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Impact of Spirulina on Exercise Induced Oxidative Stress and Post Exercise Recovery Heart Rate of Athletes in Comparison to a Commercial Antioxidant

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Abstract

Spirulina, blue green algae is known for its potential health benefits and ergogenic effects. However, benefits on post exercise recovery heart rate are not fully established. In this background, the aim of the present investigation was to study the effect of spirulina on exercise induced oxidative stress and post exercise recovery heart rate of athletes in comparison to a Commercial Antioxidant Supplement (CAS). Ninety male athletes were divided into (n = 45) two groups: (1) Aerobic Exercise Group (AEG) and (2) Mixed Exercise Group (MEG). The exercise groups were further randomly divided into (i) Control Group (CG), (ii) Experimental Group I (EGI), and (iii) Experimental Group II (EGII) with 15 subjects in each group in a double-blind fashion. The CG did not receive any supplement. Spirulina (3g/day) for EGI and CAS (1 capsule/day) for EGII were administered for 60 days. During supplementation, the control and experimental groups performed the same volume of training and dietary intake. Anthropometric measurements and dietary intake were assessed. A blood test was done to determine the pre- and post-levels of Malondialdehyde (MDA). Cardiopulmonary exercise testing was done pre- and post-supplementation to assess the physiological transients. Supplementation of spirulina and CAS both showed significantly improved recovery heart rate (EGI MEG Before: 125±1.46; After 120±1.03, p<0.01; EGI AEG Before: 117±3.40; After: 115±2.79 p<0.01; EGII MEG Before: 127±2.17; After 123±2.01, p<0.01; EGII AEG Before: 121±3.33; After: 113±2.06, p<0.01) and reduced peak lactate (EGI MEG Before: 11.8±0.56; After 10.9±0.56, p<0.01; EGI AEG Before: 14.7±0.89; After: 12.6±0.64 p<0.01; EGII MEG Before: 11.4±0.45; After 10.1±0.37, p<0.01; EGII AEG Before: 14.1±0.85; After: 12.5±0.66, p<0.01) and malondialdehyde (EGI MEG Before: 1.57±0.08; After 1.16±0.02, p<0.05; EGI AEG Before: 2.3±0.11; After: 1.37±0.07 p<0.05; EGII MEG Before: 1.54±0.05; After 1.14±0.02, p<0.05; EGII AEG Before: 2.34±0.11; After: 1.34±0.06, p<0.05) in both the exercise groups and didn't show any significant difference between EGI and EGII. Short term supplementation of spirulina and CAS will improve recovery; attenuates the protection against exercise induced oxidative stress.

Keywords: Antioxidants; Exercise Performance; Oxidative Stress; Spirulina

Introduction

Strenuous physical exercise results in an enhanced uptake of oxygen, leading to increased metabolism, which in turn increases the production of active oxygen radical species produced by the electron transport system [1]. Active oxygen species, including hydroxyl radicals, superoxide radicals, hyper oxides and aldehydes are known to be toxic, mutagenic and carcinogenic to cells [2].

Antioxidants produced by the body act in concert with their exogenous mainly dietary, counterparts to provide protection against the ravages of reactive oxygen as well as nitrogen species [3]. The metabolic rate may increase as mush a tenfold during physical exercise, enhancing leakage of oxygen from the mitochondria to the cytosol. The rise in oxygen free radical concentrations could exceed the protective capacity of cell antioxidant defense systems [4]. Antioxidant supplementation is likely to provide beneficial effects against exercise induced oxidative tissue damage. There has been considerable interest in the antioxidant properties of various vitamins and minerals [5]. This interest was fueled in part by studies demonstrating that nutrients antioxidants, including vitamins C, vitamin E and beta carotene, have a role in protecting cells from oxidative free radical damage. However, there are many conflicting reports concerning antioxidant supplementation and exercise performance in athletes [6-11]. In this context, it becomes rather difficult to come to a consensus and generalize the results pertaining to sports nutrition to wide population of the respective field. Accordingly, the present study was undertaken to focus the impact of antioxidant supplementation on exercise performance of athletes.

