

Effect of Antioxidant Supplementation on Hematological Parameters, Oxidative Stress and Performance of Indian Athletes

Geetanjali Kelkar^{1*}, K. Subhadra¹ and Rana K. Chengappa²

1. *Department of Food, Nutrition & Dietetics, College of Home Science, Nirmala Niketan, Mumbai, Maharashtra, India*
*Mobile: 9845389853, *E-mail: gkelkar@rediffmail.com
2. *Sports Science Cell, Army Sports Institute, Pune, Maharashtra, India*

KEYWORDS Antioxidants. Supplements. Oxidative Stress. Hematological Parameters. Half Marathon Race

ABSTRACT Strenuous endurance exercise has a deleterious effect on metabolism. Exercise is known to increase oxidative stress and affect haem status, which in turn affects performance. With the hypothesis that antioxidants can lower oxidative stress, the present study was undertaken to analyze the effect of antioxidant supplementation on a study cohort (8 male runners, age 24 to 26y) competing in the Pune International Marathon, 21 Km race in December 2004. Two weeks prior to the race, the subjects were supplemented with Spirulina (4g) and antioxidants drink divided in 2 doses. The total antioxidant composition of the supplement was β Carotene 7600mcg, Vitamin A 400IU, Vitamin E 80 IU, Vitamin C- 320mg, Zinc-2.7mg and Selenium- 40mcg. Body composition (fat, lean, water) of the subjects was analysed using BIA, hematological parameters- Hemoglobin (Hb), Packed cell volume (PCV), Red blood cells (RBC), were evaluated before supplementation, one day prior to the race and the day after the race. Marker for oxidative stress serum malondialdehyde (MDA) was assessed before supplementation and day after the race. One week prior to the race the runners were given a modified carbohydrate loading dietary regimen. Training was tapered as per the needs of competition. Data analyzed using paired t test, Pearson's correlation analysis. After 2 weeks of antioxidant supplementation a significant ($p=0.000$) improvement in Hb, PCV and RBC was seen prior to the race and the levels were marginally reduced after the race. The serum MDA level was remarkably reduced ($p=0.003$) after supplementation. The average time taken to complete the race was 1:06:31. The study concluded that antioxidant supplementation combats oxidative stress; improves hematological status and performance in runners.

INTRODUCTION

Exercise is a strenuous activity and results in a number of metabolic changes in the body. Endurance athletes experience hematological disturbances due to mechanical hemolysis, intestinal bleeding, haematuria, sweating, iron deficiency and poor absorption of iron (O'Toole et al., 1988; Chatard et al., 1999). Poor hematological status necessitates an increase in cardiac output to maintain oxygen delivery to tissues (Pate, 1982), decreases the blood gas transport and muscle enzyme activity (Smith, 1995).

Exercise increases the oxygen uptake 10 to 20 times, and promotes generation of free radicals, reactive oxygen species that attack cellular components, damage carbohydrate, protein, lipid and nucleic acids (Clarkson, 1995; Ji, 1995; Sen, 1995). Free radicals, local acidosis and hypoxia aggravate hemolysis and iron release from transferrin (Lachant and Tanaka, 1987; Wohlbarsht and Fridovich, 1989). These metabolic changes increase stress and affect performance.

To augment the exercise induced oxidative stress, the organism is well equipped with

antioxidant defense systems with compounds like glutathione peroxidase, superoxide dismutase, catalase and glutathione reductase (Demopoulos, 1984). The non-enzymatic line of defense include substances such as reduced glutathione (GSH), vitamin A, C, E, selenium, zinc, that can efficiently neutralize the damaging effects of free radicals and protect the body (Clarkson and Thompson, 2000).

A subtle balance exists between pro and antioxidant activities of some micronutrients (Abuja, 1998). To counteract the chronic effects of exercise antioxidant supplementation may be effective.

Taking into consideration the above aspects and the paucity of research on the oxidative stress of Indian athletes the present study was undertaken to analyze the effect of antioxidant supplementation on hematological parameters, oxidative stress, in Indian long distance runners in response to a half marathon race.

