ORIGINAL PAPERS

Adv Clin Exp Med 2012, **21**, 3, 313–320
ISSN 1899–5276

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The Effect of Exhaustive Exercise on Oxidative Stress Generation and Antioxidant Defense in Guinea Pigs*

Wpływ wyczerpujących ćwiczeń na wywoływanie stresu oksydacyjnego i obronę antyoksydacyjną u świnek morskich

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Abstract

Background. Exercise induces a multitude of physiological and biochemical changes in blood that can affect its redox status. Exercise causes an increase in oxygen consumption by the whole body and particularly by the exercising muscle. As a result of this process there is a rise in the production of reactive oxygen species (ROS), which are capable to trigger a chain of damaging biochemical and physiological changes known as lipid peroxidation and oxidative stress. Since the early work of Dillard et al. in 1978, and findings of increased lipid peroxidation following acute aerobic exercise, the topic of exercise-induced oxidative stress has received considerable attention.
Objectives. The aim of this study was to examine how swimming to the point of exhaustion affects oxidative stress generation and nonenzymatic antioxidant activity in an animal model.
Material and Methods. The experiments were conducted on 10 male Dunkin-Hartley guinea pigs, and the swimming program used in the study, described as swimming to exhaustion, was defined by Dawson and Horvath. Peripheral blood samples were taken at rest and immediately after the exercise test, and the following parameters were evaluated: total plasma peroxide concentration (TPPC), total plasma thiols (TPT), plasma xanthine oxidase activity (XOD), plasma antioxidant capacity and total plasma proteins.
Results. No significant difference was found between TPPC measured at rest and immediately after exercise swimming test ($p = 0.138$). Two other parameters – TPT and XOD activity – showed significant differences before and after the swimming exercise test: After the test the TPT value decreased significantly ($p = 0.022$), while XOD activity increased significantly ($p = 0.039$). Comparing antioxidant activity in plasma before and after the exercise test, although the value decreased, the difference was not statistically significant ($p = 0.755$).
Conclusions. The results of this study show that exercise to exhaustion induces the generation of oxidative stress primarily by oxidative modification of protein molecules. The results also indicated that the prooxidative enzyme xanthine oxidase is an important source of ROS during exercise-induced oxidative injury (**Adv Clin Exp Med 2012, 21, 3, 313–320**).

Key words: swimming exercise, guinea pigs, free radicals, oxidative stress.

Streszczenie

Wprowadzenie. Ćwiczenia fizyczne wywołują wiele zmian fizjologicznych i biochemicznych we krwi, które mogą mieć niekorzystny wpływ na stan oksydoredukcyjny. Ćwiczenia powodują wzrost zużycia tlenu przez cały organizm, a szczególnie przez ćwiczące mięśnie. W wyniku tego procesu wzrasta wydzielanie reaktywnych form tlenu (RFT), które są w stanie wywołać łańcuch biochemicznych i fizjologicznych uszkodzeń zwanych peroksydacją lipidów i stresem oksydacyjnym. Już od czasów pracy Dillard et al. w 1978 r. i ustalenia, że zwiększona peroksydacja lipidów jest następstwem forsownych ćwiczeń aerobowych, tematowi stresu oksydacyjnego wywołanego przez wysiłek fizyczny poświęcono wiele uwagi.

* This work was partly supported by a grant from the Ministry of Science and Technological Development, Republic of Serbia (Project number III 44007).