Prolific antioxidant preparations are commercially available each year. The significance of a blue green algae spirulina as nutrient rich supplement is extensively examined. However, its role sports nutrition is not fully established. Spirulina has a high content of beta-carotene and Vitamin- E [12,13] that can improve antioxidant status in athletes [14]. Therefore, in the present context, a rationale for antioxidant supplementation to prevent free radical damage to muscles and delay fatigue during exercise has been examined. In this background, the aim of the present investigation was to study the effect of spirulina on exercise induced oxidative stress and performance of athletes in comparison to a commercial antioxidant supplement. The study hypothesized that supplementation of spirulina may reduce serum malondialdehyde, thus improve exercise performance in terms of improved recovery heart rate, exercise time and aerobic capacity.

Materials and Methods

Subjects

The subjects selected were Indian athletes undergoing train-

ing at the centre of excellence and state training centre, Nethaji Subhas National Institute of Sports (NSNIS), Patiala, Punjab. The sampling procedure adopted was purposive sampling. All the subjects were healthy and declared medically fit by a sports medicine expert. The subjects were briefed on the purpose of the study and the experimental protocol and their written consent was taken. The procedures were in accordance with the Helsinki Declaration of 1975 and approved by the institutional review board.

Ninety male athletes in the age range of 15-21 yrs were recruited as subjects for the study. Among them, 45 subjects in the cycling and running athletic category were assigned to Aerobic Exercise Group (AEG) and 45 hockey players to the Mixed Exercise Group (MEG). The aerobic and mixed exercise groups were further classified as Control, Experimental I and Experimental II groups with 15 subjects in each group. The subjects were controlled for sex, age and anthropometry.

Study Design

The investigation was conducted in three phases. In the first phase, initial data on anthropometric measurements such as height, weight and skin fold measurement were obtained and body mass index, body fat and lean body mass were calculated. Serum malondialdehyde level was measured. The subjects performed an exercise test till exhaustion. The physiological transients such as exercise heart rate, recovery heart rate, total exercise time, maximum aerobic capacity and peak lactate were measured.

In the second phase, the Experimental Group I (EGI) received 3g/day of spirulina (Sunova, Dabur Pvt. Ltd) and the Experimental Group II (EGII) received 1 capsule/ day of Commercially Available Antioxidant Supplement (CAS) (Selace Forte, Universal Medicare Ltd) for 60 days. The dose of spirulina was fixed through matching its beta carotene content with that of the commercial antioxidant. In the third phase, after 60 days of supplementation, both the exercise groups performed graded cycle ergometric test till exhaustion. A repeat estimation of Serum malondialdehyde and cardiopulmonary responses was conducted.

Anthropometric Measurements

Height was measured using stadiometer (Seca model 220, UK). Recordings were made nearest to 0.1 cms. Weight was measured using electronic weighing machine with the subject wearing minimum clothing and recordings were made nearest to 0.1kg (Seca Alpha 770, UK). Body mass index was calculated from Quetlet's equation (wt/ht2) (kg/m2). A skinfold caliper (Holtain Limited, UK) was used to assess the body fat percentage following standard methodology [15]. The skinfold was taken from four different sites of the body (biceps, triceps, subscapular, and suprailiac) using the skinfold caliper on the right side of the body. LBM was computed using the formula given by Durnin and Rahman (1967) [16].

Estimation of Malondialdehyde

Participants were instructed to fast at least 8-10 hours prior to their assessment. A venous blood was drawn from each subject for the determination of MDA. It was measured by the thiobarbituric acid method [17].

Exercise Testing

The subjects underwent cardiopulmonary exercise testing, to assess the physiological transients such as heart rate, maximum aerobic capacity, endurance capacity and peak lactate.

