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ORIGINAL PAPER

The effect of acute physical exercise on the antioxidant status of the skeletal and cardiac muscle in the Wistar rat

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RADU MARIUS-DANIEL, SCHIOPU STELIAN, COPREAN DRAGOMIR
Department of Physiology, Faculty of Natural and Agricultural Sciences,
"Ovidius" University of Constanta, 124 Mamaia Blvd, Romania
Contact address- phone:+40768763200 and email: marius_radu_ursu@yahoo.com

Abstract

Oxygen reactive species are usually produced by the body metabolism. Physical exercise increases the synthesis of oxygen reactive species, besides promoting muscular injury and inflammation. The present study aimed to analyze the effects of physical training at high intensities on aerobic conditioning and oxidative stress biomarkers (superoxide dismutase, catalase and reduced glutathione) in rats. The results suggest the effects of acute exercise on various oxidative stress parameters and antioxidant defense systems.

Keywords: acute exercise, oxygen free radicals, oxidative stress, tissue damage

Introduction

In the past 30 years, the research regarding the physiological effects of physical activity have rapidly crossed the border from organ to cell, concentrating in the past decade on the subcellular and molecular domain [1], [2]. Starting with 1987 the research in the field of the physiology of physical effort have evolved fast and at present the scientific community is talking about a new term, "the molecular science of physical exercise", a phrase which defines the knowledge about the current state of the physiological mechanisms of physical exercise [3],[1].

It is well known that the regular physical exercise reduces mortality by decreasing the incidence of cardiovascular diseases, diabetes and cancer [4], [5]. Paradoxically, physical exercise represents a veritable source of free radicals, as a result of the intensification of metabolic processes and oxygen consumption. [6], [7].

The cells produce free radicals of secondary oxygen continually, as a result of the metabolic processes. The free oxygen radicals are very reactive molecules or transitory molecular fragments of a considerable complexity, which contain one or more odd electrons.

Because of the chemical instability, the reactive species of oxygen react almost with all active bio-molecules altering the morpho-structural and functional status [6],[8], [9], [10].

The reactive species of oxygen- a general term which refers not only to "the central oxygen radical" but also covers other chemical reactive species of oxygen named "false radicals" [11].

The free oxygen radicals are: superoxide radical, hydroxyl radical, hydrogen peroxide radical (oxygenated water) and single oxygen.

In the normal physiological or cellular references limits, the free oxygen radicals fulfil many roles in the biological systems such as: the regulation of gene expression, the regulation of cellular signalling, the regulation of sanguine flux, and also the control of the superior nervous activity. There are many potential sources for the generation of free oxygen radicals during physical exercise and the specialized literature cites in this regard: the self-oxidation of catecholamines secreted in excess during intense physical exercise, the

activation of PMN as a result of the micro-tears caused by the intense physical effort, the mitochondrial electrons transport chain, the xanthine-oxydase in the last stage of the catalysis reaction, the oxidation of oxy-haemoglobin and oxymyoglobin, and in ischemia reperfusion process [6],[12].

The free oxygen radicals can be neutralized by the antioxidant cellular defensive system constituted from enzymes such as: superoxide- dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and from numerous non-enzymatic antioxidant substances like: vitamins A, C and E, reduced gluthatione, flavonoids, and ubiqinone [12],[6].

Superoxide dismutase (SOD) was discovered in 1969 by McCord and Fridivich I, being part of the first defence line of cells against free oxygen radicals.

SOD catalyses the dismutation reaction of superoxide radicals resulting as reaction product of the hydrogen peroxide radical. Tests accomplished on many tissues indicated that SOD is very largely distributed in the mammalian organism, being identified as 3 isoforms:

- SOD-Cu/Zn localized in cytoplasm (SOD 1);
- SOD-Mn localized in mitochondria (SOD 2);
- SOD-EC localised in extracellular matrix (SOD 3).

The analysis of the seleno-proteins with enzymatic role identified in the mammal organisms 5 isoforms of glutathione peroxidase named GPX1,….GPX5, located differently at cellular level [13],[14]..

