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Oxidative Stress And Enzymatic Antioxidant Defense Systems With Special Reference To Vitamin E Supplementation To The Sedentary Exercising Females.

Authors:

1. **Chandana Bera**, Assistant Professor, Department of Physiology, NC Medical College, Panipat, Haryana, India. Email:berachandana24@gmail.com
2. **Dr. Manila Jain*,** Professor & HOD, Department of Physiology, Index Medical College, Hospital and Research Center, Indore, MP, India.Email:gbubai90@gmail.com
3. **Subarna Ghosh**, Assistant Professor, Department of Physiology, NC Medical College, Panipat, Haryana, India. Email: gbubai@rocketmail.com
4. **Bijay Kumar Mahaseth**, Assistant Professor, Department of Biochemistry, NC Medical College, Panipat, Haryana, India. Email: gbubai82@gmail.com

***Corresponding Author:**

Dr.Manila Jain, Professor & HOD, Department of Physiology, Index Medical College, Hospital and Research Center, Indore, MP, India.

gbubai90@gmail.com

+91 8930971149.

Abstract :

Background: Exercise increases the production of free radicals, while vitamin E acts as a scavenger of free radicals, while vitamin E acts as a scavenger of free- radicals. Thus exogenous vitamin E supplementation has been shown to be effective against exercise- induced oxidative stress.The study aims to investigate the effect of vitamin E (400mg) supplementation on the endogenous antioxidant systems of the body viz. superoxide dismutase (SOD), glutathione peroxidase (GP_X) and catalase (CAT) of hundred healthy sedentary females of 18-21 years age group and comparable height and weight. **Material & Methodology:** Endurance capacity of each subject was determined by exercising them on Magnetic Break Bicycle Ergometer at a fixed workload of 600KgM/ min till exhaustion. SOD, GP_X and CAT levels were analyzed at pre and post exercise levels. The subjects were divided into two groups --- control group (n=50) and the group receiving 400 mg of vitamin E (Evion 400) (VE) supplementation for 15 days. The same experimental procedure was repeated after the supplementation tenure. **Results :**Results indicates that exercise caused a significant decrease in the CAT level ($P<0.05$) and an increase in SOD level ($P<0.001$) and GP_X level ($P< 0.05$). However, these changes in the level of SOD, GP_X and CAT were minimized after exogenous vitamin E supplementation.**Conclusion:** The entire result signifies that vitamin E is highly effective in combating exercise- induced oxidative stress in sedentary females.

Key Words: Exercise, female, endurance capacity, antioxidant enzymes, vitamin E.

Introduction:

Oxidative stress is the result of an imbalance between the generation of reactive oxygen species (ROS) and their elimination by antioxidant mechanism.[1] Physical exercise is characterized by the whole body. This leads to a decrease in antioxidant levels that could promote both an increase in the makers of lipoprotein peroxidation and damage to the erythrocyte membrane with consequent modification of the membrane fluidity.[2]

Exercise increases oxygen utilization. 2 to 5% of this oxygen from oxygen containing free radicals such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl (OH^-) radical, due to electron "lekage" at various stapes in the electron transport chain.[3]

The production of free radicals can be countered by natural defense system present within the cell. This defense includes protective enzymes, for example super oxide dismutase SOD, glutathione peroxidase GP_X, catalase CAT and use of dietary antioxidants like tocopherol (Vitamin E) etc.[4]

Vitamin E is an essential nutrient that was discovered in the 1920s. Many of physiological functions of vitamin E, including its antioxidant effects have studied for nearly 100 years.[5] Vitamin E shows numerous beneficial effects to and beyond its antioxidant properties, consequently, vitamin E is expected to prevent degenerative diseases.[6] Therefore, vitamin E supplementation has been shown to be effective against exercise induced oxidative stress.[7]

It has been observed that the inducible antioxidants like GP_X, CAT and SOD are highly influenced by the dietary intake of non-inducible antioxidant like vitamin E.[8]

Thus, the present study aims to elucidate the correlation between the immediate effect of physical exercise on the enzymatic antioxidant system and the first-hand effect of vitamin E supplementation in combating exercise-induced oxidative stress.

Materials and Methods:

Ethical Clearance: This study was conducted in Index Medical College, Hospital and Research Center, Department of Physiology. Ethical clearances were obtained from the Institutional Ethical Committee before carrying out the study.

The entire study procedures were explained and written consent was taken from each volunteer, individually for doing the study. Consent was also taken for publishing this article.

Hundred (N=100), physically fit (no cardiorespiratory disorder, no menstrual disorder) , females of Index Medical College, Hospital and Research Center students of comparable height and weight and aged 18 to 21 years were selected for the study. The subjects were subdivided into two groups – the first group (n=50) served as the control with placebo supplementation for 15 days and the second group was experimental group (V_E) (n=50) was given vitamin E 400mg (Evion 400, MeRCK Ltd.) supplementation in capsular form for the same period of time. The subjects had no history of any major disease, were not undergoing physical conditioning training.

