



Oxygen free radicals and exercise: mechanisms of synthesis and adaptation to the physical training*

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ABSTRACT

The interest about the mechanisms of generation and adaptation of oxygen free radicals (OFR) to exercise has increased significantly from the demonstration of its relation with the oxygen intake. The OFR are formed through the incomplete reduction of oxygen, generating species presenting high reactivity to other biomolecules, especially lipids and proteins of the cell membranes and even DNA. The injuries caused by the oxidative stress present accumulative effects, being related to several diseases such as cancer, arteriosclerosis and diabetes. The acute physical exercise furthers the increase on the formation of OFR in function of the increment on the oxygen intake. However, the physical training generates adaptations able to soften the harmful effects caused by OFR. These adaptations are related to several systems, among which the most important are the enzymatic system, composed by the superoxide dismutase, catalase and glutathione peroxidase; and the non-enzymatic system, composed by the ceruloplasmine, the sexual hormones, co-enzyme Q, uric acid, thermal shock proteins, among others. Such adaptations, despite the controversies about the mechanisms involved, further a higher tissue resistance and oxidative challenges such as those provided by long-duration high intensity exercises. The evaluations techniques of the oxidative stress, most times are not able to detect injuries in short-duration exercises. Thus, studies of physical efforts performed for long periods or until exhaustion have been conducted. New lesion markers by OFR action have been discovered and new techniques for its determination have been created. The objective of this work is discuss the formation mechanisms of OFR and the adaptations to the chronic oxidative stress caused by physical training.

INTRODUCTION

The increase on the oxygen intake as well as the activation of specific metabolic paths during physical exercise results in the formation of oxygen free radicals, substances simply known as free radicals⁽¹⁻³⁾. These molecules are increased in high-intensity^(4,5) and exhausting⁽⁶⁾ exercises and from the 80's decade were related to a large number of diseases such as pulmonary emphysema, inflammatory diseases, arteriosclerosis, cancer and aging^(7,8). On the other hand, it is known that psychical activity is a source of stress, and the chronic exposition to this source of stress, called as physical training, is able to release adaptations in responses to a higher production of these free radicals. In this context, the newest studies establish the role of the physical activity in the prevention and control of several diseases such as colon cancer and possibly breast and prostate cancers⁽⁹⁾, diabetes and hypertension⁽¹⁰⁾, dislipidemy and arteriosclerosis⁽¹¹⁾, among others. This paper provides a review-

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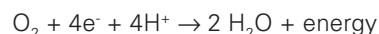
ing with regard to the mechanisms of the free radicals generation through exercise as well as the adaptive processes and the respective consequences induced by physical training.

FREE RADICALS

The oxygen free radicals (OFR) are naturally produced in our organism through oxidative metabolic processes, many times being extremely useful as in situations where the activation of the immunologic system is required (for example, macrophages use the hydrogen peroxide to destroy bacteria and other strange elements); in drugs detoxification and in the production of the endothelium-derived relaxing factor, the nitric oxide, extremely important in processes that unchain the relaxation of the blood vessels⁽¹²⁾.

According to Halliwell⁽¹⁾, the oxygen (O₂) that we breathe is metabolized in our organism as follows: approximately 85 to 90% is used by mitochondria through the electrons transportation chain and the 10 to 15% remaining is used by several oxidase and deoxidase enzymes as well as by direct oxidation chemical reactions. At the terminal part of the electrons transportation chain, the enzyme cytochrome oxidase (reaction 1) removes one electron from each one of the four cytochrome reduced molecules, oxidizing them and add the four electrons to the O₂ in order to form water (around 95 to 98% from the 85 to 90% mentioned above). The 2 to 5% remaining is univalently reduced into metabolites called reactive species of oxygen.

Reaction 1 – tetravalent oxygen reduction



FORMATION OF REACTIVE OXYGEN SPECIES

Due to the electronic configuration, the oxygen presents strong tendency of receiving one electron at the time. The univalent conversion of oxygen into water is given as follows:

(a) The addition of one electron to one oxygen molecule in its fundamental state generates the formation of the superoxide radical (O₂^{•-}) (reaction 2).

