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# Effect of Vitamin Supplementation on Exercise Induced Oxidative Stress in Trained Elite Indian Cyclists

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## Abstract

It has been established that intense physical exercise is associated with an increase in free radical production. Our body has defense system to combat the deleterious effect. But, when the body's natural defense system against free radicals are overwhelmed, oxidative stress increases. In the present investigation, we studied the effect of vitamin supplementation (composed of 400 IU of vitamin E and 500 mg ascorbic acid for 2 months) on oxidative and enzymatic exercise stress markers during endurance training activity in 50 trained elite Indian cyclists attending national camp at Sports Authority of India, Netaji Subas National Institute of Sports (SAI-NSNIS), Patiala, India. Serum concentrations of ascorbic acid, alpha tocopherol, malondialdehyde (MDA), superoxide dismutase (SOD) and catalase were measured before supplementation and after completion of 2 months of antioxidant supplementation. Antioxidant supplementation led to significant increase in serum alpha-tocopherol and ascorbic acid from pre-supplementation to post-supplementation stage. Serum MDA concentration and SOD activity decreased significantly after 2 months of antioxidant treatment. There was increase in serum catalase activity after supplementation compared to before supplementation. The results of the present study suggest that antioxidant supplementation may strengthen the antioxidant defense system, thus reducing the oxidative stress produced after endurance training in elite Indian cyclists.

**Keywords:** Antioxidant supplementation; Ascorbic acid; Alpha tocopherol; Malondialdehyde (MDA); Superoxide dismutase (SOD); Catalase

  

## 1. Introduction

Exercise has been known to increase oxygen consumption. 95-98% of the oxygen consumed is reduced to water during aerobic metabolism, but the remaining fraction may be converted to oxidative by-products like reactive oxygen species (ROS). During exercise, when volume of oxygen consumption ($VO_2$) is elevated to 10-15 folds above rest, it is very likely that free radicals are produced to a greater extent, compared with the rest. Considering that thousands of radicals are produced in each resting cell every day, it is tempting to speculate on the number of free radicals, which may be produced as a result of elevated metabolism [1].

ROS may damage body tissues, if their production is not controlled precisely and adequately. Irreversible oxidative damage to certain vulnerable by-molecules is thought to contribute to the degenerative process associated with cell breakdown and aging. It is now widely accepted that free radical generation is enhanced during strenuous exercise [2]. To combat the deleterious effects of free radicals and ROS, our body has some complex internal protective mechanisms like enzymatic defenses, which include primary antioxidative enzymes like SOD, catalase, glutathione peroxidase and non-enzymatic defenses like vitamin C, vitamin E, ubiquinol co-enzyme Q-10 and reduced glutathione. During exercise, the pro-oxidant/antioxidant balance shifts in favor of the former, with the rate of radical and ROS production exceeding their rate of removal by the antioxidant defense mechanisms [3].

Therefore to avoid or minimize deleterious affects of exercise induced oxidative stress the antioxidant capacity of the cell must be increased. Therefore this increased capacity may be achieved through appropriate training, diet and most importantly through the use of antioxidant supplementation. In this study we studied the effect of vitamin supplementation on exercise induced oxidative stress in elite trained cyclists.

## 2. Material and Methods

Fifty elite cyclists (male-32, female-18) participated in this investigation. They all were homogenous population with regard to age and life style patterns. Cyclists had experience of several years of long distance cycling and trained with a mean weekly cycling distance of 90 km. In selecting the cyclists, a treadmill test (spiroergometry) was performed to determine the performance capacity and $VO_2$ max uptake. Performance level on the treadmill started with a speed of 8 km/h and increased every 3 min. by 2 km/h up to exhaustion of the cyclists. The nature and purpose of the study and the risks involved were explained verbally and in writing. Before the investigation a written informed consent to participate was obtained.

**Body Composition:**

Body weight was measured in Kilograms employing a calibrated beam scale. Height was measured in centimeters with a scale-mounted anthropometer. The descriptive data for age and anthropometric measures for the test group is presented in Table 1.

**Table 1. Age and anthropometric measures for the test group**

| | **Cyclists** |
|---|---|
| ***Age (Years)*** | 21.2±10.43 |
| ***Height (cms)*** | 168.36±11.02 |
| ***Weight (Kg)*** | 63.14± 12.51 |

All values are Mean ± S.D

**Antioxidant supplementation:**

Each subject in the experimental group was supplemented with 400IU/day of vitamin E (purchased from M/S. E. Merck, Darmstadt, Germany) and 500mg/day of vitamin C (purchased from M/S Glaxo SmithKline, Brentford, UK) for a period of two months. None of the participants had, however, received vitamin E or vitamin C or any other antioxidant

  

supplement within two months of entry into the study. The subjects had a standardized diet during the course.