MATERIALS AND METHODS

The study cohort, 8 well trained male long distance runners (24-26y) were enrolled in the

study purely based on their willingness to participate and ethical approval by the Sports Sciences Cell, Army Sports Institute. The subjects were receiving training at the Army Sports Institute, Pune and were preparing to participate in the Pune International Marathon- 21Km race in December 2004. They acted as self-controls for the supplement intervention trial and had not consumed any antioxidant supplements other than dietary antioxidants one month prior to supplementation. The subjects and the coach were educated about the need and importance of antioxidant supplementation and carbohydrate loading. The subjects followed a similar dietary pattern and ate in the Sports Mess of the institute and were instructed to refrain from making any drastic changes in the diet and to abstain from any other nutritional supplements. Antioxidant supplements comprising of Spirulina (4g), 4 x 500mg capsules in the morning with breakfast and at bedtime. Antioxidant rich drink (200ml) was given one at midmorning and one after evening training. The total antioxidant composition was β Carotene 7600mcg, Vitamin A 400IU, Vitamin E 80 IU, Vitamin C- 320mg, Zinc- 2.7mg and Selenium- 40mcg. The antioxidant supplement intervention was started two weeks prior to the actual date of the race. One week prior to competition the athletes were given a modified carbohydrate loading regimen. The food intake was recorded by weighment method for 3 consecutive days in the normal training phase before supplementation and 3 days prior to competition. Dietary nutrient intake (macro and micro nutrients) was calculated using Nutritive value of Indian Foods (Gopalan et al., 2000) and Digest software package. Body mass was measured nearest to 0.1Kg using a standardized digital scale and height with a stadiometer nearest to 0.1cm. Body composition (fat, lean, water) was assessed using bioelectrical impedance analysis (BIA) with Body stat (UK) following a standard protocol. The hematological parameters- Hemoglobin (Hb), Packed Cell Volume (PCV) and Red blood Cell (RBC) was assessed using standardized procedures. The parameters were assessed before supplementation, after supplementation one day prior to the race and the day after the race. The product of lipid peroxidation serum malondialdehyde (MDA) was assessed by Satoh (1978) method with Shimadzu U V Spectrophotometer. TBARS was assessed

before supplementation and day after the race. The coach set the training schedule. In the week preceding the pre marathon (competition) week the athletes were given normal training and ran 183-km/ week. In the pre marathon week the training was tapered to 126km. The subjects were considered as a support group to the athletes running the 42.2km race on the day of competition. Paired t test and Pearson's correlation were used to analyze the data using Statistical software package (SPSS –10).

RESULTS AND DISCUSSION

Subjects in the present study were well-trained marathon runners with the following characteristics as indicated in Table 1.

Table 1: Subject characteristics

Parameters	Mean $\pm$ SD
Age (years)	25.7 $\pm$ 1.90
Body mass (Kg)	59.17 $\pm$ 3.37
Height (cms)	172 $\pm$ 5.91
Body fat (%)	16.63 $\pm$ 1.36
Lean Body mass (%)	83.36 $\pm$ 1.36
Water (%)	59.68 $\pm$ 1.18

Mean $\pm$ standard deviation (SD)

Nutrient intake of elite athletes is a critical determinant of their performance and ability to compete. Table 2 and 3 indicate the dietary nutrient intake. The caloric intake significantly ($p < 0.01$) correlated with the weekly training load. In the normal training phase the caloric distribution was 59%carbohydrates, 14% protein and 26 % fat. Endurance athletes consume a high carbohydrate diet to pack muscles with glycogen during the competitive phase. Singh et al (1993) has reported an increase in energy supplied from carbohydrate about 54.2 ± 2.3 to 60.1 ± 2.4 % in