Exercise test was performed on an electronically operated computerized bicycle ergometer (ER 900: Erich Jaeger, Germany), using a test protocol that consisted of graded cycle ergometry. The subjects were asked to warm up and cycle at 60 RPM for two minutes without any load. This constituted the reference phase. The test phase followed next. The initial load in the test phase was fixed at 50 watts, and increased by 50 watts every 2 min till exhaustion, consequent to which, the subjects were asked to cycle briskly at a rate of 60 RPM without any load during the entire period of recovery for a total period of 3 minutes (active rest: recovery phase). The time taken till exhaustion was recorded to determine the maximal endurance performance. The test was carried out in an air-conditioned room, with temperature and humidity levels controlled at 230 C+20C and 55+5 % respectively. The tests were carried out at least 3 hours after last meal.

All measurements were carried out using portable metabolic analyzer, Cosmed K4 (Cosmed srl, Italy). The heart rate was recorded through heart rate sensor (Polar HR sensor, USA). Total exercise time till exhaustion was recorded to determine the maximal endurance performance. Blood samples were drawn from a forearm vein after exercise to analyze the peak blood lactate. Peak blood lactate was analyzed using lactate analyzer (1500, YSI, USA).

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 16.0 was used for the analysis. Mean and standard error was applied for all the variables. Two -Way Analysis of Variance (ANOVA) was applied to study the differences between the control and the two experimental groups and between aerobic and mixed exercise groups. Significant differences were determined by the Duncan Multiple Range Test (Post hoc). Differences were considered significant at p<0.05.

Results

The physical profile of the subjects (Table1) reveals that both the groups (AEG and MEG) differ for almost all the anthropometric parameters and indexes calculated using anthropometric measurements. The height, weight, body mass index and lean body mass were significantly higher (p<0.01) and body fat percentage was significantly lower (p<0.01) in AEG than MEG. This could

be due to demand of the particular sport, further the weights may differ according to the energy intake and expenditure patterns set during training campaign.

	Mea			
Parameter	MEG (N=45)	AEG (N=45)	't' Value	
Age (yrs)	17.40±0.16	18.69±0.162	5.52*	
Height (cms)	171.46±0.72	175.33±1.20	2.25*	
Weight (kg)	59.87±0.66	66.06±1.053	4.96*	
Body Mass Index (BMI)	20.30±0.221	21.47±0.254	3.93*	
Body Fat (%)	17.96±0.320	14.88±0.366	6.33*	
Lean Body Mass (kg)	48.10±1.10	56.18±0.895	5.67*	

MEG: Mixed Exercise group; AEG: Aerobic Exercise Group

*Significant at p < 0.05

Table 1: Anthropometry and Body Composition of Subjects.

Before supplementation, the physiological parameters such as maximum heart rate (Max HR), recovery heart rate (Rec HR) at 180 seconds, maximum oxygen uptake (VO2 max), total exercise time and peak lactate did not show a significant difference between control and experimental groups, but a significant difference was found between AEG and MEG. The VO2 max (Table 2), total exercise time (Table 3) and peak lactate (mean difference: Figure 3) of AEG was significantly higher (p<0.05) and Rec HR was significantly lower (p<0.05) than that of MEG (Table 4).

	Before Supplementation		After Supplementation			
Group	Category Mean±SE		ANO- VA	Category Mean±SE		ANO- VA
	MEG	AEG	Value	MEG	AEG	Value
CG (N=15)	53.7°± 1.98	59.3 ^b ± 1.24	F _{Group} = 1.29NS	53.5 a±1.89	60.4 ^b ± 1.21	$F_{Group} = 2.30 \text{ NS}$
EGI (N=15)	55.9°± 1.68	59.6 ^b ± 1.46	F_{Category} $= 6.49^*$	56.2 a±1.21	60.5 ^b ± 1.45	F _{Category}
EGII (N=15)	54.6°± 2.05	55.6 ^b ± 1.27	$=6.49^{*}$	55.6°± 1.48	56.1 ^b ± 1.16	= 9.17*

MEG: Mixed Exercise group; AEG: Aerobic Exercise Group; CG: Control Group; EGI: Experimental Group I, EGII: Experimental Group II

* Significant at p < 0.05, NS Not significant

Means having the same superscript do not differ significantly at p < 0.05

Table 2: Maximum Oxygen uptake (ml/kg/min) of MEG and AEG Subjects before and after Supplementation.