Reaction 2: $\text{O}_2 + \text{e}^- \rightarrow \text{O}_2^{\bullet-}$

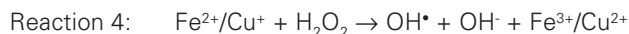
(b) The superoxide that receives more than one electron and two hydrogen ions forms hydrogen peroxide (H₂O₂) through the process called as dismutation⁽¹³⁾. This reaction is catalyzed by the enzyme superoxide dismutase (SOD) found in high amounts in the mammals cells and accelerates the reaction up to 10⁴ times the frequency for spontaneous dismutation in physiological pH (reaction 3).

Reaction 3: $2 \text{O}_2^{\bullet-} + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2$

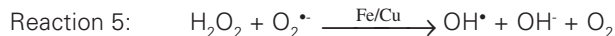
(c) When H₂O₂ receives more than one electron and one hydrogen ion, the hydroxyl radical is formed (OH[•]), which is the most reactive of the intermediate ones, once it may react and change

any near cellular structure, thus influencing enzymes, membranes or nucleic acids⁽⁶⁾.

The hydroxyl radical may be formed when H₂O₂ reacts with iron or copper ions (reaction 4). This reaction is known as Fenton Reaction.



The ions of transition metals may as well catalyze the reaction between H₂O₂ and superoxide, leading to the production of hydroxyl radical (reaction 5), the called Haber-Weiss Reaction.



The superoxide and hydroxyl radicals present unpaired electrons in its outer orbit and, therefore, called as free radicals. The hydrogen peroxide is not a free radical, however, it represents a partly reduced oxygen metabolite. Other reactive species of interest are the singlet oxygen, which are spin-altered forms of oxygen. These oxygen-derived metabolites, if considered as a whole, are called as oxygen reactive species (ERO) in function of their increased reactivity with regard to biomolecules⁽¹⁴⁾, that generally change the size and shape of compounds that they interact with.

Besides, the superoxide radical may react directly with the nitric oxide (NO). A free radical centralized in the nitrogen, generating peroxynitrite. This compound may lead to the formation of an oxidant agent with hydroxyl radical features (reaction 6).



Each ERO has its own characteristics, showing different reactivity and half-life times^(13,15).

OXIDATIVE STRESS

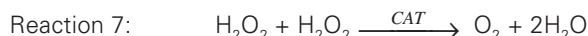
The term oxidative stress is used under circumstances in which the "challenge" for free radicals results in tissue damage or in the production of compounds toxic or harmful to tissues. One may say that an organism is found under oxidative stress (OE) when an unbalance between the pro-oxidant and antioxidant system occur in such way that the pro-oxidant system prevail⁽¹⁶⁾. One of the main lesion mechanisms is the lipoperoxidation (LPO), in other words, the oxidation of the cellular membrane lipidic layer. Besides, the EO may generate damage to protein and DNA, causing several alterations in the cellular function and consequently to tissue.

ANTIOXIDANT DEFENSE

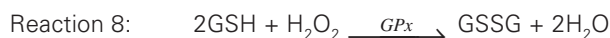
As the ERO are continuously formed in small amounts by the metabolism regular processes, all cells present mechanisms to soften their harmful effects. It is worthy emphasizing that the composition of the antioxidant defenses is distinguished from tissue to tissue, from type of cell to type of cell and possibly from cell to cell of the same type in a given tissue⁽¹⁾.

The antioxidant defense system is divided into enzymatic and non-enzymatic. The first one included the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

The catalase plays important role in the elimination of H₂O₂, furthering its catalysis into water.



The GPx also acts as mechanism of protection against the oxidative stress, converting the reduced glutathione (GSH) into oxidized glutathione (GSSG), removing H₂O₂ and forming water (reaction 8)⁽¹⁷⁾.



Thus, both the CAT and the GPx avoid the accumulation of the superoxide radical and hydrogen peroxide so that the hydroxyl radical is not produced, against which there is no defense enzymatic system⁽¹³⁾.

