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Effects of Vitamin-E Supplementation on Platelet Aggregation and Endurance Capacity in Different Menstrual Phases of Female Athletes

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Abstract. Thirty six female subjects (Athletes=18, Sedentary=18) were recruited in the study to evaluate the effects of Vit-E supplementation (at a dose of 400mg per day for 7 days) on platelet aggregation and endurance capacity. Platelet aggregation was and endurance capacity were measured by Optical absorbance technique and exercising on bicycle ergometer, respectively. The post-supplemental values of endurance capacity (min) in sedentary females were higher than the pre-supplemental values in different phases of menstrual cycle. Female athletes also exhibited significantly higher values of post-supplemental endurance capacity than the pre-supplemental values in follicular phase, luteal phase and flow phase of menstrual cycle. Vit-E also significantly decreased the platelet aggregation in all the phases of menstrual cycle in both the groups especially during the follicular phase. From the present investigation it can be concluded that Vit-E significantly inhibited the platelet aggregation and improved the endurance capacity in all the phases of menstrual cycle in female athletes as well as in sedentary female subjects. Accordingly the study establishes the beneficial effects of Vit-E supplementation on endurance performance of the athletes.

Keywords: platelet aggregation, endurance capacity, athletes, menstrual cycle.

1. Introduction

Platelets play a key role in physiological haemostatic process and pathologic thrombosis by forming platelet plug [1]. Available evidences suggest that exercise evokes multiple effects on blood haemostasis in healthy normal subjects. A single bout of exercise is associated with a transient increase in blood coagulation [2]. Heavy physical exercise also augments platelet aggregation [3]. Evidences strongly suggested that platelet aggregation and functions are augmented with exercise [4]. Platelet count, plateletcrit and mean platelet volume are increased after a resistance exercise and all of these occur in parallel with an in vivo activation of platelet as manifested by an increase in platelet aggregation Ahmadizad et al. [5]. However, moderate intensity of exercise suppresses shear induced platelet activation as well as subsequent polymorphonuclear leukocyte adhesion to platelets and thus reduces the risks of vascular thrombosis and inflammation [6]. Saldeen et al. [7] indicated that α and γ tocopherol decrease platelet aggregation and delay the intra-arterial thrombin formation by increasing the endogenous antioxidant activity. However mixed tocopherols were found more potent in platelet aggregation than α-tocopherol alone [8].

Recent epidemiological studies indicated the potential benefits of utilization of antioxidants in the prevention of coronary diseases [9]. Vitamin E (Vit-E), a natural antioxidant has the characteristics that should have made it as an excellent choice to inhibit latelet aggregation [10]. Unchern et al. [11] indicated that Vit-E reduced platelet reactivity that resulted a delayed hypoxaemia and pulmonary occlusion that commonly occurs in spleenectomized β-thalassaemia or haemoglobin E patients.

Researchers suggested that Vit-E induced inhibition of protein kinase C leads to decreased platelet pseudopodia formation upon stimulation by agonists – a process that is instrumental in reducing platelet adhesion [12]. Vit-E also inhibits collagen induced platelet activation by blunting the formation of hydrogen peroxide [13]. Vit-E also enhances the bioactivity of nitric oxide 14].

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Female athletes have unique physiology and kaleidoscope of changing hormonal profiles throughout their reproductive life. Platelet function varies during different phases of menstrual cycle. Previous studies exhibited that strenuous exercise increases ADP-induced platelet aggregation in the mid-follicular phase of menstrual cycle [15]. Depending on the relative proportions of circulating estrogen and progesterone, there can be variation in cardiovascular parameters, which alter the vascular tone and thus effect the platelet functions. Estrogen is specifically known to alter the fibrinolytic activity and causes a detrimental increase in platelet aggregation and thrombosis.

Thus, inhibitory effect of Vit-E on platelet aggregation is quite established. But the literatures concerning the variation in platelet aggregation in different phases of menstrual cycle, especially among female athletes are scanty. Exploration on specific alteration in platelet aggregation in different phases of menstrual cycle is also insufficient. Moreover, Vit-E induced alteration in platelet aggregation in different phases of menstrual cycle among Eastern Indian female athletes has not been attempted before. Therefore the present study was conducted to evaluate the effects of Vit-E supplementation on endurance capacity and platelet aggregation in different phases of menstrual cycle of Eastern Indian female athletes.

2. Methodology :

2.1. Subjects

State level female athletes (N=18) with at least 8 years of active participation in athletic training were selected from different sports academies of Kolkata, India. Age matched female sedentary subjects (N=18) for control group with similar socio-economic background were recruited from the postgraduate students of the University of Calcutta. They were undergoing no medication and/or vitamin supplementation from three months prior to the study till the end of the investigation. Their menstrual cycle was regular with normal length (28-30 days). Sedentary subjects did not participate in any physical conditioning programme except some recreational sports. Their age was calculated from the date of birth as recorded in the University or Sports Academy Register. Body height and body weight were measured by standard weighing machine (Avery India Limited) fitted with height measuring rod.

