Short Communication

Effect of Regular Training on Plasma Thiols, Malondialdehyde and Carnitine Concentrations in Young Soccer Players

Gökhan Metin[1], M. Koray Gümüştaş[2], Ezel Uslu[2], Ahmet Belce[2] and Abidin Kayserilioglu[3]

[1]*Department of Physiology, Cerrahpasa Faculty of Medicine*
[2]*Department of Biochemistry, Cerrahpasa Faculty of Medicine*
[3]*Department of Sports Medicine, Istanbul Faculty of Medicine*
Istanbul University
Istanbul, Turkey

Abstract

Physical activity is known to induce oxidative stress in individuals subjected to intense exercise. Contrarily, there are enzymatic and nonenzymatic defence systems against oxygen radicals in aerobic organisms. Sulphydryl groups such as thiol and glutathione (GSH) can be given as an example to non-enzymatic low molecular weight antioxidants. Carnitine may be related to the performance enhancement in high intensity intermittent exercises and might probably improve the aerobic capacity by stimulating lipid oxidation in muscle cells during long term exercise. But, the effects caused by this supplement during physical activity have not been fully described in the literature. The aim of the study was to compare plasma thiols (PSH), malondialdehyde (MDA) and carnitine levels and maximal oxygen uptake (VO_{2max}) of the soccers under regular training with the values of the healthy controls. Our results demonstrates that soccers seem to be under less oxidative stress, as their MDA levels were significantly lower ($P < 0.001$) when compared with the control group while their PSH levels were significantly elevated ($P < 0.001$), during resting condition. In addition, the plasma carnitine concentrations of the soccer group yields lower values while the VO_{2max} yields a higher value when compared with the control group. The differences between the soccer and the control groups are significant (for both, $P < 0.001$). The present research reveals the fact that regular soccer training shows beneficial effect on decreasing of lipid peroxidation levels. Furthermore; the sportsmen who are under intense training programs have low plasma carnitine values which do not cause negative effect on their sportive performance.

Key Words: soccer, MDA, thiols, carnitine, VO_{2max}

Introduction

Physical activity results in an increased production of free radicals and reactive oxygen species (ROS), and it is well known to induce oxidative stress in individuals subjected to intense exercise. In addition, during exercise, the process of delivering the oxygen to working muscles may actually result in oxidation of polyunsaturated fatty acids in the mitochondria. Furthermore, growing evidence implicates cytotoxic ROS as an underlying cause in exercise-induced disturbances in muscle redox status that could result in muscle fatigue or injury (21).

Contrarily, there are enzymatic and nonenzymatic defence systems against ROS in aerobic organisms. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-px) are known to be the primary antioxidant enzymes. Moreover sulphydryl groups such as thiol and glutathione can play as nonenzymatic low molecular weight antioxidants (15, 24). Additionally, it is showed that plasma thiol levels can be considered as an oxidative

Corresponding author: Dr. Gökhan Metin, 19 Mayis Mah. Sinan Ercan Sok. No: 18 C/22 Kazasker, Kadıköy, 81090 Istanbul, Turkey. Tel: +90-212-5861523, Fax: +90-212-5861581, E-mail: gmetin@istanbul.edu.tr
Received: September 12 2002; Revised: November 21, 2002; Accepted: November 25, 2002.

stress marker in experimental studies (17). The feature of most thiols is their ability to act as reducing agent. ROS have a strong tendency to transfer electrons to other species but reducing agents such as thiols may act as prompt electron acceptors (23).