Cel pracy. Zbadanie, jak pływanie aż do wyczerpania sił wpływa na wywoływanie stresu oksydacyjnego i nieenzymatyczną aktywność przeciwutleniającą w modelu zwierzęcym.
Materiał i metody. Badania przeprowadzono na 10 świnkach morskich szczepu Dunkin-Hartley płci męskiej, a program pływania zastosowany w badaniu, określony jako pływanie aż do wyczerpania, został opisany przez Dawsona i Horvatha. Pobrano próbki krwi obwodowej w spoczynku i bezpośrednio po próbie wysiłkowej oraz oceniono następujące wskaźniki: całkowite stężenie nadtlenku w osoczu (TPPC), całkowite stężenie tioli w osoczu (TPT), aktywność oksydazy ksantynowej w osoczu (XOD), potencjał antyoksydacyjny osocza i całkowitą zawartość białka w osoczu.
Wyniki. Nie stwierdzono istotnej różnicy między TPPC mierzonym w spoczynku i bezpośrednio po teście pływania ($p = 0{,}138$). Dwa pozostałe parametry – TPT i aktywność XOD – wykazały istotne różnice przed i po próbie wysiłkowej. Po wysiłku wartość TPT znacznie zmniejszyła się ($p = 0{,}022$), a aktywność XOD znacznie wzrosła ($p = 0{,}039$). Porównując działanie antyoksydacyjne w osoczu przed i po teście wysiłkowym, wartość zmniejszyła się, ale różnica nie była istotna statystycznie ($p = 0{,}755$).
Wnioski. Wyniki tego badania wykazują, że ćwiczenia aż do wyczerpania wywołują powstawanie stresu oksydacyjnego głównie przez oksydacyjną modyfikację cząsteczek białka. Wyniki wskazują również, że prooksydacyjny enzym oksydaza ksantynowa jest ważnym źródłem RFT podczas powysiłkowego uszkodzenia oksydacyjnego (**Adv Clin Exp Med 2012, 21, 3, 313–320**).

Słowa kluczowe: pływanie, świnki morskie, wolne rodniki, stres oksydacyjny.

One of the most frequent definitions of free radicals defines them as any species capable of existence, containing one or more unpaired electrons. Both the radical and nonradical species created via interaction with free radicals are collectively referred to as reactive oxygen and nitrogen species (RONS). Free radical reactions occur physiologically and pathophysiologically and are responsible for a number of key biochemical events in aerobic life forms, such as prostaglandin synthesis, phagocytosis, and endoplasmic reticulum hydroxylation reactions [1].

Exercise induces a multitude of physiological and biochemical changes in blood that may affect its redox status. As Nikolaidis and Jamurtas pointed out, some of the events that arise during exercise include "increases in blood temperature and blood lactate concentration and decreases in blood pH and blood oxygen partial pressure. All these exercise associated homeostasis disruptions are able to modify blood redox status" [2]. Since the early work of Dillard et al. in 1978 [3] and findings of increased lipid peroxidation following acute aerobic exercise, the topic of exercise-induced oxidative stress has received considerable attention. As Lamprecht et al. noted: "The state in which the accumulation of free radicals and other reactive species overcharges the antioxidative defense capacity of the system [has become] an interesting topic not only for scientists but for trainers and other specialists [in] sport and exercise as well" [1]. This increased interest can be explained both by an enhanced awareness of the role of RONS in the pathogenesis of different human diseases on the one hand, and on the other hand by interest in promoting exercise as a means to improve health [4, 5].

Exercise-related oxidant production is based on number of potential pathways. It has been estimated that exercise can cause the active muscle fibers' oxygen utilization to increase to 200 times resting levels. It appears that superoxide production rises along with this major increase in the flow of oxygen through muscle mitochondria; this process has been viewed as causing the release of superoxide and hydrogen peroxide by the muscle cells and local hydroxyl radical formation [6] A very important mechanism involved in free radical production during and after strenuous exercise is based on ischemia-reperfusion injury with two essential elements: cellular hypoxia and reoxygenation. The amount of superoxide anion produced by reoxygenated cells depends on the duration of both anoxia and reoxygenation [7] The prooxidative enzyme xanthine oxidase catalyzes the conversion of hypoxanthine and xanthine to uric acid. This enzyme is also involved in the production of superoxide radicals, which are potentially toxic to cellular structures [8]. As Dröge wrote: "Neutrophils are the principal effector cells of reperfusion injury, and the inhibition of neutrophil adhesion to the endothelium attenuates the process of free radical generation" [9]. Xanthine oxidase is also considered a source of superoxide radicals during inflammatory events in skeletal muscle, where, as in most other tissues, it is localized in the vascular walls [10]. As Fisher-Wellman and Bloomer wrote, the initial increase in RONS production during exercise "can lead to additional secondary generation of prooxidants via phagocytic respiratory burst, a loss of calcium homeostasis and/or the destruction of iron- containing proteins" [5]. Autooxidation of oxyhemoglobin to methemoglobin also results in the production of superoxide and the rate of formation of methemoglobin can increase with exercise [6].

The blood antioxidant defense is a complex system consisting of various molecules, both enzymatic (catalase, superoxide dismutase, glutathione peroxidase) and nonenzymatic (α-tocopherol, ascorbic acid, β-carotene, glutathione and uric acid). These antioxidants are crucial to protecting against oxidative damage during exercise. There have been numerous reports of increases in lipid, protein, DNA and glutathione oxidation after strenuous aerobic exercise in both humans and animals, which indicates that different tissues and cellular components are left vulnerable when the antioxidant system is depleted [5].

