Biology of Sport, Vol. 25 N°2, 2008

ACUTE EXERCISE INDUCED OXIDATIVE STRESS IS PREVENTED IN ERYTHROCYTES OF MALE LONG DISTANCE ATHLETES

S. Dane[1], S. Taysi[2], M. Gul[1], F. Akcay[2], A. Gunal[3]
Depts. of [1]Physiology and [2]Biochemistry, Faculty of Medicine, Ataturk University, Erzurum, Turkey; [3]College of Physical Education and Sports, Ataturk University, Erzurum, Turkey

Abstract. The aim of this study was to investigate the redox status in blood of long distance running athletes if it is favourably affected, and help to prevent acute exercise-induced oxidative stress. Nineteen sedentary males and 20 male long distance runners, volunteered to participate in this study. Acute exercise was applied as treadmill run, which was continued until the heart rate of the subject has reached 80-90% of the maximum and stopped after 5 min. Acute exercise increased the hematocrit percentage in sedentary males but not in male athletes. It decreased the number of erythrocytes and also Hb level in sedentary males, but not in male athletes when they were adjusted to the changes in hematocrit level. There was no difference in erythrocyte malondialdehyde levels between sedentary males and male athletes at rest. Acute treadmill run increased the erythrocyte malondialdehyde level in sedentary males, however, it did not affect it in male athletes. Erythrocyte glutathione peroxidase and superoxide dismutase activities were not affected by acute exercise in both groups. Our results show that erythrocytes in long distance male athletes are better protected against acute exercise-induced oxidative stress compared with the ones from sedentary counterparts. ***(Biol.Sport 25:115-124, 2008)***

Key words: Long distance runner – Exercise – Erythrocyte – Malondialdehyde - Antioxidant enzymes

Introduction

During strenuous exercise, metabolic rate in the skeletal muscle increases up to 100 times above resting levels [9]. Increased superoxide anion production in the mitochondria due to increased oxygen consumption during exercise [5,15] may lead to subsequent formation of other reactive oxygen species (ROS), such as

Reprint request to: Mustafa Gul, M.D., Associate Professor, Dept. of Physiology, Faculty of Medicine, Ataturk University, 25240, Erzurum, Turkey.
Tel: +90-442-2316615; Fax: +90-442-2360968; E-mail: mgul@atauni.edu.tr

hydrogen peroxide (H_2O_2) and hydroxyl radical. Exercise, whether it is moderate [1,12,16], or exhausting [18,22,27] may increase ROS production overwhelming the capacity of antioxidant defence mechanisms. Erythrocytes are susceptible to oxidative damage because of their continuous exposure to oxygen and their high concentrations of polyunsaturated fatty acids and heme iron [4]. Increased lipid peroxidation in erythrocytes after acute exhaustive treadmill exercise in rats [22,27] and men [21] has been reported. Erythrocytes have antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase, as well as nonenzymatic antioxidants such as glutathione and vitamin E to defend themselves against oxidative stress [4,28]. SOD catalyses the dismutation of the superoxide anion into hydrogen peroxide. H_2O_2 can be transformed into H_2O and O_2 by catalase or H_2O by GPX. GPX is a selenoprotein, which reduces lipidic or nonlipidic hydroperoxides as well as H_2O_2 while oxidizing glutathione [10].

Favourable effects of endurance training on antioxidant defense mechanisms in various tissues of rats have been reported by swim [17,35] and treadmill-trained normal [26] and diabetic [11] rats. Attenuation of the exercise-induced oxidative stress in erythrocytes of endurance trained rats [22] and men [21], decreased serum thiobarbituric acid reactive substances (TBARS) level in long distance runners [24], and children after 4 week swim training [7], and a decrease in susceptibility of erythrocytes to peroxidative hemolysis in trained men compared with untrained men after exercise [29] have been reported. On the other hand, there are also studies where endurance training was not found to affect antioxidant defenses in treadmill-trained rats [19,32] and men [33] or may even have harmful effects such as increased conjugated dienes (CD) level in heart tissue of the diabetic rats [8], increased plasma malondialdehyde (MDA) level in marathon runners [20], increased TBARS and CD in blood of sportsmen, who are university students of physical education and sports [3]. Hence, the aim of this study was to look at the redox status in blood of long distance running athletes if it is favourably affected, and help to prevent acute exercise-induced oxidative stress in these male athletes in comparison with sedentary counterparts.