Table 2: Dietary Intake of Macronutrients and Percent calorie distribution

Phase	Energy Kcal	Protein g	Carbohy- drates g	Fat g
Training phase	3885 $\pm$ 348.48	140.81 $\pm$ 29.19	565.55 $\pm$ 37.46	112.05 $\pm$ 15.22
Percent caloric intake	(14%)	(59%)	(26%)	
Competitive phase	3113 $\pm$ 164	84.32 $\pm$ 6.09	526.567 $\pm$ 29.80	74.19 $\pm$ 8.98
Percent calorie Intake	(12%)	(76%)	(12%)	

Mean $\pm$ SD

Table 3: Average daily vitamin and mineral intake of the subjects

Nutrients/d	Training phase dietary intake	Training phase after supplementation	Competitive phase dietary intake	Competitive phase supplementation	RDA*
Vitamins					
βCarotene(μg)	4278.91±1698.89	11878.91±1698.89	3589.18±1276.65	11189.18± 1276.65	NA
Vitamin A(IU)		400	-	400	1080-2500 μg
Vitamin E(IU)	NA	80	NA	80	10-20
Vitamin C(mg)	249.72 ± 72.06	569.72 ± 72.06	189.50± 61.77	509.5 ± 61.77	80-150
Thiamin(mg)	2.66 ± 0.55	2.66 ± 0.55	1.50± 0.22	1.50± 0.22	3-6
Riboflavin(mg)	3.18 ± 0.85	3.18 ± 0.85	3.37± 0.67	3.37± 0.67	3-6
Niacin(mg)	26.74 ± 5.46	26.74 ± 5.46	10.98 ± 0.93	10.98 ± 0.93	30-60
Minerals					
Calcium(mg)	1706.9 ± 325.47	1706.9 ± 325.47	1406.41±219.34	1406.41±219.34	1500-3000
Iron(mg)	35.0 ± 9.93	36.09 ± 9.93	24.81±5.45	27.21± 5.45	50-85
Magnesium(mg)	616 ± 87.8	616 ± 87.8	393.89± 31.5	393.89± 31.5	NA
Zinc(mg)	11.54 ± 2.27	14.24 ± 2.27	8.67 ±1.69	11.37 ±1.69	NA

*Ref: Satyanarayana et al., 1985 N.A.=Not available

the pre race period. Carbohydrate loading in the present study increased the carbohydrate intake to 76%.

High carbohydrate intake is known to increase body mass, and the extra weight may actually negate the potential benefits derived from the high glycogen storage (Wilkinson and Liebman, 1998). The body mass did not show any variation in normal training and competitive phase, this could be because of the routinely consumed high carbohydrate diet of Indians. Although the percent carbohydrate intake was elevated (Table3), the total amount of carbohydrate and calories was less in comparison to normal training phase in conjunction with the tapered pre competition training. The dietary intake of iron ($p=0.014$), calcium ($p = 0.008$), B3 ($p=0.000$), Magnesium ($p=0.000$) and other antioxidants was reduced indicating the need for supplementation.

Foods high in carbohydrates tend to be low in Vitamin E and other antioxidants. So people who habitually ingest high carbohydrate, low fat diets have marginally low Vitamin E intake (Bendich and Machlin, 1988). Carbohydrate supplementation attenuates the cortisol and catecholamine response after exercise. However, no reduction in oxidative stress was evident in marathon runners running 3 h at 70% VO₂ max. (Mc Anulty et al., 2003).

The antioxidant enzymes have a prominent adaptive response to chronic exercise in skeletal muscles provided an adequate nutritional status is maintained. It is dependent on dietary intake and is susceptible to deficiency (Berger, 2005).

Body composition of the subjects (Table 4) i.e. percent lean body mass; fat and water did not show a remarkable variation prior to

supplementation, prior to race and after the race. The average loss of body mass after the competition was 0.8 to 1.0 Kg. Earlier studies on antioxidant capacity and supplementation in response to half marathon race/ extreme endurance stress have indicated varied results. The rise in total antioxidant capacity did not prevent exercise induced lipid peroxidation and muscle damage due to elevated MDA and Creatine kinase (CK) level in response to simulated half marathon. The antioxidant defense system was thus inadequate (Child et al., 1998). Supplementation of alpha tocopherol (400 IU), ascorbic acid (200mg) per day 4.5 weeks prior to marathon race indicated reduction in blood CK and TBARS (Rokitzki et al., 1994). Maughan et al (1989) exposed the subjects to 45 min downhill running and found that neither plasma TBARS nor CK showed any increment post exercise. But the TBARS significantly elevated 6 hours and CK peaked at 24 h post exercise.

