

Exercise induce oxidative stress in rats kidney

Radu MD¹, Schiopu S¹, Tută Liliana-Ana², Chirică R²

¹Faculty of Natural and Agricultural Sciences, Ovidius University of Constanta, Romania

²Faculty of Medicine, Ovidius University of Constanta, Romania

Abstract. Oxygen free radicals play an important role in metabolic and phenotypic adaptation of skeletal muscle to exercise. Recent experimental studies have shown that oxygen free radicals influence the functional activity and thus adaptive metabolic organs, involved in post-exercise recovery and disposal of products of metabolism, plus nerve control structures, gonads and digestive system. Our experimental model aims to quantify the biochemical phenomenon of oxidative stress induced by acute exercise on kidney Wistar rats at 24 hours post-exercise. Male Wistar rats were divided into two groups (control group and the experimental group who performed a single workout, swimming for 90 min), and were sacrificed at 24 hours post-exercise, with the protection of laboratory animals. After slaughter samples were collected from the renal parenchyma and the biochemical parameters followed were: superoxide dismutase activity (SOD) and catalase (CAT) and tissue levels of reduced glutathione (GSH). From the experimental data (superoxide dismutase activity – $6.48 \pm 0.19 \uparrow^{**}$; catalase activity- $3.80 \pm 0.25 \uparrow^*$; Reduced glutathione levels- 1.63 ± 0.24 NS) that at 24 hours post exercise installs the phenomenon of mild oxidative stress on kidney.

Key words: exercise, oxidative stress, kidney.

Introduction

Physical inactivity is a leading cause of death globally in recent years. The World Health Organization puts sedentary on fourth place in the hierarchy of medical phenomena that induce death worldwide (1). Inactivity is the main cause of obesity, cardiovascular diseases and tumor diseases.

Exercise is the essential component for a healthy life of modern man. Moderate physical effort is usually recommended in the treatment of cardiovascular diseases and chronic obstructive pulmonary disease and in the prevention of diseases such as atherosclerosis, type 2 diabetes, colon cancer and breast cancer (2-6).

By the energy requirements, exercise increases oxygen consumption by 20-25% compared to basal physiological conditions, an event that will lead to the generation of oxygen free radicals.

Free radicals are chemical species, of reactive oxygen species which may react chemically with any biomolecules (7). The main ways of producing oxygen free radicals during exercise are: mitochondrial activity, neutrophil activation, as a result of polymorphonucleate tissue activity, cell reperfusion after an ischemic phenomenon, and autooxidation of catecholamines (8-11).

In addition to the ability to generate oxygen free radicals as a result of the cell's metabolic processes, the cell dispose biochemical mechanisms of defense against oxygen free radicals.

By their nature, biochemical cellular defense mechanisms against oxygen free radicals, were grouped into enzymatic and non-enzymatic mechanisms.

The category of enzymatic mechanisms, include the following enzymes: superoxide dismutase, catalase, glutathione peroxidase, tyreoredoxine, glutaredoxyne and peroxiredoxyne and the category of non-enzymatic antioxidant mechanisms include: vitamins A, E, C, reduced glutathione (GSH), coenzyme Q10, bilirubin, uric acid, α -lipoic acid, β -carotene and many phytochemicals (12-16).

In basal physiological conditions there is a balance between the rate of generation of reactive oxygen species and antioxidant neutralizing capacity for the system overall.

In certain situations, such exercise or different pathophysiological phenomena can be easily tilted the balance in favor of oxygen free radicals, called oxidative stress phenomenon (17, 18).

capacity of the antioxidant to neutralize the increased level of oxygen free radicals. Oxidative stress, through changes in redox equilibrium tissue / cell influence athletic performance.

Recent experimental studies have shown that oxygen free radicals play a role in the adaptation signal of the body to exercise, by activating antioxidant mechanisms, biogenesis of mitochondria and increased rate of absorption of glucose in skeletal muscle (19).

In addition to changes in metabolic and biochemical events, associated rate, during exercise blood is sent to the muscles, especially in business.

The blood flow during exercise, is mainly sent to the muscles in activity, and to other organs such as: intestine, liver, kidney and brain (20-22). Those organs go through short periods of hypoxia during exercise, post event will lead to a hyper-oxygenation effort of body, and hence the production of oxygen free radicals that can induce oxidative stress phenomenon.

Exercise can directly influence redox status and functional activity of skeletal muscle, and indirect, redox status and functional activity of organs involved in rebuilding post-exercise, metabolic products of metabolism, and elimination of control nerve structures.

Our experimental model quantify biochemical phenomenon intensity of oxidative stress induced by acute exercise in the kidney.

Material and Method

Biologic material used in our experimental model were Wistar albino rats, males 16 weeks of age and weighing 200 ± 20 g.

The animals were individually housed in thermostatic ($22 \pm 2^\circ\text{C}$) windowless stainless steel 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.

Our experimental model consisted of two groups: a control group and an experimental group, each group consisting of 6 animals, albino male rats of Wistar line.

- Control group (M) male - animals in this group did not perform exercise, serving as a

reference for the experimental group.

