VETERINARSKI ARHIV 77 (3), 219-227, 2007

Oxidative stress in thoroughbreds during official 1800-metre races

Giuseppe Piccione*, Francesco Fazio, Claudia Giannetto, Anna Assenza, and Giovanni Caola

Department of Morphology, Biochemistry, Physiology and Animal Productions, Section of Veterinary Physiology, Faculty of Veterinary Medicine, University of Messina, Italy

PICCIONE, G., F. FAZIO, C. GIANNETTO, A. ASSENZA, G. CAOLA: Oxidative stress in thoroughbreds during official 1800-metre races. Vet. arhiv 77, 219-227, 2007.

ABSTRACT

The aim of the present study was to find possible relationships between physical exercise and oxidative stress in thoroughbreds during official races. For this purpose six healthy and trained thoroughbreds were used. Blood samples were collected from all horses at rest immediately after the race, and 30 and 180 min. after the race. The following parameters were assessed on obtained serum using the spectrophotometer method: lactate dehydrogenase, creatine kinase, reactive oxygen species, thiol antioxidant barrier and antioxidant barrier. Obtained results showed the highly significant effect of exercise on some of the studied parameters: LDH, $F_{(3,15)} = 49.85$, $P<0.0001$; ROMs, $F_{(3,15)} = 60.71$, $P<0.0001$; Oxy-adsorbent, $F_{(3,15)} = 393.70$, $P<0.0001$. No statistical significant differences were observed for CK and SHP. Our results appear to indicate that during official races thoroughbreds generate free radicals, but are unable to determine a significant lyses of skeletal muscle cells.

Key words: physical exercise, oxidative stress, reactive oxygen species, athletic horse

Introduction

Free radicals are active chemical structures defined as any species capable of independent existence that contain one or more unpaired electrons (AUROMA, 1994). Free radicals are chemically highly reactive because the unpaired electron attempts to stabilize itself by pairing with another electron. It should be noted that the terms "reactive oxygen species" (ROS) and "free radicals" are often used interchangeably in the literature. They may be formed in the course of physiological and pathological processes in aerobic organism, and the combination of a free radical with a cellular component may result in cellular dysfunction. Consequently, ROS have the ability to cause skeletal muscle damage at either the site of origin, or elsewhere (MATSUO and KANECO, 2000). Physical

*Contact address:

Prof. Giuseppe Piccione, Department of Morphology, Biochemistry, Physiology and Animal Productions, Section of Veterinary Physiology, Faculty of Veterinary Medicine, Polo Universitario dell'Annunziata, 98168 Messina, Italy, Phone: +39 90 350 3584; Fax: +39 90 350 3975; E-mail: giuseppe.piccione@unime.it

ISSN 0372-5480
Printed in Croatia

exercise is associated with a dramatic increase in oxygen uptake by the whole body and particularly by the skeletal muscle. The production of reactive oxygen species (ROS) is believed to be the underlying mechanism for a series of biochemical and physiological changes that occur during exercise and are indicative of oxidative stress. In thoroughbred race horses, physical activity gives rise to an imbalance between free radical production and antioxidant agents, leading to oxidative stress (SIES, 1991; SIES et al., 1992). This stress can produce damage in several bio molecules (NATTA et al., 1992; BRENT and RUMACK, 1993) creating metabolic alterations affecting physical performance. The increased free radical production, identified as semiquinone, coincides with a series of cellular disorders, such as lipid peroxidation, loss of sarcoplasmatic reticulum latency, and mitochondrial uncoupling. Little progress has been made in the past decade in direct measurement of free radical generation during exercise in the horse (CHIARADIA et al., 1998; WHITE et al., 2001; WILLIAMS et al., 2004; de MOFFARTS et al., 2004; KINNUNEN et al., 2005a; KINNUNEN et al., 2005b). The aim of this study was to find possible relationships between physical exercise and oxidative stress in thoroughbred during an official 1800-metre race. In particular, we assessed: levels of lactate dehydrogenase and creatine kinase in order to evaluate possible muscle fibre damage, and reactive oxygen species, thiol antioxidant barrier and antioxidant barrier in order to evaluate oxidative stress.