The aim of this study was to examine how swimming to the point of exhaustion affects oxidative stress generation and nonenzymatic antioxidant activity in an animal model.

Material and Methods

The Experimental Animals

The experiments were conducted on 10 male Dunkin-Hartley guinea pigs initially weighing 500–550 g. The animals were housed in a temperature controlled (22 ± 2°C) and ventilated room, under natural lighting conditions, and they had free access to standard food and water.

The animals were handled in accordance with the European Community Guidelines (86/609/EEC) and the American Physiological Society's "Guiding Principles for Research Involving Animals and Human Beings" [11]. All procedures were approved by the Ethical Committee of the University of Pristina Medical School in Kosovska Mitrovica (Serbia).

The Experimental Protocol

The swimming program used in the study (swimming to exhaustion) was defined by Dawson and Horvath [12, 13]. The swimming test was conducted in a cylindrical tank with a diameter of 100 cm and a depth of 50 cm, containing water at 30–32°C. The animals were manually supported below their thorax, placed in the water tank and allowed to swim. The point in time at which a guinea pig remained below the water surface for 10 seconds was recorded as the exercise endurance capacity. The average endurance capacity of the guinea pigs in this study was 45 ± 5 min.

The Biochemical Analysis

Peripheral blood samples were taken by lateral saphenous venipuncture at rest and immediately after finishing the exercise test. The following parameters were evaluated: total plasma peroxides as a marker of the lipid peroxidation process, total plasma thiols, plasma xanthine oxidase activity, plasma antioxidant capacity and total plasma proteins.

Determination of Total Peroxides

Total plasma peroxides were assessed by the ferrous-oxidation xylenol orange method (FOX2), based on the ability of various types of peroxides to oxidize ferrous ion in an acidic medium containing the dye xylenol orange [14]. The resulting ferric-xylenol orange complex can be measured at 560 nm. In brief, 90 μL of plasma was mixed with 10 μL of HPLC-grade methanol, or 10 μL of triphenylphosphine (TPP) solution (20mM in methanol), in 1.5 mL microcentrifuge vials and left in the dark at room temperature for 30 minutes before adding 900 μL of FOX solution (250 μM ammonium ferrous sulfate, 100 μM xylenol orange, 25 mM sulfuric acid, and 4 mM butylated hydroxytoluene in 90% (v/v) methanol in a final volume of 100 mL). The samples were further incubated at room temperature for 30 minutes, during which they were vortex mixed every 10 minutes, and spun (10 minutes at 10,000 g). Absorbance readings were taken at 560 nm on a UV/VIS spectrophotometer (SAFAS 2, Monaco) against an appropriate blank probe prepared with FOX-blank reagent in which the xylenol orange was omitted. The total plasma peroxide concentration (TPPC) was calculated as the difference between untreated and TPP-treated samples, and calibrated with the H_2O_2 standard curve in the concentration range of 0–20 μmol/L. The results were expressed as nmol/g protein.

Total Plasma Thiols Assay

The concentration of total plasma thiols (TPT) was determined by Ellman's reagent [15]. An aliquot of 20 μL of plasma was mixed with 800 μL of sodium phosphate buffer (Na_2HPO_4; 0.3 M) and 200 μL of Ellman's reagent (40 mg of 5,5'-dithiobis-(2-nitrobenzoic acid) in 100 mL of 1% (w/v) sodium citrate). After 10 minutes the absorbance was measured at 412 nm against reagent blank. The amount of TPT was calculated using the molar absorbance of 1.36×10^4 L × M^{-1} × cm^{-1}. The results were expressed as μmol/g protein.

Measurement of Plasma Xanthine Oxidase Activity

The measurement of plasma xanthine oxidase (XOD) activity was carried out at 25°C in

a quartz-glass spectrophotometer cuvette, by the rate of the oxidation of xanthine to uric acid [16]. All reagents were pre-warmed at 25°C. In brief, 0.3 mL of TRIS-HCl buffer (50mM; pH 7.4), 0.3 mL copper (II) sulfate (10mM) and 50 μL of freshly prepared substrate (14 mg xanthine and sodium salt in 3 mL of 0.1 M NaOH) were added to a cuvette followed by 100 μL of plasma sample; these were mixed and diluted with water to make up a volume of 3 mL. After a 15-second lag phase, the formation of uric acid was continuously measured at 293 nm for 120 sec and corrected for pre-existing uric acid. One unit of XOD activity was defined as 1 μmol/min of uric acid formed at 25°C, calculated using the molar absorbance of 1.26×10^4 L × M^{-1} × cm^{-1}. The results were expressed as milliunits per gram of protein (mU/g protein).