The perfect balance between the antioxidant enzymes (CuZn-SOD, MnSOD, CAT, GPx) is of great importance for the maintenance of the cellular integrity.

The non-enzymatic system includes compounds synthesized by the human organism such as bilirubin, ceruloplasmine, sexual hormones, melatonin, co-enzyme Q, uric acid and others ingested through regular diet or through supplementation such as ascorbic acid (vitamin C), α -tocopherol (vitamin E), β -carotene (precursor of the vitamin A) and phenol groups of plants (flavonoids).

In studies developed in our laboratory^(18,19) using hearts isolated from rats in a coronary perfusion model (Langendorff), we demonstrated that both the vitamin A and the Trolox (vitamin E hydro soluble analogue) acted by reducing the lipoperoxidation levels and the H₂O₂-induced inotropic, chronotropic and lusitropic negative effects. This is due to the *quencher* capacity of the singlet oxygen of both vitamins. A *quencher* is a molecule that receives the excitation energy from the singlet oxygen to itself, leading it to the fundamental state, becoming excited⁽¹⁾.

FORMATION MECHANISMS OF THE OXYGEN REACTIVE SPECIES

During muscular activity, the energy demand may exceed 35 times the rest demand⁽²⁰⁾. Thus, during the performance of the muscular activity, the oxygen intake is greatly increased, mostly due to the increase of the muscular work. Because ERO are produced through intermediate metabolism, the exercise causes increases on its production. As an example, suppose an adult man with 70 kg in rest uses 3.5 mL O₂.kg⁻¹.min⁻¹ or 352.8 L.d⁻¹ or 14.7 mol.d⁻¹. If 1% generates O₂^{•-} it means 0.147 mol. d⁻¹ or 53.66 mol.year⁻¹ or \approx 1.7 kg.year⁻¹ (of O₂^{•-}). Now during physical exercise, with the increase on the oxygen intake, it may increase up to ten to fifteen times⁽¹⁾.

According to Viña *et al.*⁽²¹⁾, the oxidative stress and muscular damage degrees do not depend on the exercise absolute intensity, but rather on the exhaustion degree of people who performs the exercise. Besides, knowing the free radicals' formation mechanisms with exercise is important in order to avoid the oxidative stress and the damage associated with exhaustive physical activity. The mechanisms are the following:

(1) temporary interruptions of the calcium-dependent (Ca⁺⁺) ATP pumps lead to increases on the calcium intracellular concentrations, what may activate the path of the xanthine oxidase during exercises. Increased concentrations of intramuscular calcium during periods of high intensity exercises may activate the calcium-dependent proteases, which convert xanthine dehydrogenase into xanthine oxidase. The xanthine oxidase uses the molecular oxygen instead of NAD⁺ as electrons acceptor, thus generating the superoxide radical;

(2) periods of intense exercise may increase the oxidative stress due to the temporary hypoxia and re-oxygenation occurring in the exercised muscle in function of the contractions and relaxations cyclically established. During contraction, the vascular compression establishes an ischemia condition and, therefore, hypoxia. In relaxation, the reperfusion occurs and consequently re-oxygenation. Under hypoxia condition, the reduced equivalent may accumulate within the mitochondria electrons transportation chain, resulting in a phenomenon known as reductive stress. In the re-oxygenation a burst of mono-electronic reductions may convert molecular oxygen into superoxide radical;

(3) the leukocytes activation may stimulate the production of free radicals to improve the host defense mechanism in response to the muscular damage induced by exercise. In particular, the neutrophilous may reduce the molecular oxygen into superoxide radical through NADPH oxidase, which is inactive in cells in rest. Similar processes have been observed in monocytes and eosinophils;

(4) increases on the Ca^{++} concentration may activate the enzyme phospholipase A_2 , which releases the arachidonic acid from the phospholipids. The cicloxygenase reacts with the arachidonic acid to form the hydroxyl radical;

(5) hypoxic conditions have also been shown in the increase on the nitric oxide synthase activity (NOS), leading to the formation of nitric oxide radicals. These radicals may perform a weak pro-oxidant effect by themselves or to combine with the superoxide to form a more powerful oxidant agent, the peroxynitrite⁽²²⁾, as already demonstrated (reaction 6).