Compared with the limited capacity of the human body to store carbohydrate, endogenous fat depots are large and represent a vast source of fuel for exercise. However, fatty acid (FA) oxidation is also limited, especially during intense exercise (13). Thus, many athletes and active people consume a large variety of supplements in order to increase their performance in competitions. The enzyme (4-butyrobetaine hydroxylase) of the last carnitine biosynthesis step is present only in liver, kidney and brain where synthesis is limited within those tissues. The kidney plays a major role in carnitine biosynthesis, excretion and acylation. The carnitine synthesis requires ascorbate, Fe^{+2}-dependent hydroxylase enzymes and lysine rich protein. Carnitine is critical for skeletal muscle bioenergetics and long-chain fatty acid oxidation, and it also shuttles accumulated acyl groups out of the mitochondria. Muscle requires optimization of these metabolic processes during intense exercise. Theoretically, carnitine availability may become limiting for either fatty acid oxidation or the removal of acyl-CoAs during exercise (3).

Carnitine seems to be related to the enhancement in high intensity intermittent exercises and might probably improve the aerobic capacity by stimulating lipid oxidation in muscle cells during long term exercise (11). But, the effects caused by this supplement during physical activity have not been fully described in literature. On the other hand, different kinds of studies showing the influence of carnitine supplementation in reducing lipid peroxidation are encountered (12, 25). Available studies designed to assess the effect of carnitine on exercise performance in healthy humans or athletes have not permited definite conclusions yet.

The aim of the study was to compare plasma thiols, malondialdehyde, carnitine levels and maximal oxygen uptake of the soccers under regular training with the values of the healthy controls.

Materials and Methods

The group of soccer (n=26) was former soccer players who are under regular training, between the ages of 17-20, and weighting between 68-87 kg. The subjects in the control group were the young students (n=17), who had a sedentary lifestyle and did not practise any sport regularly. Prior to testing, all subjects read and signed a consent form that was approved by the University's Policy and Review Commitee on Human Research.

Anthropometry

Body mass was measured on a balance with medical scale near at 0.1 kg. Stature was measured on a portable stadiometer to an accuracy of ± 0.5 cm.

Experimental Protocol

The maximal oxygen uptake was measured in order to establish its functional capacity. Exercise test of soccers was performed within 4 days at the end of the competitive season (after the league season). Each subject first underwent a 12 lead electrocardiogram (ECG) record at first and then a full 10-electrode, 12-lead ECG monitor during the maximal exercise test. In all cases, a Quinton 5000 recorder and lead system (Quinton Instrument company, Seattle, WA) were utilized to monitor and record the ECG. Heart rate was monitored electrocardiographally. Blood pressure was monitored using standart cuff manometry. Subjects performed pulmonary function tests before exercise test and abstained from strenuous exercise, alcohol, tobacco, and caffeine for at least 24 hours prior to the exercise trial.

Exercise Testing

All the subjects exercised on a treadmill ergometer with Bruce-type protocol. Prior to the test subject was acclimated to the treadmill with a 2-min walk/run and instructed to walk or run without the aid of the handrail. The Bruce treadmill test is a commonly used exercise test to assess exercise capacity and the electrocardiographic response to exercise stress in adults (6). The test protocol is suitable for stressing patients, healthy induviduals or trained athletes. The test was stopped at maximal exercise level. It was considered maximal if the subject achieved any two of three test criteria. The criteria included a respiratory exchange ratio of 1.10 or higher, a maximal heart rate within ± 10 beats min^{-1} of age-predicted maximum and plateau of oxygen consumption with increasing work load.

Direct Measurements of Maximal Oxygen Consumption

The peak VO_2 values reflect true VO_{2max} as the limits of oxygen delivery (22). Expired gases (O_2 and CO_2) were analyzed on a SensorMedics 2900 C Metabolic Measurement Cart (Sensormedics Corporation, Anaheim, CA) and the peak VO_2 was determined from expired gas measurements. O_2 and CO_2 content were analyzed by zirconia oxide and infrared analyzers, respectively. The system was calibrated before each test with standart O_2 and CO_2 gases with known concentrations.

Table 1. Physical characteristics and aerobic capacity in soccer players.

	Control Group (n=17)	Soccer Group (n=26)
Age (year)	17.94±0.18	18.38±0.21 (NS)
Height (cm)	177.9±2.0	178.9±1.5 (NS)
Body Weight (kg)	75.88±1.56	76.69±0.95 (NS)
VO_{2max}(ml.min^{-1}.kg^{-1})	46.02±0.52	56.69±0.60*

Values are expressed as mean ± SE. Comparisons were performed by using Student's-*t* test. *P < 0.001, in comparison with control group. NS = not significant.