	В	efore Supplementati	on			
Group	Category Mean±S.E		ANOVA Value	Category Mean±S.E		ANOVA
	MEG	AEG		MEG	AEG	Value
CG (N=15)	8.02°±0.28	9.04b±0.23	$F_{Group} = 0.024^{NS}$	7.87°±0.26	9.11 ^b ±0.22	F _{Group} = 0.70NS
EGI (N=15)	8.35°±0.21	8.79b±0.18	$F_{\text{Category}} = 12.11^*$	8.48°±0.18	8.93b±0.14	$F_{\text{Category}} = 16.98^*$
EGII (N=15)	8.29°±0.25	8.77b±0.17		8.51°±0.18	8.84 ^b ±0.17	

MEG: Mixed Exercise group; AEG: Aerobic Exercise Group, CG: Control Group; EGI: Experimental Group I, EGII: Experimental Group II,

Means having the same superscript do not differ significantly at p < 0.05

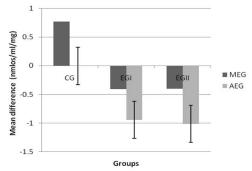
Table 3: Total Exercise Time (min) of MEG and AEG Subjects before and after Supplementation.

	Before Supplementation			After Supplementation		
Group	Category Mean±S.E		ANOVA	Category Mean±S.E		ANOVA
	MEG	AEG	Value	MEG	AEG	Value
CG (N=15)	129°±2.45	119 ^b ±2.72	$F_{Group} = 1.44NS$	131°±1.62	123b±2.22	F _{Group} = 14.97*
EGI (N=15)	125°±1.46	117 ^b ±3.40	$F_{Category} = 16.13^*$	120°±1.03	115 ^b ±2.79	$F_{\text{Category}} = 22.20^*$
EGII (N=15)	127°±2.17	121 ^b ±3.33		123°±2.01	113b±2.06	

MEG: Mixed Exercise group; AEG: Aerobic Exercise Group, CG: Control Group; EGI: Experimental Group I, EGII: Experimental Group II

Means having the same superscript do not differ significantly at p < 0.05

Table 4: Recovery Heart Rate Responses at 180 Seconds (bpm) of MEG and AEG Subjects before and after Supplementation.



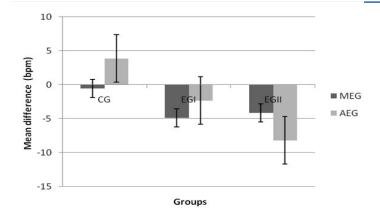
MEG: Mixed Exercise group; AEG: Aerobic Exercise Group, CG: Control Group; EGI: Experimental Group I, EGII: Experimental Group II

Figure 3: Mean Change in Malonaldehyde Status (nmoles/ml/mg) of the Subjects after Supplementation.

After supplementation both the exercise groups (AEG & MEG) showed no significant difference between control and experimental groups for Max HR, VO2 max and total exercise time, but a significant difference was observed for Rec HR at 180 sec and peak lactates. The Rec HR at 180 seconds of AEG and MEG showed a significant difference between control and experimental groups. The recovery, in experimental groups was significantly faster than that of control group. However, EGI and EGII of AEG and MEG did not differ significantly. After supplementation, the Recovery was significantly faster in AEG (p<0.05) than MEG (Figure 1). The peak lactate was significantly lower in both the experimental groups than control groups in both AEG and MEG (Figure 2).

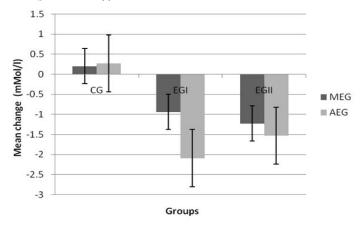
^{*} Significant at p < 0.05, NS Not significant

^{*}Significant at p < 0.05, NS Not significant



MEG: Mixed Exercise group; AEG: Aerobic Exercise Group, CG: Control Group; EGI: Experimental Group I, EGII: Experimental Group II

Figure 1: Mean Change in Recovery Heart rate at 180seconds (bpm) of the Subjects after Supplementation.



