Bulletin of the *Transilvania* University of Braşov
Series VI: Medical Sciences • Vol. 5 (54) No. 2 - 2012

ANTIOXIDATIVE SYSTEMS AND PHYSICAL EXERCISE

N. TAUS[1] M. POTROVIȚĂ[2] L. TAUS[3]

Abstract: *Intense physical exercising increases oxygen consumption and causes disturbances of the antioxidant balance. A rupture of this balance between antioxidant substances and the pro-oxidant ones (excess of free radicals (ROS) leads to oxidative stress. Reactive oxygen species (ROS) are an important menace to the antioxidant defense system in the cells.They can diminuish the deposits of antioxidant vitamins and glutathione, making the tissues more vulnerable to oxidative damage. The objective of this study was to analyze the oxidative stress of skeletal muscle, to demonstrate the existence of the effort-induced oxidative stress, throughout the detection of superoxide dismutase variations in race horses, and to observe how these variations evolve with aging.*

Key words: *oxidative stress, SOD, free radicals.*

1. Introduction

Superoxide-dismutase is the most important antioxidant enzyme in oxygen breathing organisms. It catalyses a dismutation reaction, through which O2 is reduced to H2O2 [1].

There are 3 different types of SOD, depending on the metal ion they contain: Cu/ZnSOD, MnSOD and FeSOD [2-4].

Part of the SOD`s activity takes place outside the cell, but its most important role is played inside the cell. The enzyme is very active in the mitochondrion (MnDOS) and in the cytosol (Cu/ZnSOD) [5].

The concentration of the SOD varies in the different parts of the body: the highest levels can be found in the liver, adrenal glands, kidney and spleen [6-8].

2. Objectives

To demonstrate the existence of the effort-induced oxidative stress, throughout the detection of superoxide dismutase variations in race horses. To observe how these variations evolve with aging.

3. Materials and methods

We have measured the superoxide dismutase (SOD) levels in plasma obtained from race horses, before and after intense physical effort.

The SOD levels would be an expression of the cells' antioxidant capacity.

The experimental study has been made on 20 Lipitan race horses, age 6, 12 and 15. The serum has been analyzed in the DSV Brasov laboratory.

[1] Centre "Advanced Research on Mechatronics", *Transilvania* University of Braşov.
[2] Organization of appurtenance for the second author.
[3] Emergency Clinical Hospital of Braşov.

The O_2 intake in a horse, during effort increases over 30 times, making the horse an excelent study model.

The study was made according to the laws about the use of animals in scientific purposes, with permission from the Ethics Commitee.

The horses have been placed in 3 batches:

Batches of horses Table 1

	Age (years)	No. of horses
Batch 1	6	8
Batch 2	12	7
Batch 3	15	5

The horses have been subjected to a 30 minutes daily training, 5 days a week.

The exercise test itself: submaximal effort, meaning a 60 minutes run.

Blood samples have been taken from each horse, before and after the test, centrifuged 1000 x g for 10 min. at 4° C and the plasma was separated.

The SOD activity was detected using an automatic microplate reader (ELISA TECAN sunrise). The readings have been made at every 5 seconds, at 450 nm.

The SOD activity could be measured both directly and indirectly.

The indirect method uses nitroblue tetrazolium (NBT) and is very easy and convenient.

Its disadvantages are the low solubility of NBT and the interaction with reduced xanthine-oxidase.

A better technique uses the SOD Assay Kit-WST, containing a water soluble tetrazolium salt.

The salt solution produces a colored soluble compound as the superoxide is reduced so that the reaction can be measured colorimetrically.

Content of the work kit (Fluka) Table 2

WST solution	5 ml
Enzyme solution	100 µl
Tampon solution	100 ml
Tampon dilution solution	50 ml
Superoxide dismutase (SOD)	

3.1. Preparation of the Solutions Used:

First is the WST work solution, by mixing 1 ml of WST with 19 ml tampon solution. Second is the enzyme solution. The enzyme solution tube is centrifuges for 5 seconds, then 15 µl of enzyme solution are diluted with 2,5 ml dilution solution.

3.2. Methods

Following the succesive stages:

- Add 20 µl of solution to be analyzed in well no. 1 (M - wittness) and well no. 3 (sample 2 – Blanck 2);
- Add 20 µl double distilled water (ddH2O) in well no. 2 (sample1-Blanck 1) and well no. 4 (sample 3 – Blanck 3);
- Add 20 µl tampon dilution solution in each of blanck 2 and 3;
- Add 20 µl enzyme solution in M-Blanck and Blanck 1.