Table 2. Plasma carnitine, malondialdehyde and thiols levels between contol and soccer groups.

	Control Group (n=26)	Soccer Group (n=17)
Carnitine (mol/L)	36.0±01.9	25.58±1.06*
Malondialdehyde (mol/L)	2.38±0.06	1.98±0.07*
Thiols (mol/L)	614.43±50.43	670±60.24*

Values are expressed as mean ± SE. Comparisons were performed by using Student's-*t* test. *P < 0.001, in comparison with control group.

Biochemical Measurements

The PSH levels as one of the indicators of the antioxidant defense, plasma MDA levels as the parameter of lipid peroxidation and plasma carnitine concentrations as an essential factor for fatty acid oxidation in mitochondria were measured. Intravenous blood samples were collected in heparinazed test tubes from the subjects fasting at least 8 h. Collection of blood samples of soccers was performed at the beginning of under resting conditions. The color caused by the reaction between MDA and TBA (thiobarbituric acid) was determined by Buege & Aust's modified method (7). Plasma free carnitine levels were measured by enzymatic method (Boehringer Mannheim, Germany), Cat. No: 1242008, (20). After the plasma has been separated by centrifugation at 1500 × g for 15 minutes at 4°C, PSH levels were measured using a spectrophotometric technique (10). The absorbance of colour reaction which occurred by interaction of plasma thiol groups with 5,51 dithiobis 2-nitrobenzoic acid (DTNB) in phosphate buffer (pH = 8) is determined by spectrophotometric measurement at 420 nm wave length. All results were given as μmol/l.

Results are given as means ± S.E. Student's-*t* test was used in comparing two means whereas a value of P < 0.05 was considered significant.

Results

The subjects in both groups were age, sex and weight matched (Table 1). The mean VO_{2max} values of each group are also shown in Table 1. Plasma PSH, MDA and carnitine levels are shown in Table 2.

According to our MDA and PSH results, long term trained sportsmen seem to be under less oxidative stress, as their MDA levels were lower than those in control group and their PSH levels were elevated, during resting condition. The differences between groups are significant (P < 0.001), (Table 2). In addition, the plasma carnitine concentration of sportsmen group yields lower values while the VO_{2max} yields a significantly higher value when compared with the control group. The differences in groups are significant (P < 0.001).

Discussion

Soccers are subjected to regular training programmes to improve functional capacity, including power, sprint and endurance exercises, during competitive season. The oxidant stress that can be produced by these types of exercise, was shown to raise the organism's antioxidant capacity for subsequent physical efforts at higher levels (18). Due to the elevation of oxidative stress caused by constant strenuous exercise in sportsmen, the organism keeps its own protective antioxidant mechanisms more active. In various studies, it was shown that, thiol groups were essential in the protection against the deleterious effects of ROS (2). In this study, regular soccer training has also shown induced antioxidant defence like PSH. Marker of the oxidative stress changed significantly, with a reduction in the MDA levels of soccers. The decline of MDA levels may be caused by increased antioxidant status. Brites F.D. *et al*. found a general increase in antioxidant status of soccer players (5). Contrarily, they could not find any difference between triacylglycerol and total cholesterol levels of soccer players when compared with the controls (5). We studied PSH level as an antioxidant parameter. In a such high antioxidant environment, it is naturel to find lower levels of MDA.

In addition, VO_{2max} values were significantly increased in the sportsmen group, as was expected in aerobic training exercises. Previous result has shown a positive corelation between oxygen uptake and antioxidant defence enzyme activity (16). Further data also demonstrated that the ability to quench free

radicals in serum was increased in relation to the maximum ability to consume oxygen (8).

