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Increased oxidative stress status in rat serum after five minutes treadmill exercise

Research Article

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Abstract: Although it is accepted that an important correlation exists between the physical exercise and the oxidative stress status, the data regarding the levels of the main oxidative stress markers after physical training have been difficult to interpret and a subject of many controversies. There are also very few studies regarding the effects of short-time exercise on the oxidative stress status modifications. Thus, in the present report we were interested in studying the modifications of some oxidative stress markers (two antioxidant enzymes- superoxide dismutase and glutathione peroxidase, a lipid peroxidation parameter – malondyaldehide, the total antioxidant status and protein carbonyl levels), from the serum of rats that were subject to one bout of five minutes exercise on a treadmill, when compared to a control sedentary group. In this way, we observed a decrease of superoxide dismutase specific activity in the rats which performed the exercises. Still, no modifications of glutathione peroxidase specific activity were found between groups. In addition, increased levels of malondyaldehide and protein carbonyls were observed in the rats subjected to exercises. In conclusion, our data provides new evidence regarding the increase of the oxidative stress status, as a result of a 5-minutes bout of treadmill exercising in rats, expressed through a decrease in the SOD specific activity and the total antioxidant status and also an increase of the lipid peroxidation and protein oxidation processes.

Keywords: *Exercise • Rat • Oxidative stress • Treadmill*

1. Introduction

Today is well established that there is an important correlation between physical exercise and the oxidative stress process, which is defined as the imbalance between the organism antioxidant capacity on one hand and the overproduction of the reactive oxygen species (ROS) on the other hand [1].

In this way, there are previous reports describing a direct relationship between the muscle oxidative stress status and the isometric force production [2]. Thus, it seems that a perfectly balanced equilibrium between ROS production and the enzymatic/non-enzymatic antioxidants will result in an ideal skeletal muscle force [3,4].

However, while some authors demonstrated an increase in the oxidative stress status as a result of physical exercise [4-6], there are also reports describing a so-called adaptation of the antioxidant system to physical exercise and training [7], which will result in an increase of the various types of antioxidant defenses.

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These aspects led to numerous contradictions in the current literature regarding the levels of some oxidative stress markers after physical training. In this way, the swimming effort in rats has been reported to result in a decrease of glutathione (GSH) levels [8], which is an important antioxidant, while other studies showed that some different physical exercise generated increased levels of plasma GSH [9,10] or increased GSH concentration in several muscles after endurance exercises in dogs [11]. Similar aspects were also reported in the case of superoxide dismutase-SOD (the first line of defense against the nocive effects of ROS), which was showed to express both increased [12-14] and decreased [15, 16] specific activity after the physical exercise was performed, as well as in the cases of the glutathione peroxidase – GPX [17,18] and catalase – CAT [7,19], the other two important antioxidant enzymes.

Still, there are very few studies regarding the effects of very short-time exercise on the oxidative stress status modifications. In the present study we were interested to see the modifications of some oxidative stress markers (two antioxidant enzymes- superoxide dismutase and glutathione peroxidase, a lipid peroxidation parameter – malondyaldehide, the Total Antioxidant Status-TAS and protein carbonyl levels), from the serum of rats that were subject to one bout of 5 minutes exercise on a treadmill. In fact, according to the best of our knowledge, this is the first time when these markers have been determined after only one bout of 5 minutes exercise.

2. Material and methods

2.1. Animals

Adult male Wistar (n=14) rats, weighing 200-250 g, were kept in a room with controlled temperature (22°C) and a 12:12-h light/dark cycle (starting at 08:00 h), with food and water ad libitum.The animals were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). This study was approved by the local Ethics Committee and also efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Experimental design

All the rats (n=14) were acclimatized to the experimental room for 1 week before the exercise testing.

They performed one bout of driven rodent treadmill running 5 minutes in one day, 0% grade, at 10:00 am.

The rats were divided into 2 groups of 7 rats each: a control (sedentary) group and one group which performed the physical exercise. In order to obtain a minimal difference in the experimental manipulation of controls, the sedentary rats were placed for the same amount of time (5 minutes) on the switched-off treadmill.

Immediately after that the rats were anesthetized with pentobarbital and sacrificed. Also, the age-matched control animals, which were kept sedentary in their cages, were sacrificed using the same procedure. All animals were fasted for 12 h before death. After the blood was taken, it was allowed to clot and centrifuged for 15 min at 3000 rpm. Serum was then aliquoted into plastic tubes and stored at −40°C until measurement.