After adding the enzyme solution into the wells, the superoxide reacts very quickly. A multi-channel pipette is necessary to avoid the differences between reaction times.

The microplate is then mixed for 20 minutes at 37ºC. Finally, the absorption at 450 nm is read by the Elisa reader.

Work solutions Table 3

	Blanck-M	Blanck-1	Blanck-2	Blanck-3
Serum	20 µl		20 µl	
dd H2O		20 µl		20 µl
WST solution	200 µl	200 µl	200 µl	200 µl
Enzyme solution	20 µl	20 µl		
Tampon solution			20 µl	20 µl

Fig. 1. *Image of . ELISA TECAN sunrise microplate reader*

4. Results

Our results show that mean (± SEM) SOD values in the 6 years old horses was significantly decrease after the effort, meaning the existence of oxidative stress.

Table 4

Mean (± SEM) SOD values in the 6 years old horses

No. of horses	SOD before effort	SOD after effort
1	118±1	86±1
2	129±0.8	83±1
3	136±0	99±1
4	142±1	98±0.1
5	134±0.8	97±0.9
6	126±1	81±o
7	131±0.9	96±1
8	139±1	95±0.9

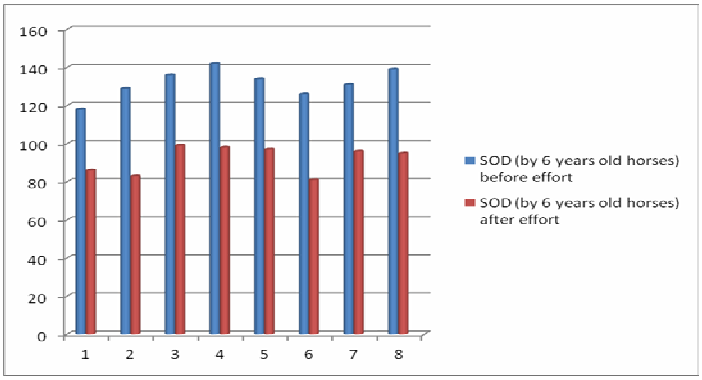

Fig. 2. *Superoxide-dismutase variations in the 6 years old horses, before and after exercise*

Mean (± SEM) SOD values in the 12 years old horses Table 5

No. of horses	SOD before effort	SOD after effort
1	136±0	97±1
2	114±1	88±0
3	139±0.1	98±0
4	140±1	93±1
5	119±1	89±1
6	137±1	99±1
7	144 ±1	102±1.1

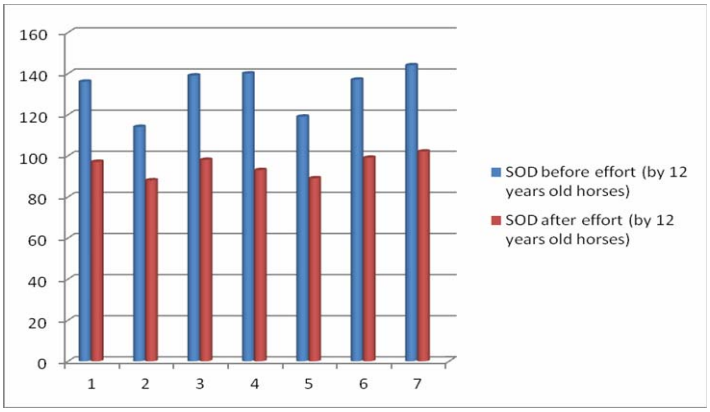

Fig. 3. *Superoxide-dismutase variations in the 12 years old horses, before and after exercise*

Mean (± SEM) SOD values in the 15 years old horses Table 6

No. of horses	SOD before effort	SOD after effort
1	137±1	101±0
2	144±1	122±0.6
3	121±0.1	109±0.2
4	105±0.2	83±1
5	129±0.1	99±1