Materials and Methods

Subjects and exercise protocol: Nineteen sedentary males and 20 male long distance runners were recruited for the study after giving their informed consent. Ages, heights and weights of them are given in Table 1. The long distance runners were the athletes who were training regularly in Erzurum, Turkey, and had taken part in both national and international races. Sedentary males had not performed

regular exercise or any job requiring heavy physical activity. The acute exercise was performed as running on a treadmill (Powerfit 1000, Sports Art) as described before [34]. The treadmill run continued until the heart rate of the subject reaches 80-90% of the maximum (220-age±10) and stopped after 5 min reaching the heart rate maximum. The study was performed in accordance with the ethics standard laid down in the Declaration of Helsinki, 1984.

Table 1
The ages, heights and weights of the sedentary males and male athletes

	Sedentary males	Sedentary males
n	19	20
Age (year)	18.8±1.9	18.6±2.0
Height (cm)	174.1±6.1	178.7±7.9
Weight (kg)	72.3±6.4	76.0±6.3

Values are means ±SD

Complete blood count and determination of erythrocyte malondialdehyde (MDA) level, and the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX):

The blood samples were collected by puncturing a peripheral arm vein just before and after exercise. Blood samples were collected in vacutainer tubes with K_3-EDTA as anticoagulant (1/10, v/v) and complete blood count, including counting erythrocytes, hemoglobin (Hb) measurement and estimating percentage of hematocrit level, was made by a GEN-S counter hematology analyzer. Then, blood samples were centrifuged at 3000 x g for 15 min and plasma was removed by a Pasteur pipette. Then, erythrocytes were washed with 0.9 % NaCl solution three times, and washed erythrocytes were hemolysed by diluting with deionized water (50-fold). The hemolysate was kept in –80 ^{o}C until biochemical determinations.

Erythrocyte MDA level was estimated by the method described by Jain *et al.* [14] based on thiobarbituric acid reactivity. MDA, an end product of fatty acid peroxidation, reacts with TBA to form a colored complex that has maximum absorbency at 532 nm. For this purpose, 0.2 ml hemolysate was suspended in 0.8 ml phosphate-buffered saline (pH 7.4) and 0.025 ml butylated hydroxytoluene. Thirty percent trichloroacetic acid (0.5 ml) was added. Tubes were vortexed and allowed to stand in ice for at least 2 h. They were then centrifuged at 2000 rpm for

15 min. One ml of each supernatant was transferred to another tube, and 0.075 ml 0.1 mol/l EDTA and 0.25 ml 1% TBA were added. Tubes were mixed and kept in a boiling water bath for 15 min. Absorbency was read at 532 and 600 nm (600 nm reading is for preventing hemoglobin interference), after tubes were cooled to room temperature. Butylated hydroxytoluene, an antioxidant, was added to prevent MDA formation during the assay, which could result in falsely elevated TBA activity. Absorbency at 600 nm was subtracted from absorbency at 532 nm. The concentration of MDA was calculated using $1.56x10^5$ $cm^{-1}mol^{-1}$, the absorbency coefficient of MDA-thiobarbituric acid complex at 532 nm, and the result was expressed as nmol/g Hb.

CuZn-superoxide dismutase (SOD) activity was determined by inhibition of the reduction of nitroblue tetrazolium by superoxide anion radicals, which are produced by the xanthine-xanthine oxidase system [30]. GPX activity was measured by coupled spectrophotometric assay at 340 nm from the oxidation of NADPH in the presence of H_2O_2 used as substrate [23]. The activities of erythrocyte SOD and GPX are given as IU/g Hb.

Statistical analyses of the data: While unpaired t test was used to compare the group means of male athletes and sedentary males, paired t-test was used to compare the means of pre- and post- exercise parameters in both groups. SPSS for windows (release 10.0.1) software was used for statistical analyses. P less than 0.05 was accepted as the level of significance.

Results

Acute exercise performed as treadmill run as described above increased the hematocrit percentage in sedentary males but not in male athletes (Table 2). It decreased the number of erythrocytes and also Hb level in sedentary males, but not in male athletes when they were adjusted to the changes in hematocrit level (Table 2).