3. Biochemical estimations

3.1. Determination of SOD

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (FLUKA, 19160) according to the manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide anions) after 20 minutes of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

3.2. Determination of GPX

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (SIGMA). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity.

Regarding the units of activity for the investigated enzymes we used units/ml = micromol/min/ml.

3.3. Total antioxidant status

TAS was assayed with a chemiluminometric method, with luminol – horseradish peroxidase system (Berthold Lumat 9507 chemiluminometer). In this method,

constant light emission result from luminol degradation in the presence of a catalyst (horseradish peroxidase) with an enhancer (p-iodo-phenol) and is kinetically recorded. When a biological fluid is introduced into this system, the level of light emission decreases for a period of time proportional to the total antioxidant capacity. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble alpha-tocopherol analogue, was used as the standard. Calibration was made with Trolox (hydro-soluble vitamin E) and final results are related to Trolox equivalents. The prooxidant system which generates light, was calibrated to five million relative units of light (RLU) and serum samples were used at a dilution of 1/10.

3.4. Determination of malondialdehyde

MDA levels were determined by thiobarbituric acid reactive substances (TBARs) assay. 200 µL of serum were added and briefly mixed with 1 ml of trichloroacetic acid at 50%, 0.9 ml of TRIS-HCl (pH 7.4) and 1 ml of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100 ºC for 20 minutes. Afterward, samples were centrifuged at 3000 rpm for 10 min and supernatant was read at 532 nm. The signal was read against an MDA standard curve, and the results were expressed as nmol/ml [20,21].

Total protein was measured using Bradford dye-binding method, with bovine serum albumin as the standard [22].

3.5. Protein carbonyl determination

Plasma proteins carbonyl content was measured by forming labeled protein hydrazones derivates, using 2, 3-dinitrophenylhydrazine (DNPH), which were then quantified spectrophotometrically at 360 nm, using a molar absorption coefficient for hidrazones of 21000 mol/L.cm.

4. Data analysis

The results were statistically analyzed by using Student's t-test (two tailed, unpaired). All results are expressed as mean ± SEM. P<0.05 was regarded as statistically significant.

5. Results

We report here a significant decrease (p=0.01) for the specific activity of SOD, the first antioxidant enzyme in the way of ROS, in the exercised group, as compared to the sedentary rats (Figure 1).

However, we did not find any significant modifications (p=0.34) between the group of rats which was subjected to the 5 minutes exercise bout, when compared to the sedentary control group, in the case of GPX specific activity (Figure 2).

Asignificant decrease (p=0.02) was found for the total antioxidant status in the serum of rats which performed the physical exercise, as compared to the sedentary control rats (Figure 3).

Additionally, we observed a significant increase in the lipid peroxidation processes, as demonstrated by the increased levels of MDA (p=0.03) from the serum of exercised rats, when compared to the sedentary controls (Figure 4).

Moreover, we showed a significant increase (p=0.03) in the serum protein oxidation markers (as assessed by the levels of protein carbonyls) in the exercised rats, when compared to the sedentary control group (Figure 5).

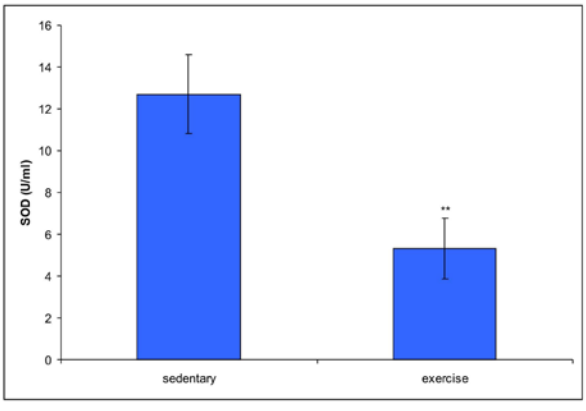

Figure 1. The effects of five minutes treadmill exercise on SOD specific activity from the rat serum. The values are mean ± SEM (n=7 animals per group). **p=0.01 vs. sedentary-control group.