Materials and methods

To this end, six healthy thoroughbreds (3 geldings and 3 mares) aged between 3 and 5 years, with a body mass of 400 ± 30 kg, fed with oats and hay, were used. All horses were trained and competed regularly at the "Mediterraneo" racetrack (Siracusa - Sicily, Italy). The horses took part in an official 1800-metre race (average speed 14.95 m/s in 2.04 min.) Blood samples were collected from all horses at rest, immediately after the race, and again after 30 and 180 min. Each animal blood sample, collected by jugular venipuncture using vacutainer tubes (Terumo Corporation, Japan) with no additive, was centrifuged at 3000 × g for 10 min. On the obtained serum, using a spectrophotometer (SEAC Slim, Italy), the following parameters were assessed: lactate dehydrogenase (LDH), creatine kinase (CK), reactive oxygen species (ROS), thiol antioxidant barrier (SHp) and antioxidant barrier (Oxy-adsorbent). Creatine kinase (CK) concentration was assessed by means of UV spectrophotometry following the Szasz et al. method (SZASZ et al., 1976); a buffer solution and a substrates and enzymes mixture (Creatine-phosphate, ADP, Glucose-6-phosphate, diadenosinepentaphosphate and AMP) were used to asses CK at 37 °C. Lactate dehydrogenase (LDH) concentration was assessed by means of UV spectrophotometry following the Wroblewski and La Due method (WROBLEWSKI and LA DUE, 1995). The reagent supplied with the kits was dissolved and mixed gently until a uniform solution containing $\beta NADH_2$, EDTA, TRIS was obtained, and with Piruvato

used as substrate. The temperature (37 °C) remained constant during the whole period of the test. The ROS test is a colometric test that assesses the level of hydroperoxideses (R-OOH) (a class of reactive oxygen species (ROS) in a serum and is based on the following principle: the reactive oxygen species (ROS) are free radicals characterised by having an uneven number of electrons in the oxygen external orbit. Due to their extreme chemical instability they form products with high chemical reactivity and a good oxidant power in the plasma and cells. These products, reacting with a buffered chromogen, develop a coloured complex measurable by photometer at 505 nm and proportional to their concentration, following the Lambert and Beer law (CESARONE et al., 1999). In order to assess ROS, a chromogenic mixture (N,N, diethylparaphenylen-diamine) (10 µL) and an acid buffered solution (pH 4.8) (1 mL) were mixed with serum (5 µL) and incubated for 75 min. at 37 °C. Absorbance was read at 505 nm wavelength. Hydroperoxides concentration, which directly correlates with detected colour intensity, is expressed as arbitrary units which are simple to use in clinical practice; Carratelli Units (CARR U) where 1 CARR U correspond to 0.08 mg/100 mL H_2O_2. The oxy-adsorbent test is a colometric determination of plasma/serum antioxidant barrier. This test evaluates the plasma/serum ability to oppose the massive oxidate action in a hypochlorous acid solution (TROTTI et al., 2001). In order to asses oxy-absordent an oxidant solution (1 mL) and a chromogenic mixture (N,N-diethylparaphenylendiamine) (10 µL) were mixed and read immediately according to the following working conditions: wavelength 505 or 546 nm and at room temperature. Finally, the SHp test is a colometric determination of plasma/serum thiol antioxidant barrier using the DTNB (5,5-dithiobys-2-nitrobenzoyc acid) (ELLMAN, 1959). In order to asses SHp a buffer solution (pH 7.6) (1mL) and a chromogenic mixture (DTNB) (20 µL) were mixed with serum (50 µL). The test can be performed according to the following working conditions: wavelength 405 nm and at room temperature. All samples were analyzed in duplicate. Samples exhibited parallel displacement to the standard curve. The overall intra-assay coefficient of variation has been calculated as <5%. The overall inter-assay coefficient of variation has been calculated as <5%. Since the intragroup variance was not significant, the statistical elaboration of data was carried out on mean values of studied parameters. On mean values we applied the analysis of variance (one-way and repeated measures ANOVA), so as to evaluate the statistical significant differences obtained by comparing the different experimental conditions (at rest *vs* immediately after the race, at rest *vs* 30 min. after the race, and immediately after the race *vs* 30 and 180 min. after the race).