2.2. Experimental Protocol

Subjects were asked to come to the laboratory early in the morning in fasting condition. The blood sample was collected from fasting subjects under aseptic condition with the help of a plastic disposable syringe to determine the platelet aggregation. Then standard light breakfast was offered to the subjects and they were asked to take rest for three hours. They were explained about the entire experimental protocol to allay apprehension. After recording the pre-exercise heart rate, subject's endurance capacity was measured. After completing the endurance exercise, subject was asked to take rest till she feels comfortable to leave the laboratory. Each subject was given Vit-E capsules for 7 days at a single dose of 400mg.day^{-1}. After finishing the 7 days of supplementation period, the subject reported to the laboratory in the morning of the 8th day and the similar experiments were conducted. To find out the variation in different phases of menstrual cycle, the whole experimental protocol was repeated during each phase of one's menstrual cycle, i.e.,

- 2nd or 3rd day of the menstrual cycle (Flow phase)
- Between 9th and 11th day of the menstrual cycle (Follicular phase)
- Between 20th and 23rd day of the menstrual cycle (Luteal phase)

2.3. Determination of Platelet Aggregation

Optical absorbance technique of Born and Cross [16] was employed to determine the platelet aggregation. Briefly, platelet rich plasma (PRP) was prepared from the collected blood sample and was centrifuged at 160g for 10 minutes. The supernatant PRP was drawn out and the rest of the citrated blood was centrifuged at 2000g for 10 minutes to obtain the platelet poor plasma (PPP). The platelet aggregation was measured as the change in optical density recorded as a percentage of the difference in optical density between PRP and PPP with the help of a semiautoanalyser (Microlab 100, Germany) by using the optical absorbance method.

2.4. Measurement of Endurance Capacity

The subject was allowed to perform endurance exercise till exhaustion on a magnetic brake bicycle ergometer (Max Plank Institute, Germany) with workload of 600 kgm.min^{-1} and 750 kgm.min^{-1} for the

sedentary subjects and athletes, respectively. The duration of their exercise was recorded and expressed in minutes (min) that reflected the endurance capacity of the respective subject.

2.5. Statistical Analysis

The values are expressed in mean ± standard deviation. Student's t-test was employed to test the significance of difference between means. The level of significance was set at $P<0.05$.

The Human Ethics Committee approved the experimental protocol. Room temperature and relative humidity were constantly monitored within a range of 30 – 32^{0}C and 65 – 70% respectively.

3. Results

The physical characteristics of the subjects are tabulated in table 1.

Table 1. Physical characteristics of the subjects.

Group	Age (Yrs)	Body height (cm)	Body weight (kg)	Heart rate (beats.min^{-1})
Athletes (N=18)	23.01±1.20	153.3±2.40	52.3±1.70	68.2±2.10
Sedentary (N=18)	22.87±1.83	148.0±4.15	46.6±2.68	85.0±1.73