Before the actual experiment, the details of the experimental procedure were explained to the subjects to allay apprehension. They were asked to refrain from eating at least for an hour prior

to the test and allowed to take complete rest for half an hour before the actual experiment, so that the heart rate could settle to a constant value.

Before the actual exercise, height(Cm), weight(Kg), oral temperature (in°F by clinical thermometer) and blood pressure(mmHg) by sphygmomanometer (Auscultatory method) and pre- exercise heart rate by feeling carotid artery pulsation in beats/min were recorded. Endurance capacity (min) of each subject was determined through exercise on a Magnetic Break-Bicycle Ergometer with a fixed workload of 600KgM/min till exhaustion.

At the end of exercise, endurance capacity, peak heart rate, recovery heart rate upto 30 minutes of recovery heart rate upto 30 minutes of recovery period were recorded. The entire procedure was repeated on each subject before and after vitamin E 400 mg supplementation.

Blood (5ml) was collected from the ante cubital vein before and after exercise for the determination of CAT[9], GP_X[10], SOD[11]. Blood Hb[12] was determined only before exercise.

The room temperature varied between 20°C- 24°C and the relative humidity was about 77%.

Statistical Analysis:

A two-tail 't' test by difference method was asked for testing the significance of difference between the sample means.

Results:

The physical parameters of the female subjects are shown in Table 1.

Endurance capacity (min) of the exercising female showed a significant increase ($P<0.001$) after vitamin E 400 mg supplementation, as shown in Table 2.

1^{st} ($p<0.001$), 2^{nd} ($p<0.001$), 3^{rd}($p<0.001$),4^{th} ($p<0.01$) and 5^{th} ($p<0.05$) min recovery heart rates showed significant decrease after vitamin E 400mg supplementation, as shown in Table 3. Recovery of heart rate after exercise is a good indicator of physical fitness and faster the recovery is, the better fitness is reflected.

There were insignificant effects of vitamin E 400mg supplementation on resting hemoglobin concentration, as shown in Table 4.

In Table 5., it was found that the exercise caused a significant ($P<0.05$) decrease in CAT level, significant increase in serum SOD ($P<0.001$) and significant increase in GP_X level ($P< 0.05$).

Post exercise CAT level significantly ($p<0.05$) changed after vitamin E (400mg) supplementationwhen compared with post exercise pre supplementation value, on the other hand the same for SOD level i.e significant changes ($p<0.001$) and GP_X level ($p<0.05$) when compared with post exercise pre supplementation value. (Table 5.). So the post exercise decrease in CAT level and increase in GP_X and SOD levels were minimized by 15 days of vitamin E(400mg) supplementation.

Discussion

To justify clinically, it has already been globally standardized in adults i.e, 400mg of Vitamin E per day. It is a global acceptance.[6] However regarding this particular vitamin supplementation, one can surely advocate that regular intake for 2-3 weeks uninterruptedly of Vitamin E – is the key factor for such better performance and not the discrete dose itself.

The study shows that the vitamin E supplementation is highly effective in reducing the change (increase in GPx and SOD level and decrease in CAT level) in the endogenous antioxidant enzymatic concentration following exhaustive exercise.

Exercise increases the free radical concentration and causes oxidative stress. Markers of oxidative and psychological stress are elevated during high-intensity exercise. Additionally, when energy intake does not match expenditure, women who actively participate in sports and exercise are at risk of developing menstrual dysfunction, infertility and osteoporosis.[13] This causes the reduction in the activity of the antioxidant enzyme CAT. Vitamin E is known to be potent scavenger of free radicals.[14] The use of dietary antioxidant like vitamin E to reduce exercise induced muscle injury.[15] Thus, Vitamin E supplementation scavenges the free radicals formed due to the oxidative stress due to exhaustive exercise, which thereby stabilizes the antioxidant enzymes and this increases their activity.

Vitamin E supplementation significantly increases the endurance capacity ($p<0.001$). It is known that the presence of reactive oxygen species (ROS) damages the capillary endothelial cells immensely, thereby reducing the microcirculation increased erythrocyte membrane rigidity.[2,16] Vitamin E scavenges ROS effectively, increasing the capillary circulation and thus, increasing O_2 availability to the working muscles, thereby increasing the endurance capacity.[16]

The study shows that SOD ($p<0.001$) and GPx ($p<0.05$) activity in erythrocyte increases significantly after the exercise. This may be due to the compensatory action of SOD and GPx to fight free radicals. Previous experiment cohesively suggested that the post-exercise endogenous GPx level increases.[17]

In this study post-exercise increase in SOD and GPx level reduced significantly (SOD; $p<0.001$, GPx; $p<0.05$) after Vitamin E supplementation. After exercise, Vitamin E combats against the negative effects of ROS and thus the endogenous antioxidant enzyme like SOD, GPx and CAT were not get over burdened. An adequate intake of vitamins and minerals through a varied and balanced diet remains the best approach to maintain an optimal antioxidant status. The differences between diet and exogenous supplementation as well as available tools to estimate effectiveness of antioxidant intake are also reported.[18]

Thus, it is evident for the present study that the effect of endogenous antioxidant (Vitamin E) supplementation on the endogenous antioxidant defense system (GPx, SOD and CAT) have helped the latter to be spread for other protective functions at the cellular level, thereby ensuring a better and improved capacity to defend against exercise induced oxidative stress stimulations in sedentary females. So, Vitamin E supplementation, at a measured dose (400mg per day for 15 days) might be advocated to the sedentary females for a healthy life and for a better performance.