There was no difference in erythrocyte MDA levels between sedentary males and male athletes at rest. Acute treadmill run increased the erythrocyte malondialdehyde level in sedentary males, however, it did not affect the erythrocyte MDA level in male athletes (Fig. 1). The antioxidant enzymes of the erythrocytes studied, GPX and SOD were not affected by acute exercise in both sedentary males and male athletes (Table 3).

Fig. 1
Erythrocyte malondialdehyde levels before and after exercise in sedentary males and male athletes; **p<0.001, paired t test, vs before exercise in sedentary males

Table 2
Erythrocyte numbers, hemoglobin and hematocrit levels in sedentary males and male athletes before and after exercise

Sedentary males	Before exercise	After exercise	
Hematocrit (%)	48.75±2.52	51.19±2.58**	
		Uncorrected	Adjusted to change in hematocrit level
Erythrocytes (10^3/mm^3)	5 629±329	5 845±356**	5 364±381**
Hemoglobin (g/dl)	16.53±0.85	17.18±0.92**	15.75±1.06**

Male athletes	Before exercise	After exercise	
Hematocrit (%)	48.23±2.52	49.35±3.14	
		Uncorrected	Adjusted to change in hematocrit level
Erythrocytes (10^3/mm^3)	5 551±485	5 668±545	5 431±556
Hemoglobin (g/dl)	16.38±0.91	16.85±1.04*	16.04±1.85

Values are means ±SD; *p<0.05, **p<0.01; vs before exercise

Table 3
Erythrocyte glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities in sedentary males and male athletes before and after exercise

	Before exercise	After exercise
Sedentary males		
GPX(IU/g Hb)	27.19±15.09	26.84±14.96
SOD (IU/g Hb)	1697.5±275.77	1718.73±304.36
Male athletes		
GPX(IU/g Hb)	28.29±11.17	29.65±12.9
SOD (IU/g Hb)	1726.05±236.59	1780.83±253.15

Values are means ±SD

Discussion

Our result, lack of differences in erythrocyte MDA levels between sedentary males and male athletes at rest, disagrees with decreased serum TBARS level in long distance runners (24), and increased plasma MDA level in marathon runners [20], increased TBARS and conjugated dienes in blood of sportsmen [3] compared with sedentary controls. These variations show that training may either decrease the lipid peroxidation by strengthening antioxidant defence mechanisms [25] or exacerbates the oxidative stress by overwhelming the antioxidant defense mechanisms [31]. In our study, acute treadmill run increased the erythrocyte MDA level in sedentary males, however, it did not affect the erythrocyte MDA level in male athletes. Parallel to this finding, attenuation of the exercise-induced oxidative stress in erythrocytes of endurance trained rats [22], decreased plasma TBARS in children after 4 weeks of swim training [7], decrease in susceptibility of erythrocytes to peroxidative hemolysis in trained men compared with untrained men after exercise [29] have been reported. Attenuation in the increase in TBARS level in erythrocytes have also been reported after acute exercise both before and after training, running at 80% maximal heart rate (HRmax) for 60 min a day, 5 days a week for 12 weeks [21]. Our finding, no change in erythrocyte MDA level after exercise in male athletes, and the results of other studies [7,22,29] show that physical training could prevent acute exercise-induced oxidative stress by strengthening antioxidant defence mechanisms.

On the other hand, the increase in lipid peroxidation after acute exercise in sedentary males might have taken part in increased hemolysis, determined as a

decrease in the number of erythrocytes adjusted to change in the hematocrit level. Supporting to this assumption, a marked reduction in resistance to exogenous oxidative stress of erythrocytes taken from sedentary rats after exhaustive exercise has been found. The treatment of these erythrocytes with H_2O_2 has resulted in significantly higher TBARS levels, which might be due to the exhaustion of erythrocyte antioxidant defense capacity during exercise [27].

The antioxidant enzymes studied in our study, erythrocyte SOD and GPX activities were not affected. Our finding, no change in erythrocyte SOD activity in male athletes compared with sedentary males agrees with no difference in erythrocyte SOD activity in long distance runners compared with controls [24]. On the other hand, increased SOD activities have been reported in marathon runners [20], sportsmen, who were students of the Physical Education and Sports Sciences [3], in men after running training [21], in children after swim training [7]. While acute exhaustive exercise decreased the erythrocyte SOD activity in sedentary rats, it increased the activity of this enzyme in trained rats in our previous study [22]. No change in erythrocyte GPX activity in male athletes compared with sedentary males found in our study is in accordance with the finding in swim trained children [7]. On the other hand, decreased GPX activity in erythrocytes in marathon runners [20], and increased erythrocyte GPX activity after training in men [21] and rats [22] have also been reported. In our study, acute exercise did not affect the erythrocyte GPX activity in both male athletes and sedentary counterparts, in line with the finding in trained athletes after exercise [6]. However, an increase in erythrocyte GPX activity has been found in young adults after 40 min of exercise at 60% of their peak oxygen consumption [2], and in sedentary but not trained rats after acute exhaustive exercise [22].