Values are mean ± SD

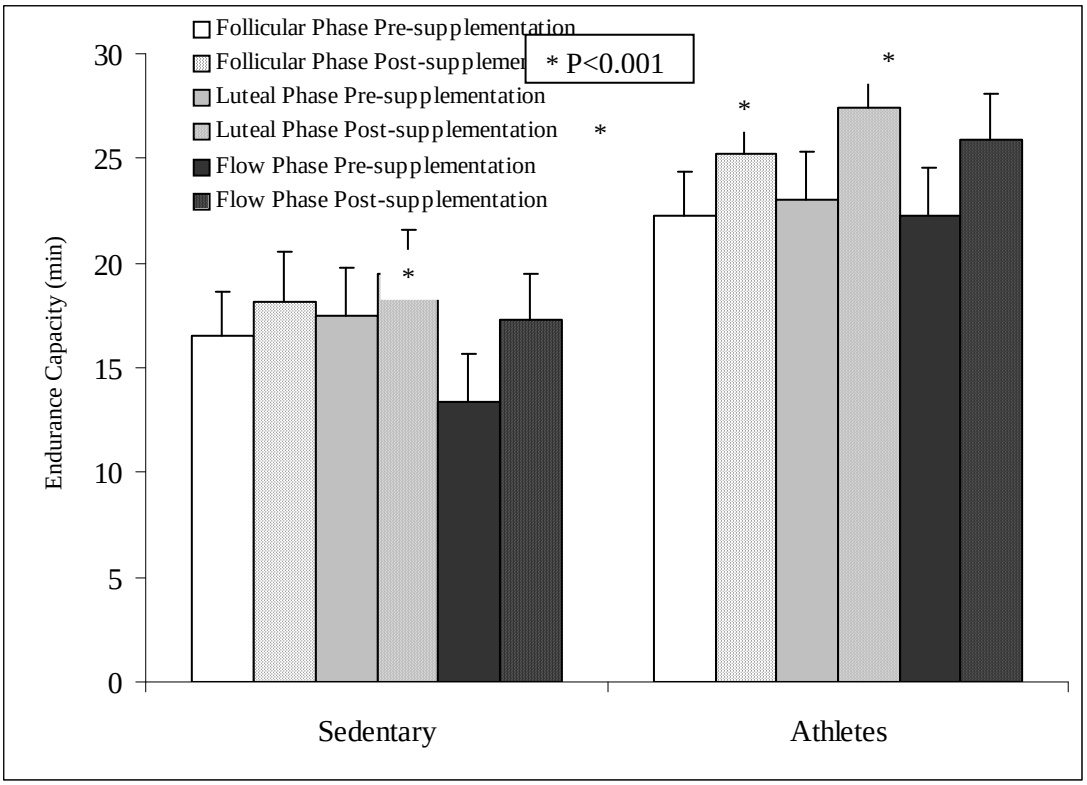

Figure 1. The endurance capacity of female athletes and sedentary females (control group) during different phases of menstrual cycle before and after supplementation of Vit-E.

The pre-supplemental mean values of endurance capacity (min) of sedentary females during follicular phase, luteal phase and flow phase were 16.50±2.1 min, 17.50±2.40 min and 13.38±2.10 min, respectively. The corresponding post-supplemental values were 22.25±3.5 min, 23.00±3.10 min and 22.25±2.60 min. It indicated that Vit-E supplementation exerted beneficial effects on endurance capacity of sedentary females during different phases of menstrual cycle. Similarly the pre-supplemental mean values of endurance capacity (min) of athletic females during follicular phase, luteal phase and flow phase were 18.13±2.0 min,

19.50±2.30 min and 17.25±2.10 min, respectively. The corresponding post-supplemental values in female athletes were 25.25±2.3 min, 27.38±2.2 min and 25.88±2.2 min. This finding also indicated that Vit-E supplementation exerted beneficial effects on endurance capacity of female athletes during different phases of menstrual cycle (Fig 1).

While analyzing the platelet function of sedentary females during three phases of menstrual cycle (table 2) it was observed that Vit-E significantly decreased platelet aggregation. The results are highly significant during all the phases of menstrual cycle especially during the follicular phase. Similarly it could be hypothesized that Vit-E significantly inhibited the platelet aggregation in all the phases of menstrual cycle in female athletes, especially during the follicular phase.

6. Discussion

Present investigation revealed that Vit-E significantly decreased exercise induced and ADP-induced rise of platelet aggregation during all the phases of menstrual cycle of female athletes and sedentary female subjects.

Table 2. Platelet function in female athletes and sedentary females (control group) during different phases of menstrual cycle before and after supplementation of Vit-E.

Group	Percentage of Optical Absorbance Following Platelet Aggregation					
	Follicular Phase		Luteal Phase		Flow Phase	
	BS	AS	BS	AS	BS	AS
Athlete (N=18)	0.069±0.005	0.099±0.008*	0.066±0.005	0.103±0.006*	0.064±0.006	0.098±0.007*
Sedentary (N=18)	0.081±0.01	0.097±0.003*	0.081±0.004	0.093±0.006*	0.079±0.005	0.096±0.003*

Values are mean ± SD. BS = Before Supplementation, AS = After Supplementation.

*P<0.001 in intra-group comparison between pre and post supplementation values.

In the present study, the platelet aggregation was decreased after Vit-E supplementation. Wang et al. [15] showed that acute exercise affects female platelet function in an intensity dependant manner in the mid-follicular phase but not in the mid-luteal phase. Leburn et al. [17] demonstrated that the estrogen at the follicular phase is specifically known to alter fibrinolytic activity and to cause a detrimental increase in platelet aggregation and thrombosis.

Moderate and strenuous exercise cause enhancement of platelet plug formation or platelet aggregation that might cause hypoxia [18]. Moreover, prolonged muscular activity increases platelet function that in turn increases plasma cortisol level which increases heart rate that imposes cardiovascular risks [19].

In the present investigation Vit-E supplementation at a dose of 400mg per day for 7 days significantly inhibited the ADP-induced and exercise induced platelet aggregation along with a significant increase in the endurance capacity in female athletes and non-athletes. Perhaps, Vit-E induced decrease in platelet plug formation might decrease the obstruction to blood flow and enabled better oxygen supply to the working muscles. In addition, Vit-E might have reduced the platelet induced increase in blood cortisol level and that in turn increased the endurance capacity. The finding of the present study might be useful in the biological preparation of endurance athletes for improving their performance. It would be perhaps because of the antioxidant role of Vit-E towards protecting the cell from exercise-induced free radical mediated oxidative stress. Therefore, from the current study it might be concluded that Vit-E significantly inhibited the platelet aggregation and improved the endurance capacity in all phases of menstrual cycle in female athletes as well as in sedentary females. So, Vit-E supplementation at a dose of 400mg per day may be recommended to female athletes as well as to the sedentary females subjected to strenuous work.

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