MEG: Mixed Exercise group; AEG: Aerobic Exercise Group, CG: Control Group; EGI: Experimental Group I, EGII: Experimental Group II

Figure 2: Mean Change in Peak Lactate (mMol/l) of the Subjects after Supplementation.

Before supplementation the exercise groups showed no significant difference in the MDA status between control and experimental groups (Figure 3). The values of AEG control and experimental groups were significantly different (p<0.05) when compared with respective groups of MEG. After supplementation, a decreased MDA was observed in both AEG and MEG experimental groups (EGI & EGII) when compared with their control groups (p<0.05). No significant differences were found between EGI and EGII for MDA. The decrease in MDA observed for EGI and EGII of AEG was greater than that of EGI and EGII of MEG.

Discussion

In the present study, beta carotene level of supplementation with spirulina and CAS (5000 μ g/d) is comparable to the supplement levels provided by other research works. Whereas, in the case

of vitamins - E and C both the supplements have a varying level of these vitamins and further when compared with other research data their supplementary levels are many times lower. Extant literature reveals no specific dosage for these antioxidant vitamins for sports persons. The dose of spirulina was fixed through matching its beta carotene content with that of the commercial antioxidant. Compositionally the two supplements are different and they are matched only with regard to beta carotene. To the EGI 3g/day of spirulina and to the EGII 1capsule/ day of CAS was given. The receptivity of these two experimental groups was compared against the control group.

Exercise results in increased amounts of MDA in blood, serves as an indicator of lipid peroxidation. The spirulina supplemented group (EG I) and CAS supplemented group (EG II) of AEG and MEG showed decreased MDA. Although the CAS and spirulina differs in composition in terms of nutrients, no significant difference was observed in decreased MDA. This shows similar effect of supplementary action with spirulina and CAS on reduction of oxidative stress induced by exercise. The increased MDA levels in the control group than antioxidant supplemented group indicated that exercise induces oxidative stress and to initiate lipid peroxidation in lymphocytes [18] Supplementation with vitamin-C, vitamin-E and other antioxidant mixtures can reduce symptoms or indicators of oxidative stress [19,20]. Gauze-Gnagne, et al. (2015) [21] found significantly decreased MDA level with supplementation of spirulina after a marathon run in athletes. Lipid peroxidation was reduced in both AEG and MEG with antioxidant supplementation than control group. Tiidus (1995) [22] observed reduced lipid peroxidation after antioxidant supplementation (500mg/d vitamin-E, 1g/d of vitamin-C and beta carotene 30mg/d of beta carotene). Reuiz, et al. (2005) [23] suggests that in moderately trained cyclists antioxidant supplementation counters oxidative stress induced by exercise.

Lipid peroxidation was high in AEG than MEG. This could be due to nature of sport events of the particular exercise group. Free radical production within the body depends on exercise intensity, resulting in considerably high muscle damage [24]. Intense exercise produces oxidative stress and cellular damage in lymphocytes. Therefore, athletes regularly participating in acute high intensity exercise may require higher intakes of exogenous antioxidants to defend against increased oxidative stress during exercise, which can be met through an adequate intake of antioxidant rich foods [25].

During exercise, the pro-oxidant/ antioxidant balance shifts in the favor of the former, with the rate of radical and respiratory oxidative species production exceeding their rate of removal by the antioxidant defense mechanisms. Therefore, to avoid or minimize skeletal muscle damage the antioxidant capacity of the cell must be increased. This increased capacity may be achieved through appropriate training, diet and the use of antioxidant nutritional

supplements. Supplementation with vitamin A, C, other dietary antioxidants and antioxidant mixtures can reduce the symptoms or indicators of oxidative damage as a result of exercise [11,26]. Before supplementation the PHR, Max HR, Rec HR, VO2 max, total exercise time and peak lactate of MEG and AEG showed no significant difference between control and experimental groups. This shows that the subjects inducted in the control, EGI and EGII were in similar cardiopulmonary fitness. This facilitates the study to examine the effect of supplementation on physiological transients of the subjects.

