) and plasma  $\beta$  TG increased from  $10.26 \pm 4.2$  to  $11.32 \pm 4.4 \text{ ng/ml}$  ( $p < 0.01$ ). In girls during Severe exercise, plasma PF4 increased from  $2.16 \pm 0.9$  at rest to  $4.82 \pm 2.1 \text{ ng/ml}$ , ( $p < 0.01$ ) and plasma  $\beta$  TG increased from  $10.26 \pm 4.2$  to  $17.81 \pm 5.8 \text{ ng/ml}$  ( $p < 0.01$ ),



**Graph 1: Comparison of changes in platelet count, Plasma Platelet Factor 4, and  $\beta$  Thromboglobulin in Moderate exercise**



**Graph 2: Comparison of changes in platelet count, Plasma Platelet Factor 4, and  $\beta$  Thromboglobulin in Severe exercise**

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**DISCUSSION:** The findings of this study support that Moderate exercise leads to increase Platelet count without Platelet activation, and strenuous exercise cause increase in Platelet count with Platelet activation as indicated by increased plasma PF4 and  $\beta$ TG.

The fact that exercise is beneficial to health is accepted by many researchers. Large number of studies has documented the beneficial effects of exercise on various aspects of physiology such as blood lipid profile, cardiovascular health, etc. with Moderate exercise. However, the influence of intensity, duration and training are yet to be emphasized. Growing evidences indicate that benefit of physical activity on cardiovascular disease may result to some extent from effects on hemostasis.<sup>4-6</sup>

The intensity of exercise should be below the individual anaerobic threshold. It is to be investigated whether a strenuous aerobic exercise might counterbalance these advantageous modifications and very little data is available on the influence of physical exercise on primary hemostasis. Moderate-intensity exercise suppresses shear-induced platelet activation and subsequent PMNs adhesion to platelets deposited at sites of vascular injury under flow, thereby reducing the risks of vascular thrombosis and inflammation.<sup>7</sup>

The present study confirms that Moderate exercise increase Platelet count without Platelet activation, and strenuous exercise cause increase in Platelet count with Platelet activation as was established by increased plasma PF4 and  $\beta$ TG which are the products released from  $\alpha$ - granules. In some earlier studies Changes in platelet reactivity were, only observed in sedentary, but not in persons who had exercise training.<sup>8</sup> Exercise training is able to prevent platelet activation. In this study the subjects did not have exercise training but were not sedentary. We observed Platelet activation during strenuous exercise. Platelet activation may lead to promotion of thrombogenesis especially in sedentary and untrained individuals. Hence strenuous exercise in sedentary and untrained individuals has to be performed with caution. Strenuous exercise may not be beneficial to sedentary people, as there would be activation of platelets and also due to the compounding effects on coagulation, both of these would lead to number of cardiovascular diseases. The present study has strongly confirmed the platelet activation during strenuous exercise which can aggravate or precipitate various cardiovascular diseases.

**SUMMARY & CONCLUSIONS:** Current recommendations with regards to regular physical activity have to be extensively studied because hemostatic response to exercise may be influenced by numerous factors such as intensity of exercise, baseline fitness, sedentary status, and whether the exercise stimulus was acute or chronic.

This study confirmed an increase in Platelet count immediately after moderate exercise, however platelets are not activated during moderate exercise, in comparison to strenuous exercise where platelets were activated. Beta-TG and PF-4 represent true in vivo platelet activation. Platelet activation has been implicated in the pathogenesis of a number of diseases, which include atherosclerosis.

Three quarters of adults do far less than the recommended amount of physical activity. Various factors probably interact with physical activity to generate the health benefits observed with exercise, for some physical activity is more or less essential to earning a livelihood, rather than being just another activity aimed toward better health <sup>9</sup>. Current recommendations, provides further evidence to support the health promotion drive to get the large number of sedentary individuals into regular physical activity, of moderate intensity, potentially reducing the large number of deaths related to CVD and at the same time prevent unaccustomed strenuous exercise.

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