Although the results of various studies are quite heterogeneous, they suggest that training generally improves the antioxidant enzyme activities, even though we could not have determined such increases in enzyme activities in male athletes in our study. It is likely that other enzymes such as catalase, glutathione reductase, glutathione S-transferase, and also levels of glutathione and other non-enzymatic antioxidants could have been increased in male athletes preventing their erythrocytes against acute exercise-induced oxidative stress.

Hematocrit level increased in sedentary males, while it did not change in male athletes after acute exercise. This may result from sweat gland acclimatization in male athletes [13]. Sweat gland acclimatization results mainly from increased aldosterone secretion by the adrenal cortex increasing the reabsorption of sodium chloride from the sweat [13].

To conclude, our results suggest that erythrocytes in long distance male athletes are better protected against acute exercise-induced oxidative stress compared with sedentary counterparts.

References

1. Alessio H.M. (1993) Exercise-induced oxidative stress. *Med.Sci.Sports Exerc.* 25:218-224

2. Atalay M., D.E.Laaksonen, L.Niskanen, M.Uusitupa, O.Hanninen, C.K.Sen (1997) Altered antioxidant enzyme defences in insulin-dependent diabetic men with increased resting and exercise-induced oxidative stress. *Acta Physiol.Scand.* 161:195-201

3. Balakrishnan S.D., C.V.Anuradha (1998) Exercise, depletion of antioxidants and antioxidant manipulation. *Cell Biochem.Funct.* 16:269-275

4. Clemens M.R., H.D.Waller (1987) Lipid peroxidation in erythrocytes. *Chem.Phys. Lipids* 45:251-268

5. Davies K.J., A.T.Quintanilha, G.A.Brooks, L.Packer (1982) Free radicals and tissue damage produced by exercise. *Biochem.Biophys.Res.Commun.* 107:1198-1205

6. Duthie G.G., J.D.Robertson, R.J.Maughan, P.C.Morrice (1990) Blood antioxidant status and erythrocyte lipid peroxidation following distance running. *Arch.Biochem. Biophys.* 282:78-83

7. Gonenc S., O.Acikgoz, I.Semin, H.Ozgonul (2000) The effect of moderate swimming exercise on antioxidant enzymes and lipid peroxidation levels in children. *Indian J.Physiol.Pharmacol.* 44:340-344

8. Gul M., M.Atalay, O.Hänninen (2003) Endurance training and glutathione-dependent antioxidant defense mechanism in heart of the diabetic rats. *J.Sports Sci.Med.* 2:52-61

9. Gul M., O.Hänninen (2002) Physiological basis of exercise. In: O.Hänninen, M.Atalay (eds) Encyclopedia of Life Support Systems (EOLSS); Physiology and maintenance. Developed under the Auspices of the UNESCO. Eolss Publ. Co. Ltd., Oxford

10. Gul M., F.Z.Kutay, S.Temocin, O.Hanninen (2000) Cellular and clinical implications of glutathione. *Indian J.Exp.Biol.* 38:625-634

11. Gul M., D.E.Laaksonen, M.Atalay, L.Vider, O.Hanninen (2002) Effects of endurance training on tissue glutathione homeostasis and lipid peroxidation in streptozotocin-induced diabetic rats. *Scand.J.Med.Sci.Sports* 12:163-170

12. Gul M., N.Oztasan, S.Taysi, K.Gumustekin, S.Akar, N.Bakan, S.Dane (2001) Short-term swimming exercise as an oxidative stress model in rat (in Turkish). *Hacettepe J.Sport Sci.* 12:26-32

13. Guyton A.C., J.E.Hall (2000) Textbook of Medical Physiology. W.B. Saunders Company, Philadelphia

14. Jain S.K., R.McVie, J.Duett, J.J.Herbst (1989) Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. *Diabetes* 38:1539-1543