All the isoforms of the GPX enzyme catalyse the reduction reactions of H2O2 and of organic hydroperoxides at H2O2, respectively alcohol (R-OH), using GSH, or in some cases tioredoxin or glutaredoxin, as electron donor [15],[16].ş

Catalase (CAT) fulfils many biochemical roles in biological systems but one main role is to catalyse the transformation of H2O2 into H2O and O2. The chemical equation by which the catalase decomposes hydrogen peroxide is:

$2H_2O$ ------- $2H_2O + O_2$ (Fita, 1985)

Catalase is a homo-tetramer with 240 kDa molecular mass, exclusively distributed in peroxisomes and not only [17].

GSH is the most important non-enzymatic antioxidant from the muscular fibre and not only [18].

At cellular level, GSH has many roles. For example, it reacts with many radicals to which it gives up one hydrogen atom (donor role).

GSH also has co-enzyme role of GPX and glutathione –S-transferase .

GSH plays a special role on the cytotoxic activity of $ONOO^-$, but the intimate protection mechanisms are still not yet known.

Besides antioxidant mechanisms mentioned above, the cells also had other enzymatic mechanisms which contributed directly or indirectly to maintaining the cellular redox equilibrium. Some examples of such enzymes with antioxidant role are: thireoredoxin, glutaredoxin and peroxiredoxin.

The term *oxidative stress* was defined for the first time in 1985 as an alteration of the balance between oxidants and antioxidants in favour of the former [19].

With the discovery of the completed molecular mechanisms which lead to the installation of oxidative stress, the definition of oxidative stress has been reformulated, so Dean Jones proposes a redefinition of this term as follows: “an interruption of redox signalling control” [20].

Whether this formulation will gain supporters or not, it anticipates the oxidative stress results.

Materials and Methods

Biologic material used: the animals used in our experimental model were Wistar albino rats, females 14 weeks of age and weighing 200±20 g.

The animals were individually housed in thermostatic (22 ± 2°C) windowless Plexiglas cages with constant humidity and controlled lighting conditions (12 h of light and 12 h of darkness per day) as well as with free access to tap water. They were fed under standard laboratory conditions.

The experiment was carried out in accordance with the Helsinki Declaration and guidelines of the Ethics Committee of the International Association for The Study of Pain. They were approved by the Animal Care Committee of the Faculty of Natural and Agricultural Sciences within "Ovidius" University of Constanta, Romania.

Experimental model used: Our experimental model comprises 2 groups with some characteristics:

- Control Group (M) (n=6) without physical training (serving as reference for the experimental group);
- Experimental Group (Exp.) (n=6) with one physical training session (swimming in a pool with water heated at 28 °C) for 60 minutes.

The animals were sacrificed with respect to the legislation of animal protection. The samples of skeletal muscle (Vastus lateralis) and myocardium tissues were taken immediately.

The analyzed parameters from the tissues were: **SOD** activity, **CAT** activity, GSH concentration and total protein concentration **(TPC)**.

The superoxide dismutase (SOD) activity assay. This method is based on the superoxide dismutase capacity to inhibit the reduction of NBT (nitro blue tetrazolium) by the free radicals. One enzymatic unit (EU) is the enzyme amount that induces a 50% inhibition in standard conditions. Free oxygen radicals are generated through riboflavine photo-reduction [21].

CAT activity was measured by the spectrophotometer method of Beers (1952), based on the decomposition of H_2O_2 [22]

GSH were analyzed in the tissue, using spectrophotometric determination, previously described by Beutler [23].

Protein concentrations in the tissue homogenates were determined by the spectrophotometrical methods described by Lowry [24].

Data were processed in the program OriginPro75. The significance threshold was set at $p \leq 0.05$.

Results and discussion

Table1. The activity of the superoxide dismutase, catalase and reduced glutathione concentration in rat myocardium.