Results

Table 1 shows average value of LDH, CK, ROS, SHp, Oxy-absorbent, expressed in their conventional units, together with the relative standard deviations and statistical significance obtained under the different experimental conditions.

Table 1. Mean ± SD of LDH, CK, ROS, SHp, oxy-adsorbent, expressed in their conventional units, together with the statistical significance obtained on the different experimental conditions in 6 thoroughbreds

Parameters	Experimental conditions			
	Rest	After race	After 30’	After 180’
LDH (U/l)	206.30 ± 13.95	298.30 ± 12.91*	220.50 ± 14.61♦	223.00 ± 18.97♦
CK (U/l)	136.00 ± 8.51	141.70 ± 6.95	138.20 ± 7.39	137.00 ± 9.96
ROS (Ucarr)	162.30 ± 5.32	192.30 ± 5.68 *	154.30 ± 6.41♦	157.32 ± 6.44♦
SHp (µmol/l)	499.20 ± 21.08	482.50 ± 25.04	515.50 ± 21.30	515.80 ± 22.89
Oxy-adsorbent (µmol HclO/mL)	433.30 ± 14.02	314.70 ± 12.48*	235.80 ± 15.63*♦	237.20 ± 13.35*♦

Significance: * *vs* rest (P<0.001); ♦ *vs* after race (P<0.001).

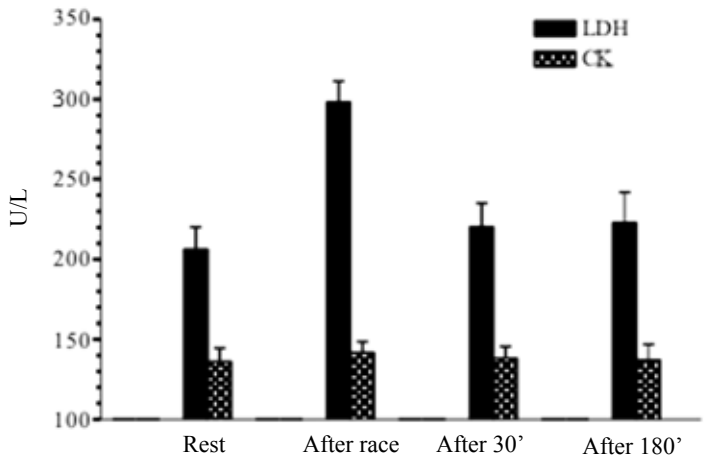

Fig. 1. Pattern of mean value and their standard deviation of LDH and CK obtained under different experimental conditions in 6 thoroughbreds