Measurement of Plasma Antioxidant Capacity

The ferric reducing ability of plasma (FRAP) test was used to determine plasma antioxidant capacity [17]. This method is based on the ability of water-soluble antioxidants to reduce ferric ions in an acidic medium containing the dye 2,4,6-tripyridyl-s-triazine (TPTZ). The working FRAP reagent was prepared daily by mixing 25 mL acetate buffer (0.3 M; pH 3.6), 2.5 ml TPTZ solution (10 mM TPTZ, 40mM HCl) and 2.5 mL ferric chloride solution (20mM). Two milliliters of working FRAP reagent, pre-warmed at 37°C, were mixed with 60 μL of plasma, and after 6 minutes of incubation at room temperature the ferrous-TPTZ complex was measured at 593 nm against acetate buffer. The FRAP value was calibrated with the ferrous sulfate standard curve prepared in the concentration range of 100–2000 μmol/L. The results were presented as μmol/g protein.

Determination of Total Plasma Proteins

The concentration of total plasma proteins was determined by the biuret method, using bovine serum albumin as a standard (50 g/L).

The Statistical Analysis

The statistical analysis was performed using a commercial software package (SPSS version 12.0 for Windows, SPSS Inc., Illinois, USA). The data are reported as means ± SE. The statistical procedures included the paired-samples Student t-test. Differences were considered significant at $p < 0.05$.

Results

All the measured parameters are shown in Table 1.

Oxidative Stress Parameters

Among the oxidative stress parameters, TPPC as a marker of lipid peroxidation showed no significant difference between the concentration measured at rest and the concentration immediately after the swimming test ($p = 0.138$). Two other parameters TPT (as a marker of oxidative protein modification) and XOD activity (as a marker of ischemia-reperfusion injury) showed significant differences before and after the swimming exercise test. Namely, the TPT value significantly decreased ($p = 0.022$), while XOD activity significantly increased ($p = 0.039$) after the test.

Table 1. Oxidative stress (TPPC, TPT, XOD) and antioxidant (FRAP) parameters in plasma before and after the swimming test

Tabela 1. Wskaźniki stresu oksydacyjnego (TPPC, TPT, XOD) i antyoksydacyjne (FRAP) w osoczu przed i po teście wysiłkowym

	At rest (Spoczynek)	After exhaustive exercise (Po wyczerpujących ćwiczeniach fizycznych)	Student t-test values (Wartość testu t-Studenta)
TPPC – nmol/g protein	256.35 ± 25.44	200.90 ± 24.39	p = 0.138
TPT – μmol/g protein	22.35 ± 3.58	15.31 ± 2.10	p = 0.022*
XOD – mU/g protein	160.61 ± 19.59	244.42 ± 29.30	p = 0.039*
FRAP – μmol/g protein	19.42 ± 2.24	18.50 ± 1.86	p = 0.755

TPPC – Total Plasma Peroxide Concentration.
TPT – Total Plasma Thiols Concentration.
XOD – Plasma Xanthine Oxidase Activity.
FRAP – Plasma antioxidant capacity (Ferric Reducing Ability of Plasma).
* $p < 0.05$ statistical significance.

Antioxidant Parameters

Although the level of antioxidant activity in plasma decreased after the swimming exercise test, the difference was not statistically significant ($p = 0.755$).

Discussion

More than three decades have elapsed since the first findings related to exercise-induced oxidative stress, and yet the topic is still of interest to researchers in different fields of science. Although experiments are conducted on both human and animal models, it is believed that the ones conducted on animal models are more controlled because of the homogeneity of the experimental groups and the possibility of measuring oxidative stress biomarkers in different biological tissues [5].