**Serum investigations:**

Prior to testing and at the same time of day (6.30-7.30 AM), blood samples were drawn, following 15 min. of sitting, from the medial cubital vein. Serum was separated from whole blood. Each sample was assayed for lipid peroxidation marker, malondialdehyde (MDA), superoxide dismutase (SOD), catalase, vitamin E and vitamin C before and after vitamin C (500 mg per day) and vitamin E (400 IU per day) supplementation.

**Malondialdehyde (MDA), SOD, Catalase, vitamin E and C estimation**

Malondialdehyde (MDA) was measured by the thiobarbituric acid method of Beuge and Aust [4]. SOD was assayed by the method of Kono [5]. This assay was based on the principle of the inhibitory effect of SOD on the reduction of Nitro blue tetrazoliukm (NBT) dye by superoxide anions which were generated by photo-oxidation of hydroxylamine hydrochloride ($NH_2OH$ .HCl). To estimate the serum catalase activity, method of Luck [6] was used. It was based on the principle that catalase catalyses the breakdown of $H_2O_2$. Vitamin E was determined employing the method of Martinek [7] and vitamin C was measured by the method of Roe and Kuether [8].

**Statistical Analysis:**

Mann-Whitney U test was employed to establish statistical significance of mean differences between different parameters. An alpha level of 0.05 was accepted as significant.

**3. Results and Discussion**

In the present study we observed a decrease in MDA ($P<0.001$) concentration after supplementation of vitamin C (500 mg per day) and vitamin E (400 IU per day) for the period of 2 months in trained subjects (elite cyclists) (Fig. 1). Our results are in agreement with the findings of Tauler *et al*. [9] and Machefer *et al.* [10], who also observed decrease in MDA concentration after cocktail supplementation of vitamin C (150.0 mg./day), vitamin E (24.0 mg/day) and beta-carotene (4.8 mg/day) to athletes undergoing marathon des sables (MDS)- that consisted of six long races in the desert. Animal experiments by Williams *et al.* [11] have also shown that in horses undergoing intense and moderate exercise, supplementation with vitamin E and vitamin C led to decrease in MDA.

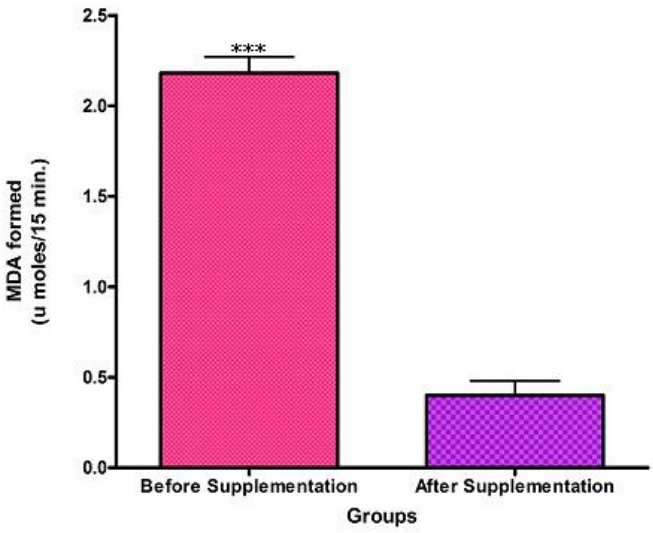


**Figure. 1.** Serum MDA levels in elite cyclists before and after supplementation of vitamin E (400 IU/day) and vitamin C (500mg/day) for two months. *** signifies $P<0.001$.

Interestingly, measurements of SOD activity, an important enzymatic parameter of antioxidant defense system, were reported to be decreased ($P<0.05$) and catalase activity was found to be increased ($P<0.05$) in post supplemented group as compare to presupplemented group of elite cyclists (Table 2). The study of Kyparos *et al.* [12] also reported that base line lymphocyte catalase activity increased and SOD activity decreased after supplementation of vitamin C (500 mg /day) for eight weeks. Studies on supplementation of cocktail of vitamin E (500 mg/d) and β-carotene (300 mg/d) for 90 days and last 15 days with ascorbic acid (1 g/day) by Sureda *et al.* [13] and after 152 mg vitamin C/day and 50 mg vitamin E/day supplementation to athletes undergoing intense physical training by Goldfarb *et al.* [15]

  

observed an increase in catalase activity and decrease in SOD activity. But the contradictory study of Rokitzki *et al*. [14] on trained long-distance runners did not show any significant difference in Catalase activity measured in erythrocyte before and after the competition in athletes supplemented with α- tocopherol (400 IU/day) and ascorbic acid (200 mg/day) during 4.5 weeks prior to marathon race. Different diet pattern, leaving conditions and different practice schedule may lead to variations in results.