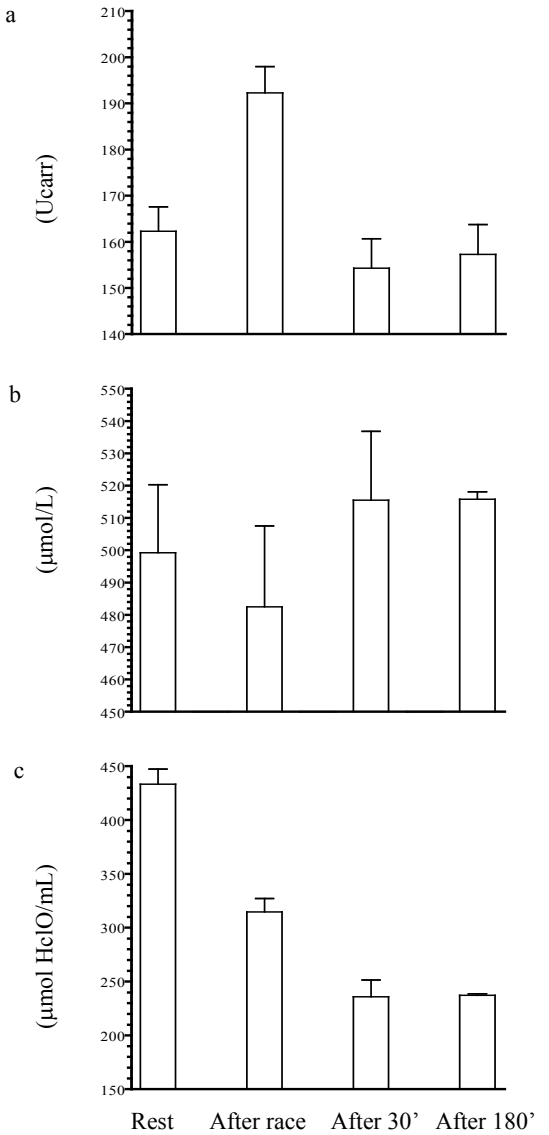

Fig. 2. The pattern of mean values and their standard deviations of (a) ROS, (b) SHP and (c) Oxy-adsorbent obtained on the different experimental conditions in 6 thoroughbred

The ANOVA for repeated measures showed a highly significant effect of exercise on some of the studied parameters: LDH, $F_{(3,15)} = 49.85$, $P<0.0001$; ROMs, $F_{(3,15)} = 60.71$, $P<0.0001$; Oxy-adsorbent, $F_{(3,15)} = 393.70$, $P<0.0001$. No statistical significant differences were observed for CK and SHP.

Fig. 1. shows the pattern of mean values and the standard deviation of LDH and CK; the pattern of mean values and their standard deviations of ROS, SHP and oxy-adsorbent are shown in Figure 2. On individual values of reactive oxygen species (ROS), and of creatine kinase (CK), a linear regression model ($y = a+bx$) was applied in order to determine the correlation degree between the studied parameters in the thoroughbreds, and the correlation coefficient (r) was determined. The application of linear regression model showed a low correlation between individual values of reactive oxygen species (ROS), and of creatine kinase (CK) in thoroughbreds ($r = 0.28$).

Discussion

In thoroughbreds, the oxidative stress associated with physical exercise would not seem to be responsible for muscular lesions. In fact, the significant increase of lactic dehydrogenates (LDH) after the race vs. rest may be due to an increase of mitochondrial membrane permeability instead of muscular lesions. The increase in permeability could be associated with the maximal physical exercise undertaken (NIMMO and SNOW, 1982). On the other hand, the lack of statistically significant variations of creatine kinase (CK) seems to exclude the insurgence of muscular lesions caused by exercise-induced oxidative stress. During physical exercise in horses there is an increase of creatine kinase (CK). This increase is not necessarily an index of insufficient fitness or muscular fatigue (KERR and SNOW, 1983). The anti-oxidant power of the plasmatic barrier would not seem to be adapted to prevent the reactive production of the reactive oxidation species (ROS) considering that, together with the statistically significant decrease of oxy-adsorbent concentration after the race vs. at rest, there is a statistically significant increase of ROS (CHIARADIA et al., 1998). The lack of correlation between ROS and CK after the race would indicate that although physical exercise causes an increase of the production of the reactive oxidation species (ROS), these are not responsible for damage at the muscular level. Hard physical exercise, characterized by a marked increase in oxygen consumption, presents a challenge to the antioxidant system because of the increased production of ROS. The enzymatic (superoxide dismutase (SOD), glutathione peroxidase (GSH), etc.) and non-enzymatic anti-oxidants (e. g. Vit. E) does not usually protect tissues from the oxidative damage (HALLIWELL and GUTTERIDGE, 1990; CHOW 1991; MICHIELS et al., 1994), but the depletion of some of these anti-oxidant systems could increase the vulnerability of tissues and of cellular components' reactive oxidation species. However, tissues seem to increase their anti-oxidant defence when exposed to a chronic activation.