Table 4: Body composition of the subjects

	Body mass	Fat%	Lean%	Water%
Before	59.17±	16.63±	83.36±	59.38±
Supple- mentation	3.37	1.36	1.36	1.18
Before	59.23±	15.46±	84.5±	59.68±
Race	3.39	0.78	0.71	2.05
After	58.46	16.1±	83.65±	59.57±
Race	±3.55	1.54	1.33	2.053

Mean ±SD

Two weeks after the supplementation a significant ($p=0.000$) improvement in Hb, PCV and RBC were seen prior to the race (Table 5). Endurance exercise tends to increase the average RBC concentration, total Hb and hematocrit by

5.3 to 5.7 % due to decreased plasma volume indicating sports anemia (Oestenberg et al., 1997). Formation of free radicals from mitochondrial leakage due to increased oxygen consumption, ischemia and reperfusion process and leukocyte activation elevates oxidative stress in erythrocytes during exercise (Lawler and Powers, 1998). Intravascular hemolysis 24 h after exhaustive exercise suggests the maintenance of destructive effects following exercise. It is attributed to the increased osmotic fragility and reduced erythrocyte deformability (Senturk et al., 2001).

Table 5: Effect of antioxidant supplementation on oxidative stress and hematological parameters

	Hemoglobin/dl	Packed Cell Volume	RBC	MD Anmol/ ml
Before Supplementation	13.4±0.65	38.87±2.03	4.6±0.36	4.40±0.88
Before Race	15.05±0.82	44.62±2.32	5.16±0.22	NE
After Race	14.43±0.80	42.87±2.23	4.88±0.23	2.68±0.32

Mean ± SD

In the present study, serum MDA concentration prior to supplementation was 4.40 ± 0.88 nmol/ml and was significantly ($p = 0.001$) lowered to 2.68 ± 0.32 nmol/ml. Oxidative stress predominates in the period just after exercise, however lipid peroxidation may increase later after exercise (Maughan et al., 1989; Lawler and Powers, 1998; Yalcin et al., 2000). The antioxidant effect is credited to the blend of antioxidants (Spirulina and antioxidant drink). The defense against oxidative stress depends on cumulative synergism between several antioxidants (Sen and Packer, 2000) because multiple sources of ROS exist in the cell (Ji, 1999; Reid, 2001). However, no significant correlation between increased antioxidant intake after supplementation and reduction in serum MDA was observed.

Thus the present study depicted a remarkable improvement in hematological parameters and reduction in MDA after 2 weeks of supplementation in response to race. Only a marginal reduction ($p=0.674$) of hematological parameters due to endurance training and oxidative stress was detected 24 h after the race. This could be attributed to the protective effect of antioxidants. Antioxidants can counteract the oxidative stress, intravascular hemolysis and prevent erythrocyte

deterioration (Senturk et al., 2001). Aguilo et al (2004) reported that antioxidant supplementation prevented the reduction of serum iron and iron saturation index in endurance athletes indicating a link between iron metabolism and oxidative stress. The remarkable improvement in hematological parameters can be ascribed to antioxidant effects of spirulina. Spirulina can improve the physiological conditions of erythrocyte membrane fluidity, and thus prevent the adverse effect of oxidative stress (Huang et al., 2003). The iron bioavailability from spirulina is comparable with beef and is considered to be a rich source of absorbable iron (Puyfoulhoux et al., 2001). The study thus reconfirmed the interrelationship between restorations of iron status after antioxidant supplementation, and reduced oxidative stress in response to half marathon.