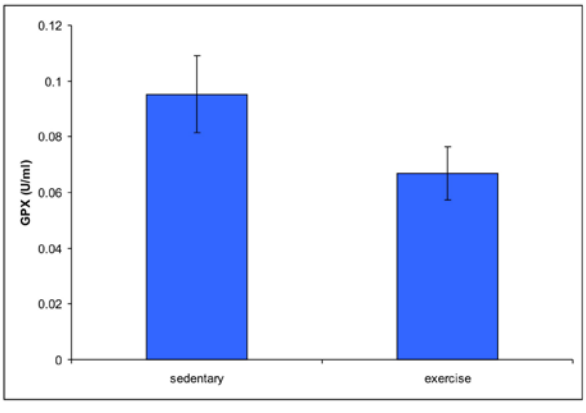

Figure 2. The effects of five minutes treadmill exercise on GPX specific activity from the rat serum. The values are mean ± SEM (n=7 animals per group).

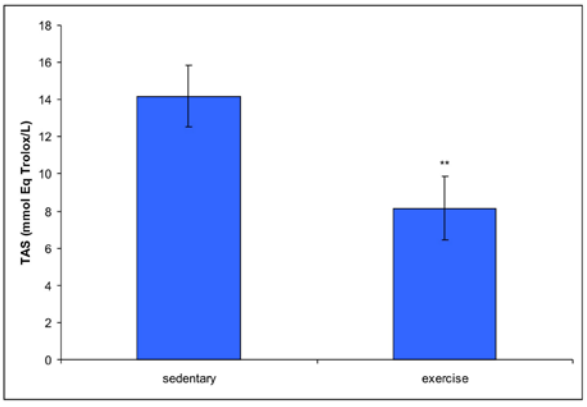

Figure 3. The effects of five minutes treadmill exercise on total antioxidant status (TAS) from the rat serum. The values are mean ± SEM (n=7 animals per group). **p=0.02 vs. sedentary-control group.

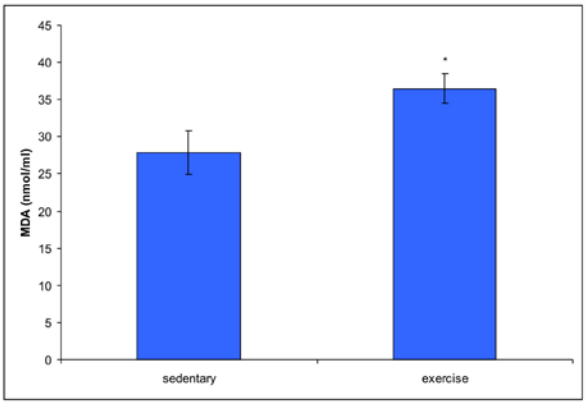

Figure 4. The effects of five minutes treadmill exercise on malondyaldehide (MDA) levels from the rat serum. The values are mean ± SEM (n=7 animals per group). *p=0.03 vs. sedentary-control group.

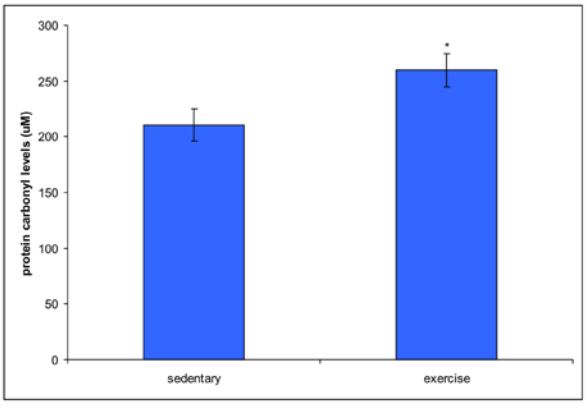

Figure 5. The effects of five minutes treadmill exercise on the levels of protein carbonyls from the rat serum. The values are mean ± SEM (n=7 animals per group). *p=0.03 vs. sedentary-control group.

6. Discussion

The results we presented here provide additional evidence regarding the increase in the oxidative stress status as a result of short-time physical exercise and expressed through a decrease in the SOD specific activity and the total antioxidant status. There was also an increase of the lipid peroxidation and protein oxidation processes, when compared to the sedentary control group. As mentioned, according to the best of our knowledge, this is the first time when these markers are determined after only one bout of 5 minutes exercise.

Similar results regarding the possible increase of the oxidative stress processes as a result of exercise have been reported as early as 1978 [23]. These results were confirmed by additional studies performed after that, such as the one of Barclay et al. that showed that free radicals which result after the contraction will generate muscular fatigue [5] or the one of Kondo that proposed the theory regarding the very important role of ROS in the degenerative processes resulting in muscular mass loss [24].