Heinonen *et al*. reported that athletes were not at risk for carnitine deficiency and did not have an increased need for carnitine (14). But, as it is seen in our results, carnitine concentrations of sportsmen declined when compared to control group. It is evident that the fatty acid oxidation and consumption must elevate with the regular training of our soccer group. The production of carnitine is not available in muscle tissue while it is limited in kidney and liver tissues. One of the explanations of the reason of low carnitine levels in soccer group may be decreasing of carnitine by the free radicals produced during exercise training. Because carnitine synthesis depends on ascorbate dependent hydroxylations. Furthermore it may be increased over-flow of short-chain carnitine esters in urine (1). Another reason for decline in plasma carnitine levels may be increased utilization by muscle cells during exercise effort.

On the other hand, the elevation VO_{2max} levels which reflects the performance criteria in soccers may indicate that the aerobic capacity was not affected from low carnitine levels. But, in a recent study, Nuesch *et al*. reported that the elevated plasma concentration of free carnitine without decrease after maximal exercise in well-trained athletes taking L-carnitine could be important in the view of the newly postulated direct vascular effects of L-carnitine in improving skeletal muscle performance (19). Despite the theoretical basis for carnitine supplementation in otherwise healthy persons to improve exercise performance, clinical data have not demonstrated consistent benefits of carnitine administration (3). In addition, some researchers also reported that carnitine supplementation does not affect the maximal oxygen uptake (4, 14).

Contrarily, it is not surprising that the use of supplementary carnitine to improve lipid peroxidation levels, due to there is some unequivocal support to this practice. Volek *et al*. reported that exercise-induced increases in plasma malondialdehyde (MDA) returned to resting values sooner during L-carnitine L-tartrate (LCLT) supplementation compared with placebo (25). Hagen et al. also reported that feeding acetyl-l-carnitine in combination with alpha-lipoic acid increased metabolism and lowered oxidative stress more than either compound alone (12). But, in our soccer group who have low carnitine levels, plasma MDA levels were found lower when compared to control group. Colombani *et al*. also reported that acute administration of L-carnitine did not affect the metabolism or improve the physical performance of the endurance-trained athletes during the run and did not alter their recovery (9).

The present research reveals the fact that regular soccer training can be beneficial in the minimization of the lipid peroxidation, although strenuous exercise may cause overproduction of oxygen radicals. So, we consider that low plasma carnitine levels of the sportsmen who have intense training programs do not effect their functional capacity and do not cause negative effect on plasma oxidative and antioxidative markers we measured.