CONCLUSION

Endurance training can aggravate a transitory lack of physiological and biochemical adaptations. Trained athletes require higher intakes of antioxidants to defend against increased oxidative stress. Diet is subjected to change in different phases of training and competition. Nutritional deficiencies can disrupt the biochemical profile and provoke increased oxidative stress. The present study revealed the need and effect of antioxidant supplementation to combat stress and initiate faster recovery from the damage generated by heavy exercise in athletes

REFERENCES

- Abuja, P.: When might an antioxidant become a prooxidant? *Acta Anaesthesiol. Scand*, **42**(Suppl 112): 229-230 (1998).
- Aguilo, A., Tauler, P., Fuentespina E., Villa, G., Cordova, A., Tur, J. A. and Pons, A.: Antioxidant diet supplementation influences blood iron status in endurance athletes. *Int. J. Sport Nutr. Exerc. Metab.*, **14**(2): 147-160 (2004).
- Bendich, A. and Machlin, L. J.: Safety of oral intake of Vitamin E. *Am. J. Clin. Nutr.*, **48**: 1088 (1988).
- Berger, M. M.: Can oxidative damage be treated nutritionally? *Clinical Nutrition*, **24**: 172-183 (2005).
- Chatard, J. C., Mujika, I., Guy, C. and Lacour, J. R.: Anaemia and iron deficiency in athletes. Practical recommendations for treatment. *Sports Med.*, **27**(4): 229-240 (1999).
- Child, R.B., Wilkinson, D.M., Fallowfield, J. L., Donnelly, A. E.: Elevated serum antioxidant