After supplementation with spirulina (EGI) and CAS (EG II) no significant difference was found for Max HR, total exercise time and VO2 max, but a significant difference was found for recovery heart rate and peak lactate. Nielson, et al. (1999) [8] found no effect of antioxidant supplementation for 6 weeks on VO2 max in triathletes. Iton, et al. (2000) [9] observed no significant difference in VO2 max and Max HR in runners receiving 1200 IU/d of vitamin -E for 6 days. Oostenburg (1997) [27] observed that supplementation of 300 IU/d of vitamin- E for 3 weeks does not improve exercise performance in endurance athletes. Powers (1987) [28] observed no measurable change in bicycling performance with the 6-week supplementation of ascorbic acid in terms of heart rate and lactate.

Balakrishna and Anuradha (1997) [10] found no beneficial effect of alpha to copherol and vitamin -C on VO2 max of athletes. Knechtle, et al. (2008) [29] found no significant difference with regular uptake of vitamin and mineral supplement for 4 weeks before the multistage race. Brytant (2003) [11] reported that 400IU/d of vitamin-E, 1g/d of vitamin-C will provide adequate protection against lipid peroxidation; however, either alone or in combination will enhance exercise performance. Clarkson and Thompson (2000) (20) reported that dietary antioxidant supplements can reduce the indicators of oxidative stress as a result of exercise, however have no beneficial effect on performance. The above studies indicated that dietary supplements either long term or short term would not enhance exercise performance. The results of the present study also in agreement with the previous studies that supplementation of spirulina or CAS had no effect on few physiological transients such as VO2 max, exercise time and maximum exercise time.

In our study, we have observed significantly decreased recovery heart rate and peak blood lactate in EGI and EGII than CG in both AEG and MEG. This shows the improved aerobic fitness with supplementation of spirulina and CAS. Intensive exercise leads to maximum activation of glycolysis. Lactate formed in this reaction diffuses into blood and enters the liver where it is converted to glucose. Thus, lactate production temporarily substitutes for the aerobic metabolism of glucose [30]. In the present study, the low blood concentration of lactate shows a high degree of aerobic fitness. Increase in oxidative stress due to eccentric exercise leads

to muscle damage, weakness [31,32] or inflammation [33,34] and recovery can be improved with antioxidant supplementation. Antioxidant supplementation may interfere with cellular signalling function of ROS, and therefore prevent the adaptations that are necessary for performance improvements [35].

No significant difference was found for recovery heart rate and peak lactate between EGI and EGII. This shows that supplementation of spirulina and CAS had similar effect and improves few cardio pulmonary transients. Schroder, et al. (2004) [6] observed a significant decrease of lactate dehydrogenase activity with supplementation of 600 mg/d of vitamin-E, 1000 mg/d of vitamin-C and 3200 μ g/d of beta carotene for 35 days in habitual training activity of basket ball players. Tauler (2004) [26] also observed decreased lactate dehydrogenase with the supplementation of 1g/d vitamin-C after the competition. Aguilo (2007) [7] found decreased blood lactate with the supplementation of vitamin-E (500mg/d), vitamin-C (1000 mg/d) and beta carotene (3000 μ g/d) for 90 days in amateur sports men.

In general, the studies on impact of antioxidant supplements on sport performance are limited. There are a wide variety of sports; and, depending on the interest of the researchers only few sport activities are studied extensively in terms of nutrition and physiological performance; most of which are from the western parts of the world. Sports nutrition in the developing and undernourished regions of the world particularly in the Asian region is still in childhood. In these contexts, it becomes rather difficult to come to a consensus and generalize the results pertaining to sports nutrition to wider population.

Conclusions

The results thus, reveal that supplementation of spirulina and commercial antioxidant does improve the cardiopulmonary fitness through better recovery, decreased fatigue and also reduces exercise induced lipid peroxidation, as evidenced through the lowered MDA status.

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