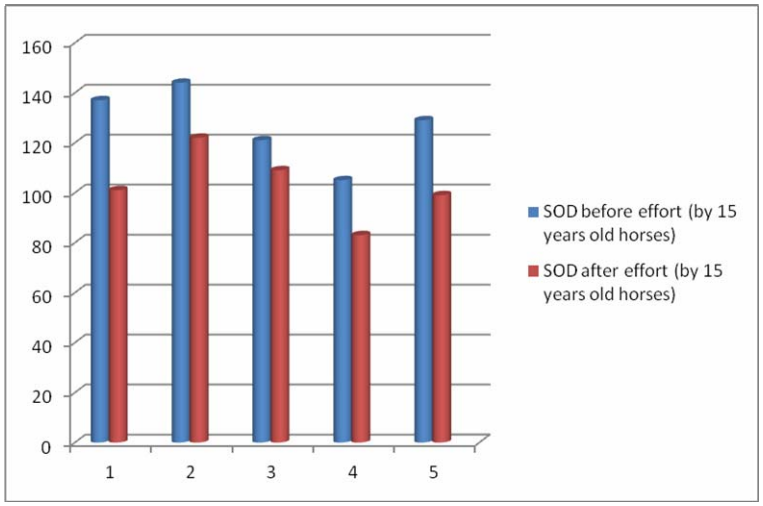

Fig. 4. *Superoxide-dismutase variations in the 15 years old horses, before and after exercise*

5. Conclusions

1. In the 6 year-old group, the mean superoxide dismutase (SOD) values before the effort, were between 119 and 143. In the same group, after the 60 minutes run, the SOD values were between 82 and 99.
2. Considering an average value before effort of 132.13, after effort, a value of 92.37, we observed a decrease with 69.90%, after the effort, meaning the existence of oxidative stress.
3. In the 12-year-old group of horses, the SOD values before the effort, were between 114.01 and 144. After the effort, in the same group, SOD values varied from 88 to 102.01.
4. Considering an average value of 132.72 before effort and one of 95.15, after effort, we have observed a decrease of the SOD values with 70.6%, showing the existence of oxidative stres.
5. In the 15 year-old group of horses, before effort, SOD values were situated between 105 and 144.01. In the same 15 year-old group, after effort, the SOD varied from 83 to 122.
6. Considering an average SOD value of 127.2 before effort and one of 102.8 after effort, we have noticed a 80 %

decrease, showing the existence of oxidative stres. Overall, in the whole batch of horses we studied, the SOD values decreased with 25.95% after the physical effort, compared with the initial, pre-effort values. Comparing the post-effort SOD values in extreme ages, we observed a value of 92.37 in the 6 year-old horses and of 102.8 in the 15 year-old horses. This means an increase of the enzymatic activity along with aging.

7. Enzymatic antioxidants have demonstrated great versatility and adaptability in response to chronic and acute exercise.

References

1. Sureda, A., Tauler, P., Aguiló, A., et al.: *Blood cell NO synthesis in response to exercise*. In: *Nitric Oxide*. 2006;15(1), p.5–12.
2. Powers, S.K., Ji, L.L., Leeuwenburgh, C.: *Exercise training-induced alterations in skeletal muscle antioxidant capacity*: a brief review. In: *Medicine and Science in Sports and Exercise*. 1999;31(7), p.987–997.
3. Kerksick, C., Willoughby, D.: *The Antioxidant Role of Glutathione and N-Acetyl-Cysteine Supplements and Exercise-Induced Oxidative Stress*. In: *J Int Soc Sports Nutr*. 2005; 2(2), p.38–44.
4. Ramel, A., Wagner, K.H., Elmadfa, I.: *Plasma antioxidants and lipid oxidation after submaximal resistance exercise in men.* In: *Eur J Nutr.* 2004; 43, p.2–6.
5. Reid, M.B., Stokic, D.S., Koch, S.M., et al.: *N-Acetylcysteine inhibits muscle fatigue in humans*. In: *J Clin Invest.* 1994; 94, p.2468–2474.
6. Matuszczak, Y., Farid, M., Jones, J., et al.: *Effect of n-acetylcysteine on glutathione oxidation and fatigue during handgrip exercise*. In: *Muscle Nerve.* 2005; 32, p.633–638.
7. Cybulski, R.J., Sanz, P., et al.: *Four superoxide dismutases contribute to Bacillus anthracis virulence and provide spores with redundant protection from oxidative stress.* In: *Infection and Immunity*. 2009;77(1), p.274–285.
8. Frankiewicz-Jóźko, A., Szarska, E.: *Anti-oxidant level to exercise in the blood of endurance horses*. In: *Biology of Sport*. 2000; 17(3), p.217–227.