- capacity and plasma malondialdehyde concentration in response to a simulated half-marathon run. *Med. Sci. Sports Exerc.*, **30(11)**: 1603-1607(1998).
- Clarkson, P. M.: Antioxidants and physical performance. *Clin. Rev. Food Sci. Nutr.*, **35**: 131-141 (1995).
- Clarkson, P. M. and Thompson, H. S.: Antioxidants: what role do they play in physical activity and health? *Am. J. Clin. Nutr.*, **72(suppl)**: 637S-646S (2000).
- Demopoulos, H. B., Santonnier, J. P., Saligman, M. L. and Pietronigro, D. D.: Free radical pathology: Rationale and Toxicology of antioxidants and other supplements in sports medicine and exercise, pp. 149-187, In: *Sports Health and Nutrition*. F. I. Katch (Ed). Human Kinetic Pub Inc Champaign, Illinois (1984).
- Gopalan, C., Rama Shastri, B.V. and Balasubramanian, S.C.: *Nutritive value of Indian Foods*. National Institute of Nutrition, Hyderabad, India (2000).
- Huang, J.M., Bai, S.M., Hu, Z.X., Yang, C.L., Zhu, D.B. and Shi, J.P.: Effects of spirulina on serum lipids erythrocyte membrane fluidity and vascular endothelial cells in tail suspended rats (abstract). *Space Med. Eng. (Beijing)*, **16(3)**: 184-186 (2003).
- Ji, L. L.: Oxidative stress during exercise: implication of antioxidant nutrients. *Free Radic. Biol. Med.*, **18**: 1079-1086 (1995).
- Ji, L.L.: Antioxidants and Oxidative stress in exercise. *Society for Experimental Biology and Medicine*, **283**: 292 (1999).
- Kanter, M.: Free radicals, exercise and antioxidant supplementation. *Proc. Nutr. Soc.*, **57**: 9-13 (1998).
- Lachant, N. A. and Tanaka, K.R.: Impaired antioxidant defense in hemoglobin E- containing erythrocytes: a mechanism protective against malaria? *Am. J. Hematol.*, **26(3)**: 211-219 (1987).
- Lawler, J.M. and Powers, S.K.: Oxidative stress, antioxidant status and, and the contracting diaphragm. *Can. J. Appl. Physiol.*, **23**: 23-55 (1998).
- Maughan, R. J., Donnelly, A. E., Gleeson, M., Whiting, P.H., Walker, K. A. and Clough, P. J.: Delayed onset muscle damage and lipid peroxidation in man after a downhill run. *Muscle Nerve*, **12(4)**: 332-336(1989).
- Mc Anulty, S.R., Mc Anulty, L. S., Nieman, D. C., Morrow, J. D., Utter, A. C., Henson, D. A., Dumke, C.L. and Vinci, D. M.: Influence of carbohydrate ingestion on oxidative stress and plasma antioxidant potential following a 3h run. *Free Radic. Res.*, **37(8)**: 835-840 (2003).
- O' Tootle, M. L., Hiller, W. D. and Roalstad, M. S.: Hemolysis during triathlon races: its relation to race distance. *Med. Sci. Sports Exerc.*, **20**: 147-160 (1988).
- Ooestenburg, G. S., Mensink, R.P., Hardeman, M.R., Vries, T. De., Brouns, F. and Hornstra, G.: Exercise performance, red blood cell deformability, and lipid peroxidation: effects of fish oil and vitamin E. *J. Appl. Physiol.*, **83**:746-752 (1997).
- Pate, R.R.: Sport's anemia and its impact on athletic performance. p. 202. In: *Nutrition and Athletic Performance*. Bull Pub Palo Alto, CA (1982).
- Puyfoulhoux, G., Rouanet, J. M., Besancon, P., Baroux, B., Baccou, J.C. and Caporiccio, B.: Iron availability from iron fortified spirulina by an in vitro digestion / Caco-2 cell culture model. *J. Agri. Food Chem.*, **49(3)**: 1625-1629 (2001).
- Reid, M.B.: Invited review: redox modulation of skeletal muscle contraction: what we know and what we don't. *J. Appl. Physiol.*, **90**: 724-731 (2001).
- Rokitzki, L., Leeann, E., Sagredos, A.N., Murphy, M., Wetzel-Roth, W. and Keul, J.: Lipid peroxidation and antioxidative vitamins under extreme endurance stress. *Acta Physiol. Scand.*, **151**:149-158(1994).
- Satoh, K.: Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chem Acta.*, **90**: 37-43 (1978).
- Sen, C.K.: Oxidants and Antioxidants in exercise. *J. Appl. Physiol.*, **79**: 675-686 (1985).
- Sen, C. K. and Packer, L.: Thiol homeostasis and supplements in physical exercise. *Am. J. Clin. Nutr.*, **72**: 653S-669S (2000).
- Sen, C. K.: Antioxidants and exercise nutrition. *Sports Med*, **31(13)**: 891-908 (2001).
- Senturk, U.K., Gunduz, F., Kuru, O., Aktekin, M.R., Kipmen, D., Yalcin, O., Bor-Kucukatay, M., Yesilkaya, A. and Baskurt, O. K.: Exercise-induced oxidative stress affects erythrocytes in sedentary rats but not in exercise trained rats. *J. Appl. Physiol.*, **91**: 1999-2004 (2001).
- Singh, A., Evans, P., Gallagher, K.L. and Deuster, P. A.: Dietary intakes and biochemical profiles of nutritional status of ultra marathoners. *Med. Sci. Sports Exerc.*, **25(3)**: 328-334 (1993).
- Smith, J. A.: Exercise training and red blood cell turnover. *Sports Med*, **19(1)**: 9-31 (1995).
- Wilkinson, J. G. and Liebman, M.: Carbohydrate Metabolism in Sport and Exercise. pp 63-99, In: *Nutrition in Exercise and Sport*. I. Wolinsky (Ed). 3rd Edition. CRC Press Boca Raton New York (1998).
- Wolbarsht, M. L. and Fridovich, I.: Hyperoxia during reperfusion is a factor in reperfusion injury. *Free Radic. Biol. Med.*, **6(1)**: 61-62 (1989).
- Yalcin, O., Bor-Kucukatay, M., Senturk, U. K. and Baskurt, O. K.: Effects of swimming exercise on red blood cell rheology in trained and untrained rats. *J. Appl. Physiol.*, **88**: 2074-2080 (2000).