Thus, during the aerobic metabolism, the possibility of oxidative lesions to occur in the tissues will depend on a precise balance between the generation of oxygen radicals and the efficiency of the antioxidant mechanisms.

PHYSICAL EXERCISE AND OXYGEN REACTIVE SPECIES

Several studies from the 80's decade presented results in which repeated loads of exercise led to accelerated damage or aging of the muscle in individuals or guinea-pigs that exercised regularly. However, Heath *et al.*⁽²³⁾, after following athletes during many years, verified that the metabolic potential and the muscular functional capacity of athletes were not impaired. Besides, Gutteridge *et al.*⁽²⁴⁾, pointed as possibility of protective mechanism the fact of finding increments on the iron and copper levels in the sweat of athletes after exercise, speculating that the excretion of such metals in the sweat would decrease the extension of the oxidative damage caused by such metals. From these studies, the possibility that the regular exercise could further an adaptive increase of the skeletal muscle defense mechanisms able to protect against lesions cause by ERO has been considered.

In 1982, Davies *et al.*⁽²⁵⁾ proposed that the formation of exercise-induced free radicals could be the initial stimulus for the mitochondrial biogenesis in a chronic training situation. In this context, Ji⁽²⁶⁾ demonstrated that in the skeletal muscle, an isolated load of exhaustive work produced an increase on the LPO and that the activity of the antioxidant enzymes glutathione reductase, GPx, SOD and CAT was significantly increased. In the same way, Alessio⁽²⁷⁾ showed increases on LPO in the fast and low muscular fibres of rats submitted to exercise loads, indicating an increase on the physical activity-induced oxidative stress. This stress was better tolerated by trained rats, suggesting an adaptation of the antioxidant systems.

Studying humans, Nies *et al.*⁽²⁸⁾, demonstrated the occurrence of damage to DNA in the circulating leukocytes after exhaustive exercise in treadmill. For the first time it was demonstrated in trained individuals, but as the damage extension was not large, the authors suggest that the adaptation to the aerobic resistance training may reduce the EO effects such as the damage to DNA. In the same year, the results of the work of Mills *et al.*⁽²⁹⁾ with racehorses showed that the exercise may induce changes on the biochemical parameters indicative of the oxidative stress and that these parameters are exacerbated in the presence of high temperatures and humidity. In an interesting work involving a training overload model, Palazzetti *et al.*⁽³⁰⁾ studied triathletes submitted to a work load increment of 21% in swimming, 51% in cycling and 44% in running during four weeks. The simple fact that the athlete was submitted to training overload already caused significant elevation of urinary adrenaline and CK plasmatic activity in rest. However, the highest differences showed when the effects of a simulated duathlon (running and cycling) were evaluated. The athletes in training overload condition presented higher lipoperoxidation indexes, evaluated through the level of substances reactive to the tiobarbituric acid (TBA-RS), CK-MB and plasmatic myoglobin, muscular lesion markers and the drop of the GSH:GSSG relation, clearly indicating that this overload impairs the antioxidant defense mechanisms related to exercise-induced response.

Margaritis *et al.*⁽³¹⁾ proposed that the improvement range of the antioxidant defense system is dependant on the training loads. The same authors also demonstrated the higher the $\dot{V}O_{2\text{max}}$ in triathletes is, the higher the activity of the antioxidant enzyme GPx will be in the erythrocytes, protecting the organism from the damage to the cellular membrane. Leewenburgh *et al.*⁽³²⁾ verified that the exercise-induced oxidative stress may release adaptations in response to training and that such adaptations would be tissue-specific, suggesting a complex regulatory mechanism. Besides, Leaf *et al.*⁽³³⁾ suggest that in healthy individuals, the physical exercise induces the lipid peroxidation transitorily, and that the LPO products are removed during the recovery phase. The work of Venditti and De Meo⁽³⁴⁾ with adult rats submitted to a regular training program with duration of one year, proved the hypothesis that such training lengthens the aerobic resistance capacity and increases the antioxidant defenses, thus limiting the tissue damage caused by free radicals. In the same way, we have demonstrated in our laboratory that the aerobic training in rats, performed through running in treadmill walking belt, increases the myocardial capacity of handling a perfusion challenge with H_2O_2 , causing lower contraction and formation of TBA-RS⁽³⁵⁾ (figures 1 and 2). In the same investigation line, 11 weeks of aerobic training in aged rats, besides causing bradycardia and increase on the glucose/insulin index (an insulin resistance marker), was associated to a reduced response to the H_2O_2 -induced oxidative stress. In this study, we demonstrated a positive correlation between basal FC and the TBA-RS levels (figure 3), in other words, the higher the basal FC is, the higher the radical lesion levels were⁽³⁶⁾. These effects may be partly explained due to the higher SOD activity found in another study from our group⁽³⁷⁾, where 21 days-old rats were submitted to training at 50%