It would appear that whereas the role of increased ROS production in exercise-induced damage is controversial, an increased production of ROS plays a major role as a signalling mechanism for adaptation of skeletal muscle following exercise. ROS produced in the days following contraction-induced damage are necessary for cellular adaptation (CLOSE et al., 2005). Thus, the application of specific training programs could improve athletic performance through an increase in anti-oxydant defence in tissue exposed to physical exercise (CHIARADIA et al., 1998).

Chronic exercise training seems to have a dual effect: it induces antioxidant enzyme and perhaps stimulates GSH synthesis, thus theoretically facilitating the removal of ROS produced during exercise. Therefore, a comparison between anti-oxidant effects and the modifications of the parameters of the athletic performance (e. g. lactic acid, heart rate, respiratory rate and O_2 consumption), both at rest and after work, is necessary to show that oxidative process inhibition during physical exercise may improve physical performance.

References

AUROMA, O. I. (1994): Free radicals and antioxidant strategies in sports. J. Nutr. Biochem. 5, 370-381.

BRENT, J., B. RUMACK (1993): Role of free radicals in toxic hepatic injury. I Free Radical Biochem. 5, 370-381.

CESARONE, M. R., G. BELCARO, M. CARATELLI, U. CORNELLI, M. T. DE SANCTIS, L. INCANDELA, A. BARSOTTI, R. TERRANOVA, A. NICOLAIS (1999): A simple test to monitor oxidative stress. Int. Angiol. 18, 127-30.

CHIARADIA, E., L. AVELLINI, F. RUECA, A. SPATERNA, F. PORCIELLO, M. T. ANTONIONI, A. GAITI (1998): Physical exercise, oxidative stress and muscle damage in racehorses. Comp. Biochem. Physiol. B. Biochem Mol. Biol. 119, 833-836.

CHOW, C. K. (1991): Vitamin E and oxidative stress. Free Radical. Biol. Med. 11, 215-232.

CLOSE, G. L., T. ASHTON, A. MCARDLE, D. P. M. MACLAREN (2005): The emerging role of free radicals in delayed onset muscle soreness and contration-induced muscle injury. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 142, 257-266.

De MOFFARTS, B., N. KIRSCHVINK, T. ART, J. PINCEMAIL, C. MICHAUX, K. CAYEUX, J. DEFRAIGNE, P. LEKEUX (2004): Impact of training and exercise intensity on blood antioxidant markers in healthy Standardbred horses. Equine Comp. Exercise Physiol. 1, 211-220.

ELLMAN, G. L. (1959): Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82, 70-77.

HALLIWELL, B., J. M. GUTTERIDGE (1990): The antioxidants of human extracellular fluids. Arch. Biochem. Biophys. 280, 1-8.

KERR, M. G., D. H. SNOW (1983): Plasma enzyme activities in endurance horses. In: Equine Exercise Physiology. (Snow, D. H., S. G. B. Persson, R. J. Rose, Eds.). Granta Editions. Cambridge. pp 432-440.

KINNUNEN, S., S. HYYPPA, J. LAPPALAINEN, N. OKSALA, M. VENOJARVI, C. NAKAO, O. HANNINEN, C. K. SEN, M. ATALAY (2005a): Exercise-induced oxidative stress and muscle stress protein responses in trotters. Eur. J. Appl. Physiol. 93, 496-501.

KINNUNEN, S., S. HYYPPA, A. LEHMUSKERO, N. OKSALA, P. MAENPAA, O. HANNINEN, M. ATALAY (2005b): Oxygen radical absorbance capacity (ORAC) and exercise-induced oxidative stress in trotters. Eur. J. Appl. Physiol. 95, 550-556.

MICHIELS, C. M., O. RAES, J. REMACLE (1994) : Importance of Seglutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Radic. Biol. Med. 17, 235-248.