Myocardium				
Group		SOD EU/mg protein	CAT EU/mg protein	GSH ng/mg protein
Control Group **(M)**	X±ES	3.26±0.50	0.70±0.08	2.29±0.30
	N	6	6	6
Experimental Group **(Exp)**	X±ES	2.89±0.79	1.59±0.28	1.76±0.49
	n	6	6	6
	t	-	3.02	3.10
	p≤	NS (>0.05)	≤0.05	≤0.05

X±ES = mean± standard error; n = the number of individual samples that represented the arithmetic mean at the end; t = the value of the "t" test taken by the student; p = the threshold of significance established on the basis of the "t" value; NS = insignificant change.

Table 2. The activity of the superoxide dismutase, catalase and reduced glutathione concentration in skeletal muscles of rats.

Skeletal muscle *(Vastus lateralis)**				
Group		SOD EU/mg protein	CAT EU/mg protein	GSH ng/mg protein
Control Group **(M)**	X±ES	6.94±0.12	0.75±0.06	3.49±0.18
	n	6	6	6
Experimental Group **(Exp)**	X±ES	8.69±0.54	2.19±0.64	2.50±0.40
	n	6	6	6
	t	3.18	4.28	4.02
	p≤	≤0.05	≤0.05	≤0.05

***** For explanation see table 1

The skeletal muscles are able to react quickly to the dynamic nature of the metabolic stimulus (e.g. food intake, changes in duration and frequency of eating) or mechanical (e.g. prolonged physical effort and high intensity).

There is not uniformity in terms of the morphology, physiology and biochemistry of the muscle groups. Because of this, each group of somatic muscles typically respond to mechanical or metabolic stimulus.

It appears that the skeletal muscles rich in type IIa muscle fibers are exposed to redox alterations with a higher frequency compared to other muscle groups where filaments of type IIx or II prevail [25, 26, 27].

The somatic muscle has an enzymatic antioxidant system represented by superoxide dismutase, catalase, glutathione peroxidase etc., and a non-enzymatic antioxidant system with vitamins, bilirubin α-lipoic acid and reduced glutathione, cells responsible for protection against the actions of oxygen free radicals. The skeletal muscle has multiple subcellular mechanisms which generate superoxide radicals and, moreover, their concentration increases during muscle contraction [3]. Although some models of chronic exercise (weeks, months) suggests that muscle SOD activity does not increase [28,29], most of the investigations show an increase in enzyme activity up to 20-112% in muscle because of physical effort [30, 31, 32].

In the case of our experimental model, as a result of performing acute physical exercise, the enzymatic activity of superoxide dismutase increases ($p \leq 0.05$) in the experimental group, compared to the control groups. SOD is an inducing enzyme, the activity increases when the concentration of superoxide radicals exceeds the reference value cell.

In regard to physical effort and the activity of catalase, opinions are divided. Some research showed an increase of CAT activity due to physical activity [33, 32], others a decrease [29, 34, 35]. This ambiguity is still a research topic. In our experimental model, catalase activity increases ($p \leq 0.05$) in the experimental group in response to physical effort. GSH (reduced glutathione) is the most important non-enzymatic antioxidant in the muscular fiber and not only [18].

In the skeletal muscle, GSH concentration varies from one muscle fiber to another. In our experimental model it is possible that the GSH level change in response to increasing concentrations of oxygen free radicals at this point. GSH reacts directly with oxygen free radicals, or using enzymes whose coenzyme it is. Many experimental data suggest that reactive oxygen species may play an important role in the pathogenesis of myocardial infarction (heart attack) [36, 37]. Regarding the metabolic parameters of oxidative stress followed in our experimental model, it is possible for the concentration of free radicals to increase in the case of heart muscle subjected to effort. This can exceed the antioxidant capacity in myocardial tissue and thus oxidative stress occurs. This statement could be supported by an increased GSH concentration and catalase activity in the heart tissue. With

regard to superoxide dismutase, we can see that activity does not alter this parameter significantly, probably due to the cooperation of the two antioxidant systems.

Conclusion

The results obtained suggest that different components of the antioxidant defense mechanisms respond differently to changes in the concentration of oxygen free radicals. Our experiment shows that physical effort by swimming for 60 minutes causes in rats an activation of the antioxidant enzymatic and non-enzymatic systems in the tissues investigated.

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