References

1. Arenas, J., Ricoy, J.R., Encinas, A.R., Pola P., D'Iddio S., Zeviani, M., Didonato, S. and Corsi, M. Carnitine in muscle, serum, and urine nonprofessional athletes: effects of physical exercise, training and L-carnitine administration. *Muscle. Nerve.* 14: 98-604, 1991.
2. Bast, A., Haenen, G.R. and Doelman, C.J. Oxidants and antioxidants: State of the art. *Am. J. Med.* 91: 2S-13S, 1991.
3. Brass, E.P. and Hiatt, W.R. The role of carnitine and carnitine supplementation during exercise in man and individuals with special needs. *J. Am. Coll. Nutr*. 17: 207-215, 1998.
4. Brass, E.P. Supplemental carnitine and exercise. *Am. J. Clin. Nutr*. 72: 618S-623S, 2000.
5. Brites, F.D., Evelson, P.A., Christiansen, M.G., Nicol, M.J., Wikinski, R.W. and Llesuy, S.F. Soccer players under regular training show oxidative stress but an improved plasma antioxidant status. *Clin. Sci.* 96: 381-385, 1999.
6. Bruce, R.A., Kusumi, F. and Hosmer, D. Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am. Heart. J*. 85: 546-562, 1973.
7. Buege, J.A. and Aust, S.D. Microsomal Lipid peroxidation. *Methods. Enzymol.* 52: 302-310, 1978.
8. Child, R.B., Wilkinson, D.M. and Fallowfield, J.L. Resting serum antioxidant status is positively correlated with peak oxygen uptake in endurance trained runners. *J. Sports. Med. Phys. Fitness*. 39: 282-284: 1999.
9. Colombani, P., Wenk, C., Kunz, I., Krahenbuhl, S., Kuhnt, M., Arnold, M., Frey-Rindova, P., Frey, W. and Langhans, W. Effects of L-carnitine supplementation on physical performance and energy metabolism of endurance-trained athletes: a double-blind crossover field study. *Eur. J. Appl. Physiol. Occup. Physiol*. 73: 434-439, 1996.
10. Ellman, G.E. Tissue sulphydryl groups. *Arch. Biochem. Biophys.* 82: 70-77, 1959.
11. Gomes, M.R. and Tirapegui, J. Relation of some nutritional supplements and physical performance. Arch. Latinoam. *Nutr*. 50: 317-329, 2000.
12. Hagen, T.M., Liu, J., Lykkesfeldt, J., Wehr, C.M., Ingersoll, R.T., Vinarsky, V. and Bartholomew, J.C., Ames, B.N. Feeding acetyl-L-carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. *Proc. Natl. Acad. Sci. USA* 99: 1870-1875, 2002.
13. Hawley, J.A., Brouns, F. and Jeukendrup, A. Strategies to enhance fat utilization during exercise. *Sports. Med*. 25: 241-257, 1998.
14. Heinonen, O.J. Carnitine and physical exercise. *Sports Med* 22: 109-132, 1996.
15. Hllaliwell, B. Lipid peroxidation, oxigen radicals, cell damage and antioxidant therapy. *Lancet* 23: 1396-1397, 1984.
16. Jenkins, R.R., Friedland, R. and Hovald, H. The relationship of oxygen consumption to superoxide dismutase and catalase activity in human skeletal muscle. *Int. J. Sports. Med*. 4: 11-14, 1984.
17. Jennings, P.E., Scott, N.A., Saniabadi, A.R. and Belch, J.J.F. Effects of gliclazide on platalet reactivity and free radicals in type II diabetic patients: clinical assessment. *Metabolism* 41: 36-39, 1992.
18. Marzatico, F., Pansarasa, O., Bertorelli, L., Somenzini, L. and Della

Valle, G. Blood free radical antioxidant enzymes and lipid peroxides fallowing long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. *J Sports. Med. Phys. Fitness*. 4: 235-239, 1997.

19. Nuesch, R., Rosetto, M. and Martina, B. Plasma and urine carnitine concentrations in well-trained athletes at rest and after exercise. Influence of L-carnitine intake. *Drugs. Exp. Clin. Res*. 25: 167-171, 1999.
20. Pearson, D.J., Tubbs, P.K., and Chase, J.F.A. Carnitine and acylcarnitines determinations In: Methods of enzymatic analysis, edited by Bergmeyer, H.U. in collaboration with K. Gawehn. New York, NY: Academic press, Inc., 1974, vol. 4, pp. 1758-1771.
21. Powers, S.K., Criswell, D., Lawyer, J., Ji, L.L., Martin, D., Herb, R. A. and Dudly, G. Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. *Am. J. Physiol*. 266: R375-R380, 1994.
22. Rowland, T.W. Does peak VO_2 reflect VO_{2max} in children?: evidence from submaximal testing. *Med. Sci. Sports. Exerc*. 25: 689-693, 1993.
23. Sen, C.K. and Packer, L. Thiol homeostasis and supplements in physical exercise. *Am. J. Clin. Nutr*. 72: 653s-669s, 2000.
24. Sun, Y. Free radicals, antioxidant enzymes and carcinogenesis. *Free. Radic. Biol. Med*. 8: 583-599, 1990.
25. Volek, J.S., Kraemer, W.J., Rubin, M.R., Gomez, A.L., Ratamess, N.A. and Gaynor, P. L-Carnitine L-tartrate supplementation favorably affects markers of recovery from exercise stress. *Am. J. Physiol*. 282: E474-4E82, 2002.