However, as we previously mentioned, there are on the other side. reports describing the possibility that the organism may generate increased concentrations of antioxidants, as a result of physical exercise, in order to cope with the new generated oxidative stress [7,25].

In this way, as we initially mentioned, in the case of SOD a significant increase of its specific activity was reported after performing some various physical exercises [26-28]. However, when other research groups performed similar testing the results could not be easily reproduced [19,25]. Moreover, in the case of CAT, another antioxidant enzyme, very different results were reported after physical training, ranging from increased specific enzymatic activity [29,30], to no modifications at all [7] or even decreased activity [19]. Similar aspects were also reported in the case of GPX [18,31].

These facts could be explained through the differences in the antioxidant enzymes isovariations, different methods in assaying the enzymatic activity (e.g spectrophotometric vs. chemiluminometric), and in some cases the different types of exercise used and the muscles that are involved in the specific types of efforts [7]. Additionally, it is important to mention that the way exercise influences the oxidative stress status, depends also on the level of training, as was demonstrated by Senturk et al. in 2001, which showed that exercise-induced oxidative stress affects erythrocytes only in sedentary rats but not exercise-trained rats [32].

Moreover, it was stated that the antioxidant enzymes could have very different patterns of the aforementioned

adaptation to physical exercises [7]. Also, it is important to keep in mind that low levels of ROS are indeed required for normal force production in the skeletal muscles [4].

Regarding the time of the physical training in our experiment we decided to use a short-time of exercise performing (5 minutes), considering that to the best of our knowledge, this is the first study using only a 5 minute set of exercise. Also, we did not find in the literature any previous use of the TAS as a parameter of oxidative stress status, after performing physical exercise. .

Additionally, considering the previous described variety of results for the oxidative stress markers and their different adaptations to the performance of exercise , we decided to use TAS as a parameter, together with the specific activity of SOD and GPX, also considering that it covers all aspects of antioxidant defending capacity (e.g. not a single enzymatic/non- enzymatic antioxidant). In this way, we obtained a significant decrease of SOD specific activity and also a decrease in the Total Antioxidant Status. Still, no significant modifications of GPX were observed. This could be perhaps explained by the fact that SOD is most sensitive to and the primary defense against ROS damage [1], exhibiting prompt compensation processes when encountering heavy oxidative injury. Therefore it is possible for the compensatory modifications of GPX to be less sensible, as compared to SOD.

Also, we observed an increase in protein carbonylation processes which are the most frequent type of protein modification in response to oxidative stress and is thought to be irreversible and destined only to induce protein degradation in a nonspecific manner [1].

Of course, all of these aspects regarding the importance of the oxidative stress status in the modifications that appear after the physical training, led to the idea of using various antioxidants as a possible solution for longer physical training or shorter recovery periods.

In this way, probably the most tested antioxidant in this area of research was represented by vitamin E, which was showed to reduce and prevent some specific tissue-damage during exercise [33]. I It also seems that the lack of vitamin E increases the risk of contraction-induced membrane damage in the muscle [34]. Also, it was demonstrated that vitamin E could delay the onset of muscular fatigue [35]. Additionally N-acetylcysteine (NAC), which is also a very well recognized antioxidant, was also reported to prevent muscle fatigue in some experimental muscle preparations [36].

However, most of the studies regarding the positive effects of the antioxidants in this area of research are controversial, since some authors showed for example that both vitamin C and E did not affect in any way the performance of the exercise [4,37]. Different results could be perhaps explained by the maximal/submaximal intensity of the physical exercise, the dosage of antioxidants, as well as the time of administration.

In fact, similar contradictory aspects were also described in terms of the effects for various antioxidants on the recovery time after the effort, with reports about some positive effects [38] in this matter, as well as with authors stating no effects at all [4,39].

In this way, future studies will need to carefully determine which antioxidants and in what dosages do they have the maximal therapeutic benefit.

There are also several limitations to our study that should be discussed. First of all, the usage of pentobarbital as an anesthetic before the killing of the animals, considering that this reagent was previously suggested to influence the levels of some oxidative stress markers [40,41]. However, pentobarbital was injected in both our study groups (the sedentary and the exercised one).

Another aspect could be represented by the relatively small number of animals we used.

7. Conclusions

The results presented here provide additional evidence regarding the increase in the oxidative stress status as a result of a 5 minute bout of treadmill exercising and expressed through a decrease in the SOD specific activity and the total antioxidant status and also an increase of the lipid peroxidation and protein oxidation processes.

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Conflict of interest statement

Authors state no conflict of interest.

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