In the process of oxidative stress, polyunsaturated fatty acids of membranes are attacked repeatedly by free radicals to form highly destructive polyunsaturated fatty acid (PUFA) radicals like lipid hydroperoxy radicals and lipid hypoperoxides. This is termed lipid peroxidation. To examine acute oxidative stress in response to exercise, most researchers have assessed various stress markers in blood and urine. Measures of lipid peroxidation include expired pentane, malondialdehyde (MDA), lipid hydroperoxides, isoprostanes and conjugated dienes [18]. Lipid hydroperoxides are considered biomarkers of early damage to lipids and proteins, induced by reactive oxygen and nitrogen species (RONS). In the current experiment no significant difference was found in the value of plasma lipid peroxide concentration after the exercise test compared to the value at rest. Similar results have been reported by other researchers [19–21], but there are also some studies noticing an increase in lipid peroxide concentration after exercise [22]. Explanations for the different findings include, as Fisher-Wellman noted, "that duration and intensity of the proposed exercise were not enough to promote reactive oxygen species production able to induce lipid peroxidation process; or that oxidative stress occurred but it did so preceding or following the sample collection, or in a different tissue other than that utilized (typically blood and urine) or resulted in oxidative damage to cellular constituents other than those measured" [5].

As Fisher-Wellman and Bloomer wrote: "Proteins are major targets for RONS because of their high overall abundance in biological systems and it has been estimated that proteins can scavenge the majority (50–75%) of RONS generated" [5]. Protein modification induced by RONS is rapid and linear, and is a more sensitive biomarker of oxidative modification of biomembranes than lipid peroxidation [23]. Thiols (sulfhydryl groups) are known to scavenge aqueous peroxyl radicals, which make them important in the regulation of both cellular redox status and antioxidant capacity. In the current study, the animals' concentration of total plasma thiols significantly decreased after the exercise test (22.35 ± 3.58 vs. 15.31 ± 2.10 μmol/g protein, $p = 0.022$). The observed decrease in plasma thiol concentration could reflect their oxidation by exercise-induced oxidative modification of protein molecules caused by RONS. These results are consistent with reported findings of increased protein oxidation [19, 24, 25]. This process is directly related to the duration and magnitude of the exercise test. As Fisher-Wellman and Bloomer noted, the concentration of total plasma thiols remained decreased for several hours after the animals performed the test; and it was also observed that "protein oxidation appears to be more affected by anaerobic exercise and that the magnitude of protein oxidation is greater following anaerobic compared with aerobic exercise" [5]. In an effort to explain oxidative damage to proteins, Fisher-Wellman and Bloomer stated that it: "can occur directly by interaction of the protein with RONS or indirectly by interaction of the protein with a secondary product (resulting from interaction of radical with lipid or sugar molecule). Modification of a protein under conditions of oxidative stress can occur via peptide backbone cleavage, cross-linking, and/or modification of the side chain of virtually every amino acid. Moreover, most protein damage is irreparable and oxidative modification of the protein structure can lead to loss of enzymatic, contractile, or structural function in the affected proteins, thus making them increasingly susceptible to proteolytic degradation" [5].

The same authors also believed "that the acute ischemia and rapid reperfusion observed during and after prolonged exercise gives rise to increased RONS formation perhaps via the radical generating enzyme xanthine oxidase" [5]. Interest in the enzyme as a source of oxidizing agents has increased markedly since it has been implicated in the pathogenesis of ischemia-reperfusion injury of tissues such as the intestine, kidney and heart. In their work Viña et al. "found not only that exercise caused an increase in blood xanthine oxidase activity in rats but also that inhibiting xanthine oxidase with allopurinol prevented exercise-induced oxidation of glutathione in both rats and in humans. Furthermore, inhibiting xanthine oxidase prevented the increases in the plasma activity of cytosolic enzymes (lactate dehydrogenase, aspartate aminotransferase, and creatine kinase) seen

after exhaustive exercise" [26]. Veskoukis et al. also examined the effects of xanthine oxidase inhibition on oxidative stress [27]. They were the first researchers to find an unexpected drop in physical performance after allopurinol-induced xanthine oxidase inhibition. That decrease "appears to implicate reactive species in muscle function during a physiological activity (swimming)" [27]. That study suggests that reactive species production at rest and during exercise is important for physical performance.