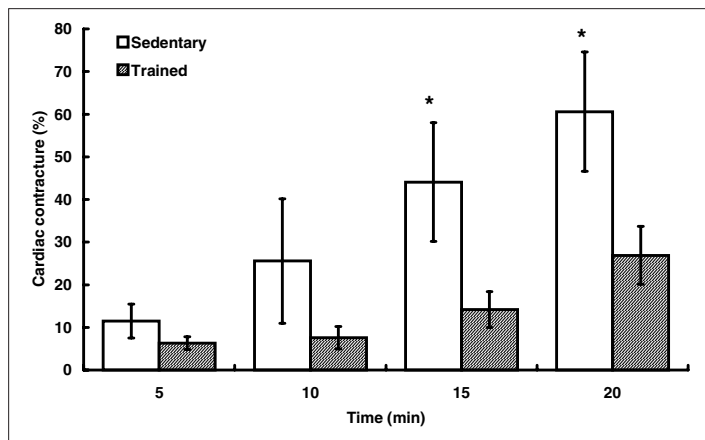


Fig. 1 – Cardiac contracture (%) in different coronary perfusion times with H_2O_2 (256 mmolar.L⁻¹) in trained and untrained rats

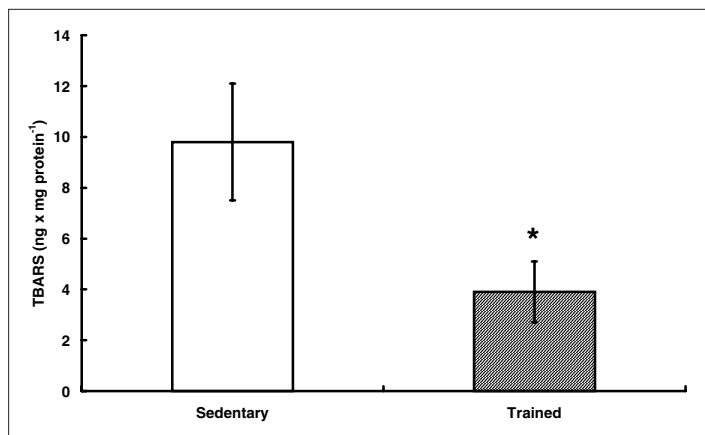


Fig. 2 – TBARS in cardiac homogenized after 20 minutes of coronary perfusion with H_2O_2 (256 mmolar.L⁻¹) in trained and untrained rats

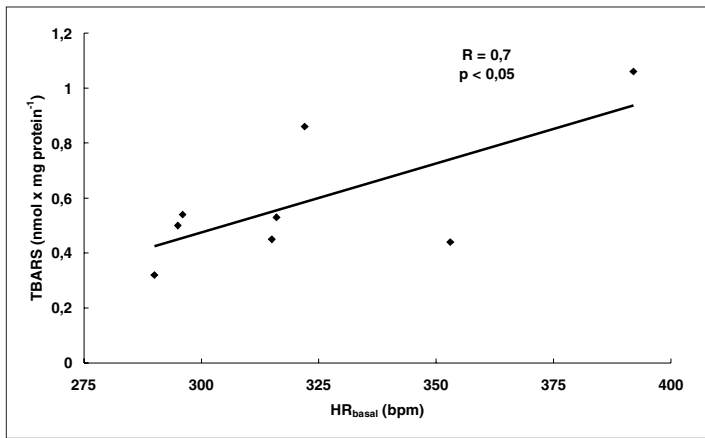


Fig. 3 – Correlation between FC and rest and the lipoperoxidation levels evaluated by TBARS in untrained aged rats and rats physically trained