MATSUO, M., T. KANEKO (2000): The Chemistry of Reactive Oxygen Species and Related Free Radicals. In: Free Radicals in Exercise and Aging. (Radak, Z., Eds). Human Kinetics. Leeds. 1-34.

NATTA, C., V. TATUM, C. K. CHOW (1992): Antioxidant status and free radical-induced oxidative damage of sickle erythrocytes. Ann. N. Y. Acad. Sci. 669, 365-367.

NIMMO, M. A., D. H. SNOW (1982): Time course of ultrastructural changes in skeletal muscle after two types of exercise. J. Apll. Physiol. 52, 910-913.

SIES, H. (1991): Oxidative stress: from basic research to clinical application. Am. J. Med. 91, 31S-38S.

SIES, H., W. STAHL, A. R. SUNQUIST (1992): Antioxidant function of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. Ann. N. Y. Acad. Sc. 669, 7-20.

SZASZ, G., W. GERHARDT, W. GRUBER, E. BERNT (1976): Creatine kinase in serum. 2). Interference of adenylate kinase with the assay. Clin. Chem. 22, 1806-1812.

TROTTI, R., M. CARRATELLI, M. BARBIERI, G. MICELI, D. BOSONE, M. RONDANELLI, P. BO (2001): Oxidative stress and thrombophilic condition in alcoholics without severe liver disease. Haematological 86, 85-91.

WHITE, A., M. ESTRADA, K. WALKER, P. WISNIA, G. FILGUEIRA, F. VALDES, O. ARANEADA, C. BEHN (2001): Role of exercise and ascorbate on plasma antioxidant capacity in thoroughbred race horses. Comp. Bioch. Physiol. A. 128, 99-104.

WILLIAMS, C. A., D. S. KRONFELDT, T. M. HESS, K. E. SAKER, J. N. WALDRON, K. M. CRANDELL, R. M. HOFFMAN, P. A. HARRIS (2004): Antioxidant supplementation and subsequent oxidative stress of horse during an 80-km endurance race. J. Anim. Sci. 82, 588-594.

WROBLEWSKI, F., J. H. LA DUE (1995): Lactic dehydrogenase activity in blood. Proc. Soc. Exp. Biol. Med. 90, 210.

Received: 11 October 2005
Accepted: 28 May 2007

PICCIONE, G., F. FAZIO, C. GIANNETTO, A. ASSENZA, G. CAOLA: Oksidativni stres u punokrvnjaka u tijeku službenih utrka na 1800 metara. Vet. arhiv 77, 219-227, 2007.

SAŽETAK

Cilj istraživanja bio je ustanoviti moguću povezanost između tjelesne vježbe i oksidativnog stresa u punokrvnjaka u tijeku službenih utrka. Istraživanje je provedeno na osam zdravih uvježbanih punokrvnjaka. Svima su uzeti uzorci krvi u tijeku odmora, netom nakon utrke te 30 i 180 minuta nakon toga. Spekrofotometrijom su određivani sljedeći pokazatelji u uzorcima seruma: laktat dehidrogenaza, kreatin kinaza, vrsta reaktivnog kisika, tiol-antioksidativna barijera i antioksidativna barijera. Rezultati su pokazali znatan učinak vježbe na neke istraživane pokazatelje: LDH, $F(3,15) = 49,85$, $P<0,0001$; ROMs, $F(3,15) = 60,71$, $P<0,0001$; oksi-adsorbens, $F(3,15) = 393,70$, $P<0,0001$. Nisu ustanovljene statistički značajne razlike za kreatin-kinazu i tiol-antioksidativnu barijeru. Rezultati pokazuju da se u punokrvnjaka u tijeku službenih utrka oslobađaju slobodni radikali, ali se ne nije mogla utvrditi značajna liza stanica kosturnoga mišićja.

Ključne riječi: tjelovježba, oksidativni stres, vrsta reaktivnog kisika, trkaći konji