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# The effect of acute physical exercise on liver and kidney in the Wistar rat

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## Abstract

*It is well known that regular physical exercise reduces mortality by minimizing the risk of cardio-vascular disorders, diabetes and cancer. Paradoxically, physical exercise represents a veritable source of free radicals, as a result of the intensification of metabolic processes and oxygen consumption. The free oxygen radicals are chemical species of high complexity which contain one or more add electrons. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase (CAT), superoxide dismutase (SOD) and numerous non-enzymatic antioxidants, including reduced glutathione (GSH).*

*The enzymatic activity was determined from rat liver and kidney after one session of intense acute physical training (swimming for 60 minutes). The results obtained show that the oxidative stress enzymes are activated at liver and kidney level as an answer of increased concentration of free oxygen radicals generated by physical training. Physical training represents an important source of free oxygen radicals for the tissues involved directly in the physical effort and also for other tissues which are not thus involved.*

**Keywords:** acute exercise**,** free oxygen radicals, oxidative stress, liver, kidney

## Introduction

The beneficial effects of regular exercise have long been known. The efficiency of regular physical activity in the prevention of chronic diseases such as cancer, hypertension, obesity, depression, diabetes, osteoporosis, and premature death is well established. However, the beneficial effects of physical activity are lost with exhaustion. It is well known that exhaustion caused by exercise, especially when it occurs sporadically, leads to structural damage or inflammatory reactions within the muscles. This damage is due, at least in part, to the production of reactive oxygen species (ROS) [1, 2]. Also, it was reported that ROS production by acute or chronic exercise may elicit different responses depending on the type of organ tissue and its level of endogenous antioxidants [3, 4, 2].

Free oxygen radicals are transitory chemical species of considerable complexity which contain one or more odd electrons and, because of the chemical instability, they react with almost all the bio molecules (proteins, glucoses, lipids, nucleic acids) [5, 6].

Oxygen is a universal electron acceptor which permits aerobe and facultative aerobe organisms to utilize the energy from nutrient.

In this catabolic process, it could generate free oxygen radicals and other reactive species of oxygen. Free oxygen radicals are: superoxide radical, hydroxyl radical and hydrogen peroxide (oxygenated water).

Superoxide radical- represents the first stage of oxygen activity in the univalent reduction way and has many unusual properties, sometimes paradoxical. In biological systems, superoxide radicals are non-enzymatic and converted to chemical species considered false free oxygen radicals.

Roth and Droge (1991) showed that superoxide radicals activate T-cell which will increase the secretion of some factors involved in the rise of IL-2 and muscular tissues [7]. Harman (1981) also showed that the superoxide radicals in vivo can be formed as a result of many incomplete enzymatic reactions [7].

Superoxide radicals react quickly with proteins rich in sulphur and NO, generating a peroxynitrite radical, which is very toxic for most biological molecules [8]. The superoxide radical formation occurs at the level of the transport chain of microsomal and mitochondrial electrons, under the action of xanthine oxydase and other flavoproteins, during catecholamines auto oxidation and interactions between haemoglobin and oxygen [6].

In the physiological pH, the superoxide radicals have a short life of milliseconds, it is not very reactive, but it has the important property of being able to penetrate bio-membranes [10].

The superoxide radicals in an organism can be generated by numerous processes and reactions, some parts being totally unknown.

Hydroxyl radical, alongside superoxide radicals, is the strongest species of oxygen. Hydroxyl radicals react with almost any organic molecule with high speed. Because of the considerable reactivity, hydroxyl radicals can form secondary radicals which are very toxic for biological systems.

The hydrogen peroxide radical is the most stable form of free radical generated by $O_2$. It is permeable for cell membranes and has a long life in the cell. It is cytotoxic and considered a very good oxidation agent. $H_2O_2$ also reacts with metallic ions (e.g. in Haber-Weiss reaction).

Keys and Tyrrell (1989) showed that $H_2O_2$ induce the gene expression which codifies hem-oxygenase HO-1. Storz et.al., (1990) show that hydrogen peroxide induces the activation of some genes from bacteria. Schreck et.al., (1991) show that in mammal cells $H_2O_2$ acts like a transcription factor for the nuclear satellites [6, 7].

NO, although not a free oxygen radical, is an important signal molecule involved in different physiological and physio-pathological processes [7, 8].

NO also reacts with some of the transitional metals and with superoxide radicals resulting in a peroxynitrite radical (ONOO-), a very reactive chemical species.

During evolution, the cells have been developing enzymatic and non-enzymatic systems of annihilation of free oxygen radicals such as: 1. catalase, the enzyme which decomposes hydrogen peroxide, 2. superoxide dismutase which catalyses the dismutation reaction of superoxide radicals, 3. glutathione peroxidase which catalyses the reduction reactions of $H_2O_2$ and organic hydroperoxides at $H_2O$ and alcohol (R-OH), using GSH as electron donor or in some cases thioredoxin and glutaredoxin [11, 12].

The GSH is the most important non-enzymatic antioxidant from the muscular fiber and not only [13].

GSH play a very important roll on the cytotoxic activity of $ONOO^-$, but the close protection mechanisms are not yet well known.