of the for 4 weeks. No increase on the TBA-RS or QL was verified in the heart of these animals, what suggests that compensatory adaptations in the tissue antioxidant system have occurred. In this context, Ramires and Ji⁽³⁸⁾ demonstrated that the physical training associated to the glutathione supplementation protects the heart of rats against oxidative lesions and depression of the cardiac function caused by *in vivo* ischemia and reperfusion. The mechanism for this adaptation was the elevation on the myocardic glutathione content and on the antioxidant capacity through the increment of the SOD, GPx, GSH reductase, and γ -glutamyl transpeptidase activities in the myocardium.

Still using the animal model, Smolka *et al.*⁽³⁹⁾ analyzed the effect of two different training protocols on the expression of HSP72 (*Heat shock protein* – 72 KDa), a stress protein with the function of maintaining and repairing the protein conformation. This protein is involved in the protection of cells against different types of injuries. Some of these injuries, as the oxidative stress, thermic stress and low pH resulting from the lactate accumulation are generated during the exercise. Besides the adaptations already described such as the increment of the citrate synthase activity, catalase and muscular GSH reductase after training, an original finding of this study was the demonstration that the HSP72 induction caused by an exercise isolated load only occurs to the group kept inactive, suggesting that it acts as a complementary protective mechanism to exercise-induced oxidative stress. Furthermore, the group of animals submitted to a short duration and high intensity protocol presented higher susceptibility to the challenge provided by the acute exercise, if compared to the group trained through continuous protocol. In the study of Child *et al.*⁽⁴⁰⁾, observed, through the measure of the total antioxidant capacity and uric acid in trained individuals submitted to a simulated half-marathon test, a higher scavenger ability (capacity of neutralizing free radicals forming less reactive compounds) on the free radical in the serum. Still, the exercise induced an increase on the malondialdehyde concentrations, suggesting that such responses were sufficient to prevent exercise-induced LPO.

Powers *et al.*⁽⁴¹⁾, assert that the usual high-intensity training required for the elite competition level is able to increase the antioxidant defenses. In this context, Halliwell⁽¹⁾ suggests that athletes present high ceruloplasmine concentrations in the plasma. The ceruloplasmine is a α -globulin involved in the copper transportation and regulation, directly reducing oxygen without known intermediates, therefore participating in the extracellular antioxidant defense system.

In 2000, the study of Selamoglu *et al.*⁽⁴²⁾ presented adaptive differences between aerobic and anaerobic exercises. The activity of the enzyme GPx in erythrocytes was found increased in long-distance runners if compared to weight lifters. In the same way, Inal *et al.*⁽⁴³⁾ analyzed the aerobic metabolism in acute swimming exer-

cise and observed that the production of free radicals was higher than the antioxidant capacity. On the other hand, Subudhi *et al.*⁽²²⁾ evaluated elite alpine ski racers after intense training and observed no change on the oxidative stress markers, suggesting that these athletes suffered a positive adaptation in their antioxidant mechanisms with training.

Recently in our laboratory, Schneider⁽⁴⁴⁾, Schneider *et al.*⁽⁴⁵⁾ and Oliveira *et al.*⁽⁴⁶⁾, found a higher erythrocyte activity of the enzyme GPx in trained triathletes if compared to untrained individuals (figure 4) and an increased total plasmatic antioxidant capacity (TRAP) after treadmill exercise in both groups (figure 5). The higher GPx activity is in agreement with several studies that show the adaptation of the enzymatic defense system^(32,41,47-49). The increased TRAP was also observed in the study of Child *et al.*⁽³⁸⁾, and it might have occurred due to a higher release of antioxidant substances such as the uric acid, as observed in the work of Mastaloudis *et al.*⁽⁴⁹⁾.