Hellsten et al. wrote: "Animal studies investigating the effect of ischemia and reperfusion on tissue damage and the level of xanthine oxidase have demonstrated an increase in tissue xanthine oxidase activity in ischaemic tissue as determined from measurements in whole-tissue homogenates" [10]. In the present study, xanthine oxidase activity was also significantly higher immediately after the swimming test than before it (160.61 vs. 244.42 mU/g protein, $p = 0.039$). The observed increase in xanthine oxidase activity could reflect the role of ischemia-reperfusion injury in oxidative stress generation. Lamprecht et al. provide a physiological explanation of this process, suggesting that: "High intensity dynamic or static exercise performance resulting in endothelial hypoxia may lead to a depletion of the cellular pool of adenosine-triphosphate. Consequently the function of the adenosine-triphosphate-dependent calcium ionic pump is impaired. The increased intracellular calcium concentration activates the calcium-dependent proteases (calmodulin or calpain). The proteases cut a peptide group from xanthine dehydrogenase which converts the enzyme to the oxidase form. The formed oxidase uses oxygen as an electron acceptor and thereby generates superoxide radicals and hydrogen peroxide as byproducts" [1].

Investigating this problem, Hellsten et al. suggested that "the location of an increase in xanthine oxidase in tissue undergoing inflammation is restricted to endothelial cells and that some leucocytes contain xanthine oxidase and thereby contribute to the increase in xanthine oxidase in muscle" [10]. This also suggests that during exercise induced inflammatory process in the muscle xanthine oxidase could cause injury to microvassels and the surrounding tissue. Suzuki et al. reported that there is "a dramatic increase in the number of leukocytes that adhere to the endothelial cells of post-capillary venuls as a critical initial step in the pathogenesis of ischemia-reperfusion injury" [28]. Hellsten et al. further noted: "As xanthine oxidase may attach to the membrane of endothelial cells via proteoglycans, circulating xanthine oxidase could theoretically bind to endothelial cells at the sites of the tissue injury. … Due to the propensity of xanthine oxidase to generate reactive oxygen species this enzyme has been considered to be a potential cause of muscle damage during exercise" [10].

The antioxidant properties of blood plasma reside primarily in the non-enzymatic systems, where antioxidant vitamins and uric acid play important roles in protecting cells and muscles from free radical damage. As Fisher-Wellman and Bloomer wrote: "Defense against cellular oxidative stress by these antioxidants consists of either forming less reactive radicals or by quenching the reaction. In response to conditions of strenuous physical exercise, the body's antioxidant capacity may be temporally decreased as its components are used to quench the harmful radical production. Thus measurement of the body's antioxidant capacity is utilized as a marker of oxidative stress" [5].

FRAP is the index of the combined antioxidant status of non-enzymic defenses. As Groussard et al. wrote: "Uric acid may act as an antioxidant both by binding iron and copper ions and by directly scavenging reactive oxygen species ... Ascorbic acid is also a powerful antioxidant, scavenging free radicals such as a hydroxyl radical and the singlet oxygen, and can also act by regenerating α tocopherol [29]. During their evolution, both humans and guinea pigs have lost one of the essential enzymes (gulonolactone oxidase), which makes the presence of ascorbic acid in the diet necessary. The most striking chemical activity of ascorbic acid is its ability to act as a reducing agent (electron donor), and it may help in detoxifying various oxygen radicals *in vivo* by a similar reduction process. The donation of one electron by ascorbate produces the semidehydroascorbate radical, which can be further oxidized to dehydroascorbate. The results of the current study showed no significant difference between the values of plasma antioxidant capacity measured beforer and after the swimming exercise test. These results are consistent with some other studies, which also reported no change in antioxidant capacity following exercise [22, 30–32], but at the same time it should be noted that a change in the ratio of dehydroascorbate and ascorbate (DHA/A – the most commonly used variable for the estimation of the antioxidant role of ascorbic acid) does not indicate a change in the total ascorbic acid concentration in plasma, which is calculated as the sum of ascorbate and dehydroascorbate On the other hand, Reichhold et al. [33] reported a significant increase in the FRAP after an Ironman triathlon, which was also associated with an increase in uric acid concentration observed immediately after the race. Since antioxidant status exhibits very different changes and magnitude in various organs and in response

to different types of exercise, changes can easily be missed if only one sample is taken immediately following exercise [5].

The results of this study show that exercise to exhaustion induces the generation of oxidative stress predominantly by oxidative modification of protein molecules. The results also point to the role of the prooxidative enzyme xanthine oxidase as an important source of ROS during exercise-induced oxidative injury.

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Conflict of interest: None declared

Received: 24.08.2011
Revised: 16.11.2011
Accepted: 6.06.2012