In some contexts (e.g. physical training), the concentration of free oxygen radicals surpasses the reference cell value and in this case the antioxidant enzymes capacity is exceeding, an oxidative stress phenomenon occurring.

The term *phenomenon of the oxidative stress* was used for the first time in 1985 as an alteration of the balance between oxidants and antioxidants, in favor of the former one [14].

When almost everything was known about the molecular mechanisms which lead to oxidative stress, the definition of oxidative stress was reformulated, therefore Dean Jones proposes a redefinition of this term as “interruption of redox signalling control” [15].

## Material and Methods

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

The animals were individually housed in thermostatic (22 ± 2$^{o}$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, Romania.

The experimental model used: Our experimental model comprises 2 groups with certain 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 (swimming in a pool with water heated at 28$^{o}$C) for 60 minutes.

The animals were sacrificed with respect to the legislation on animal protection. The samples of liver and kidney 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 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 [16].

CAT activity was measured by the spectrophotometer method of Beers, based on the decomposition of $H_2O_2$ [17].

GSH were analyzed in the tissue, using the spectrophotometric determination previously described by Beutler [18]

Protein concentrations in the tissue homogenates were determined by the spectrophotometrical methods described by Lowry [19].

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

## Results and discussion

**Table 1.** The activity of the superoxide dismutase, catalase and reduced glutathione concentration in rat liver.

| **Liver** | | | | |
|---|---|---|---|---|
| Group | | SOD<br>EU/mg protein | CAT<br>U/mg protein | GSH<br>ηg/mg protein |
| Control Group **(M)** | X±ES | 3.26±0.50 | 1.62±0.15 | 2.74±028 |
| | n | 6 | 6 | 6 |
| Experimental Group **(Exp)** | X±ES | 4.75±0.59 | 3.48±0.54 | 1.90±0.30 |
| | n | 6 | 6 | 6 |
| | t | 2.84 | 5.84 | 3.92 |
| | p≤ | ≤0.05 | ≤0.05 | ≤0.05 |

X±ES = mean ± standard error; n = the number of individual samples that represented the arithmetic mean in 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 rat kidney.

| **Kidney*** | | | | |
|---|---|---|---|---|
| Group | | SOD EU/mg protein | CAT EU/mg protein | GSH ng/mg protein |
| Control Group **(M)** | X±ES | 3.35±0.26 | 0.78±0.11 | 1.86±0.46 |
| | n | 6 | 6 | 6 |
| Experimental Group **(Exp)** | X±ES | 3.19.±0.47 | 1.80±0.38 | 2.90±0.57 |
| | n | 6 | 6 | 6 |
| | t | 1.54 | 2.14 | 2.71 |
| | p≤ | NS (>0.05) | ≤0.05 | ≤0.05 |

* For explanation see table 1

We studied the oxidative stress biomarkers and endogenous antioxidants in the liver and kidney of rats subjected to acute training. The biomarkers indicating oxidative damage included lipid peroxidation, protein oxidation, and DNA damage, while the endogenous antioxidants included ascorbic acid, ά-tocopherol, GSH and GSSG, ubiquinone, ubiquinol, cysteine, and cystine.

Training, by increasing the oxygen consumption rate, may result in oxidative stress in mitochondria. This results in an increased production of oxidants, which could be detrimental to the tissue.

The liver is the organ situated at the border between the digestive and the circulatory systems and their functions are not bound only by digestion. At the liver level many biochemical cycles occur and they can result in free oxygen radicals.

Physical training represents an important source of free oxygen radicals development at the level of the organs directly involved in the activity and at the level of other organs because of the supplementary energetic needs and also of the oxygen consumption.

In our experimental model, an increasing of SOD activity and CAT and a decrease of GSH at the liver level occur after only one training. The explanation would be that SOD is an induced enzyme and it is possible that this biosynthesis be induced by the rise of the oxygen radical species (redox signalling). SOD catalyses the dismutation reaction of superoxide radicals resulting hydrogen peroxide, the substrate on which CAT acts. In regards to the tissular concentration of GSH, it decreases in the liver.

The liver is the only organ of rodents capable of GSH biosynthesis, tri-peptide which is exported by means of blood to the other organs, where it plays the role of coenzyme or donor.

The main function of the excretory system is to maintain the pH and also the osmotic liquid. In our experimental model, there is an increase of the CAT activity and GSH concentration in renal tissue, although SOD remained unchanged in the experimental group.

During physical training, the sanguine flux is deviated to the organs directly involved in activity, the others suffering a hypoxia state at the end of the physical activity. The blood loaded with oxygen rushes into the organs temporarily deprived of blood, where the oxygen flux increases through the mitochondria. The mitochondrial electron transport chain is the one responsible for the generation of superoxide radicals.

It seems our experimental model did not observe the post-ischemic reperfusion period. This information is also supported by the SOD activity, which remains in reference physiological parameters compared to the control group.

## Conclusion

Acute physical training is an important source of free oxygen generation for the skeletal muscles, organs direct involved in activities and also for other tissues. Our experimental data show that after one single 60-minute physical training, the oxidative stress is not installed and the enzymatic and non-enzymatic antioxidant system has different answers for the two analyzed organs.

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