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Tables:

Table 1. Physical Characteristics of Female Participants (Mean±SD)			
Groups	**Age (Years)**	**Height (cm)**	**Weight (kg)**
Placebo (n=50)	21.4±0.63	154.78±3.06	60.69±5.85
V_E (n=50)	21.47±0.64	156.11±3.10	55.77±3.12

Table 2. Endurance Capacity of Female Participants before and after vitamin E400mg supplementation (Mean±SD)		
Groups	**Endurance Capacity**	
	Before Supplementation	**After Supplementation**
Placebo (n=50)	7.56±0.72	7.71±0.73 NS
V_E (n=50)	6.89±0.59	13.00±0.60**
NS = Not Significant **p<0.001 = Significant		

Table 3. Recovery Heart Rates (Beats/min) of Female Participants before and after Vitamin E400mg supplementation (Mean±SD)						
Group		**1st min**	**2nd min**	**3rd min**	**4th min**	**5th min**
Placebo (n=50)	**BS**	160.0±3.78	148.13±2.77	124.00±2.00	109.33±2.47	99.2±2.37
	AS	160.27±2.60 NS	149.20±2.70 NS	124.53±2.45 NS	109.47±2.56 NS	99.47±2.88 NS
V_E (n=50)	**BS**	158.93±3.13	148.13±3.42	122.93±2.49	110.13±2.20	101.47±3.34
	AS	149.33±2.47 **	140.07±2.02 **	120.20±1.74 **	108.73±2.05 ***	100.67±2.44 *
BS = Before Supplementation AS = After Supplementation NS = Not Significant **p<0.001 = Significant,*** p<0.01= Significant, *p<0.05=Significant						

Table 4. Hemoglobin Concentration (g%) of Exercising Female Participants before and after Vitamin E supplementation (Mean±SD)			
Groups	**Hemoglobin Concentration (g%)**		**Level of Significance**
	Before Supplementation	**After Supplementation**	
Placebo (n=50)	12.33±0.11	12.40±0.11	NS
V_E(n=50)	12.17±0.18	12.38±0.15	NS

NS = Not Significant

Table 5. Exercise induced changes in Oxidative stress Markers (Vitamin, Glutathione Peroxidase, Catalase, Glutathione Peroxidase, Catalase and Glutathione) level of Exercising Female Participants before and after Vitamin E(400mg) supplementation (Mean±SD)

Enzyme Parameters	Groups								Level of Significance
	Placebo (n=50)				V_E(n=50)				
	Before Supplementation		After Supplementation		Before Supplementation		After Supplementation		
	Pre-Exercise	Post-Exercise	Pre-Exercise	Post-Exercise	Pre-Exercise	Post-Exercise	Pre-Exercise	Post-Exercise	
GP_X Level (n mole NADPH oxidized/min/g of Hb)	24.23±2.67	28.4±2.58	24.09±2.39	28.32±2.73	24.5±2.63	28.5±2.38	24.7±2.51	26.1±2.21	1 & 2 = p<0.05 3 & 4 = p<0.05 5 & 6 = p<0.05 2 & 4 = NS **6 & 8 = p<0.05**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
CAT level (K X 10^{-2} units/g of Hb)	10.35±0.32	9.12±0.38	10.33±0.36	9.10±0.40	10.32±0.37	9.11±0.37	10.40±0.32	10.19±0.27	9 & 10 = p<0.05 9&11=NS 11 & 12 = p<0.05 13 & 14 = p<0.05 **14 & 16 = p<0.05**
	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**	
SOD level (units/mg protein)	0.32±0.06	0.56±0.06	0.31±0.07	0.53±0.06	0.33±0.05	0.54±0.06	0.33±0.06	0.46±0.07	17 & 18 = p<0.001 17&19 = NS 19 & 20 = p<0.001 21 & 22 = p<0.001 **22 & 24 = p<0.001**
	17	**18**	**19**	**20**	**21**	**22**	**23**	**24**	

NS = Not Significant, p<0.001 highly significant, p<0.05 Significant.

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Conflict of Interest:

We the authors of this study, hereby declare that no financialandnon-financialrelationshipsandactivitiesrelatedtothemanuscriptwithentitiessuchasgovernmentagencies,foundations,commercialsponsors,academicinstitutions. We also disclose that, no resources have been receivedeitherdirectlyorindirectly(viainstitution)relatedtothemanuscriptfromthetimeofinitialconceptionandplanningoftheworkto the present. We also disclose that there are no relationships or activities that are topically but not directly related to the work. This is for a complete listing thathelps to reassure readers of authors' commitment to transparency.