15. Jenkins R.R. (1988) Free radical chemistry. Relationship to exercise. *Sports Med.* 5:156-170

16. Ji L.L. (1993) Antioxidant enzyme response to exercise and aging. *Med.Sci.Sports Exerc.* 25:225-231

17. Kanter M.M., R.L.Hamlin, D.V.Unverferth, H.W.Davis, A.J.Merola (1985) Effect of exercise training on antioxidant enzymes and cardiotoxicity of doxorubicin. *J.Appl.Physiol.* 59:1298-1303

18. Khanna S., M.Atalay, D.E.Laaksonen, M.Gul, S.Roy, C.K.Sen (1999) Alpha-lipoic acid supplementation: tissue glutathione homeostasis at rest and after exercise. *J.Appl.Physiol.* 86:1191-1196

19. Leeuwenburgh C., R.Fiebig, R.Chandwaney, L.L.Ji (1994) Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. *Am.J.Physiol.* 267:R439-445

20. Marzatico F., O.Pansarasa, L.Bertorelli, L.Somenzini, G.Della Valle (1997) Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. *J.Sports Med. Phys.Fitness* 37:235-239

21. Miyazaki H., S.Oh-ishi, T.Ookawara, T.Kizaki, K.Toshinai, S.Ha, S.Haga, L.L.Ji, H.Ohno (2001) Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. *Eur.J.Appl.Physiol.* 84:1-6

22. Oztasan N., S.Taysi, K.Gumustekin, K.Altinkaynak, O.Aktas, H.Timur, E.Siktar, S.Keles, S.Akar, F.Akcay, S.Dane, M.Gul (2004) Endurance training attenuates exercise-induced oxidative stress in erythrocytes in rat. *Eur.J.Appl.Physiol.* 91:622-627

23. Paglia D.E., W.N.Valentina (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J.Lab.Clin.Med.* 70:158-169

24. Selamoglu S., F.Turgay, B.M.Kayatekin, S.Gonenc, C.Islegen (2000) Aerobic and anaerobic training effects on the antioxidant enzymes of the blood. *Acta Physiol.Hung.* 87:267-273

25. Sen C.K. (1995) Oxidants and antioxidants in exercise. *J.Appl.Physiol.* 79:675-686

26. Sen C.K., E.Marin, M.Kretzschmar, O.Hanninen (1992) Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization. *J.Appl.Physiol.* 73:1265-1272

27. Senturk U.K., F.Gunduz, O.Kuru, M.R.Aktekin, D.Kipmen, O.Yalcin, M.Bor-Kucukatay, A.Yesilkaya, O.K.Baskurt (2001) Exercise-induced oxidative stress affects erythrocytes in sedentary rats but not exercise-trained rats. *J.Appl.Physiol.* 91:1999-2004

28. Siems W.G., O.Sommerburg, T.Grune (2000) Erythrocyte free radical and energy metabolism. *Clin.Nephrol.* 53:S9-17

29. Smith J.A., M.Kolbuch-Braddon, I.Gillam, R.D.Telford, M.J.Weidemann (1995) Changes in the susceptibility of red blood cells to oxidative and osmotic stress following submaximal exercise. *Eur.J.Appl.Physiol.* 70:427-436

30. Sun Y., L.W.Oberley, Y.Li (1988) A simple method for clinical assay of superoxide dismutase. *Clin.Chem.* 34:497-500

31. Tiidus P.M. (1998) Radical species in inflammation and over training. *Can.J.Physiol. Pharmacol.* 76:533-538.

32. Tiidus P.M., M.E.Houston (1994) Antioxidant and oxidative enzyme adaptations to vitamin E deprivation and training. *Med.Sci.Sports Exerc.* 26:354-359

33. Tiidus P.M., J.Pushkarenko, M.E.Houston (1996) Lack of antioxidant adaptation to short-term aerobic training in human muscle. *Am.J.Physiol.* 271:R832-836

34. Ucok K., S.Dane, H.Gokbel, S.Akar (2004) Prevalence of exercise-induced bronchospasm in long distance runners trained in cold weather. *Lung* 182:265-270

35. Venditti P., S.Di Meo (1996) Antioxidants, tissue damage, and endurance in trained and untrained young male rats. *Arch.Biochem.Biophys.* 331:63-68

Accepted for publication 9.06.2005