**Table 2. Serum superoxide dismutase (SOD) and serum catalase activity in elite cyclists before and after supplementation of vitamin E (400 IU/day) and vitamin C (500mg/day) for two months.**

| Group | SOD activity (Units/mg protein) | Catalase activity (μ moles of $H_2O_2$ consumed/mg protein/ min) |
|---|---|---|
| Control (Prior supplementation) (N - 50) | 14.6 ± 0.87 | 53.0 ± 6.10 |
| Test (After Supplementation) (N – 50) | *13.24 ± 3.21 | *65.23 ± 4.48 |

*P<0.05, All values are Mean ± S.D

We observed that vitamin E and C supplementation for two months reported to increase the vitamin E (P<0.05) and vitamin C (P<0.001) levels of elite Indian cyclists as compare to their prior supplementation values (Fig. 2 and 3). These findings are in accordance with the finding of Rokitzki *et al.* [14] and Goldfarb *et al.* [15], in which they also reported an increase in vitamin E and C concentration after supplementation. However there is paucity of literature regarding the effect of vitamin C and E supplementation on exercise induced oxidative stress in elite Indian cyclists.

In this study, a drastic decrease in lipid peroxidation marker (MDA) after vitamin E (400 IU /day) and vitamin C (500mg/day) supplementation for two months was observed. This suggests that vitamin E and C supplementation may lead to a decrease in exercise induced oxidative stress. In addition low activity of SOD as less free radicals were available for dismutation and higher levels of vitamin E and C were observed which indicates a sign of improved antioxidative system after vitamin E and C supplementation for two months. The results of the present study bring out that supplementation with vitamin C and vitamin E improves the antioxidant system which may lead to improved performance in elite cyclists.

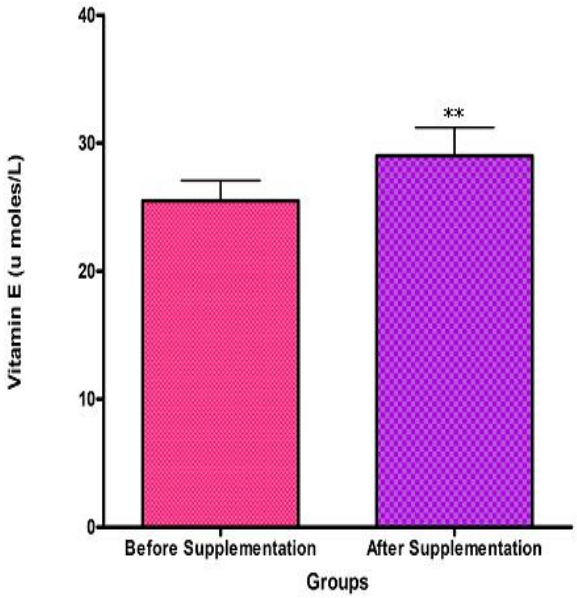


**Figure. 2.** Vitamin E levels in elite cyclists before and after supplementation of vitamin E (400 IU/day) and vitamin C (500mg/day) for two months. ** signifies P<0.05

  

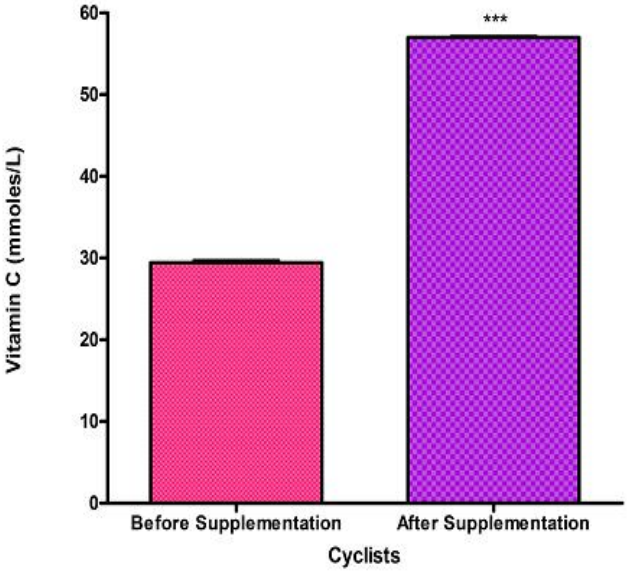


**Figure. 3.** Vitamin C levels in elite cyclists before and after supplementation of vitamin E (400 IU/day) and vitamin C (500mg/day) for two months. *** signifies P<0.001

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