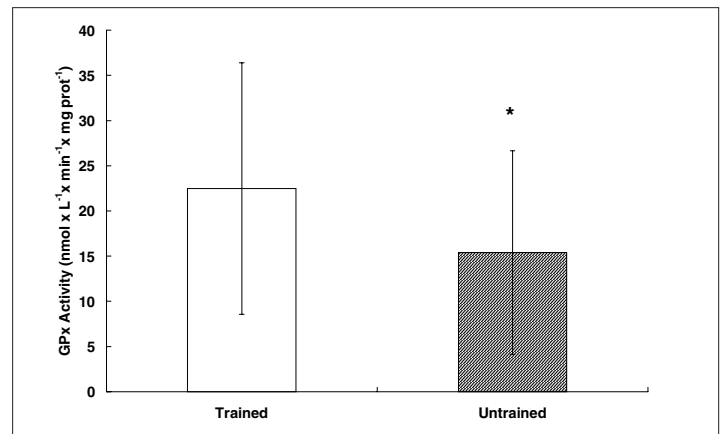


Fig. 4 – GPx activity in erythrocytes of trained (triathletes) and untrained individuals

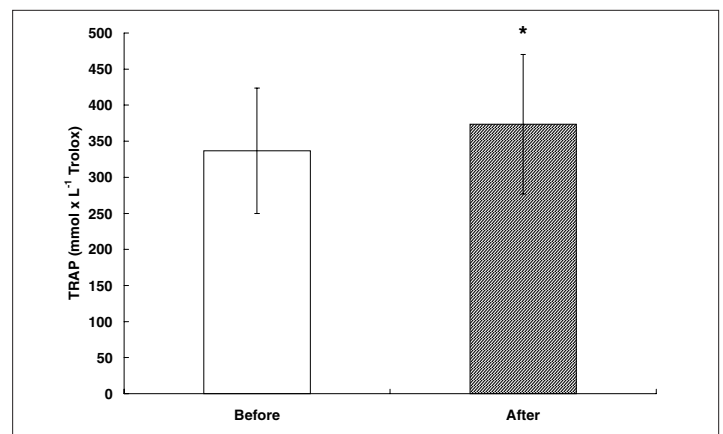


Fig. 5 – Total plasma antioxidant capacity of trained (triathletes) and untrained individuals after 40 minutes of exercise

FINAL CONSIDERATIONS

According to what has been exposed, we observed that the most important factors in the formation of the oxidative stress are the intensity and consequently the exhaustion level of the individual submitted to exercise and, therefore, the exposition to a higher flux of oxygen. Maybe some works are not able to demonstrate the unbalance between the pro-oxidant and antioxidant systems in function of the short time of exposition to exercise. Besides, we could observe several exercise protocols, generally based on a maximal percentile of oxygen intake, in other words, not making the working loads of the study subjects relative, as well as different oxidative stress detection techniques.

The physical training adaptive process is able to protect trained individuals in most situations of exercise exposition. A failure in detecting any change in the lipoperoxidation or in any other target of damage may suggest that some compensatory changes in the antioxidant system might have occurred. The results point to an up-regulation in relation to enzymes GPx and SOD in the skeletal muscle and erythrocytes, but with regard to the enzyme catalase, the results are conflicting. It is interesting observing that several works that evaluated the human antioxidant defense system analyzed the glutathione system, the GPx and SOD, the total antioxidant capacity, but none of them included the activity of the enzyme catalase.

Furthermore, the HSPs activation in acute and chronic exercise participates in the antioxidant protection process. This mechanism has deserved attention in the last years.

Finally, as alternatives of study, we could suggest the utilization of protocols that include long-duration exercises or until exhaustion associated to a diet rich in antioxidant nutrients or to the supplementation of vitamins and enzyme co-factors on the exercise-induced oxidative stress as well as the study of the genic expression of antioxidant enzymes, protein oxidation and DNA from more sensible techniques.

All the authors declared there is not any potential conflict of interests regarding this article.

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