- Experimental group (Ef) male - animals in this group were subjected to a single exercise (swimming in a pool of water at $20^\circ \pm 2^\circ\text{C}$, 90 minutes). The animals were slaughtered at 24 hours after the exercise, with the protection of laboratory animals. After slaughter samples were collected from renal parenchyma.

Biochemical assays. Biochemical parameters were:

- superoxide dismutase activity (SOD);
- catalase (CAT);
- tissue concentrations of reduced glutathione (GSH).

Determination of the enzymatic activity of superoxide dismutase (SOD) was made by the method of Winterbourne (1979) (23).

Biochemical analysis method is the colorimetric method and the enzymatic activity was related to milligrams of protein. Determination of total proteins was done by the method described by Lowry (1951) (24).

Determination of enzymatic activity of catalase (CAT) was done as was described by Beers and Sizer (1952) (25).

Determination of tissue levels of reduced glutathione (GSH) was done as was described by Beutler (1984) (26). Biochemical analysis method is the colorimetric method, and reduced glutathione tissue levels was relate to milligrams of protein.

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

Results

The results of our research are presented in Table I.

Discussion

Recent experimental studies have shown that oxygen free radicals are generated during exercise and play an important role in the control of muscle metabolism (27).

Skeletal muscle is a dynamic structure in terms of morphological, biochemical and endocrine adaptation strategy and the exercise part metabolic conversion and modification of active muscle mass (19).

Table I. Superoxide dismutase activity and catalase enzyme and tissue levels of reduced glutathione at the control group (M) and experimental group (Ef)

Group	Statistical Analysis	Superoxide Dismutase Activity-SOD U/mg protein	Catalase Activity-CAT U/mg protein	Reduced Glutathione- GSH mcg/mg protein
Control Group (M)	X±ES	4.89±0.12	2.67±0.14	1.46±0.17
	n	6	6	6
Experimental Group (Ef)	X±ES	6.48±0.19	3.80±0.25	1.63±0.24
	n	6	6	6
	t	6.54	3.45	-
	p≤	0.01↑	0.05↑	NS

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

Unfortunately the same thing cannot be said about the kidney. Proteinuria is an event that indicates an exercise that induced renal injury, hypothesis demonstrated and cited in the literature (28) and the research of the post effort kidney is a crucial target in sports physiology.

Exercise is recommended in patients with chronic renal failure to improve general and physiological status and to reduce mortality (29).

Paradoxically exercise is an important source of generation of oxygen free radicals. And when the concentration of oxygen free radicals exceeds the antioxidant defense system inactivates basal and install oxidative stress.

If skeletal muscle mechanisms for generation of oxygen free radicals witch can cause oxidative stress is known largely for kidney, but the sources that induce oxidative stress during exercise and after effort are still unclear.

A possible cause of the phenomenon installation oxidative stress in the kidney can be effort lessly, and post ischemic reperfusion phenomenon, addition the increase in glomerular filtration rate (30).

Superoxide dismutase and catalase are the antioxidant enzymes, which corrects the level of superoxide radicals and hydrogen peroxide on cellular/tissue level.

Superoxide dismutase catalyzes the conversion of the superoxide radical to hydrogen peroxide and catalase activity, using as a substrate of hydrogen peroxide. The both enzymes changes in relation to

the level of superoxide radicals to hydrogen peroxide.

Reduced Glutathione is a biologically active peptide with antioxidant and co-activator factor. This enzyme may act directly or indirectly to oxygen free radicals, by enzymes whose co-factor is.

The dynamics of the biochemical parameters in the study indicate in our experimental model of oxidative the stress phenomena installation of medium intensity at 24 hours post effort.

The enzymatic activity of superoxide dismutase and catalase, increase statistically significant and reduced glutathione tissue levels is in the range of the reference (see Table I) that indicates the installation of the dynamic phenomenon of moderate oxidative stress.

Conclusions

In our experimental model, the dynamics of the biochemical parameters in the study of the phenomenon indicates the installation of moderate oxidative stress in the kidneys in 90 minutes after (at 24 hours) physical effort on Wistar rats.

Acknowledgments. This work received financial support through the project entitled "CERO – Career profile: Romanian Researcher", grant number POSDRU/159/1.5/S/135760, cofinanced by the European Social Fund for Sectoral Operational Programme Human Resources Development 2007-2013.

References

1. Sarvas J, Sarah Niccoli, Walser E, Khaper N, Simon JL (2014). Interleukin-6 deficiency causes tissue-specific changes in signaling pathways in response to high-fat diet and physical activity. *Physiol. Rep*; 2 (7): e12064.
2. Piepoli, MF, Davos C, Francis DP, Coats AJ (2004). Exercise training meta-analysis of trials in patients with chronic heart failure (ExTra- MATCH). *British Medical Journal*; 328 (7433): 189.
3. Lacasse, Y, Brosseau L, Milne S, Martin S, Wong E, Guyatt GH, Goldstein, RS (2002). Pulmonary rehabilitation for chronic obstructive pulmonary disease. *Cochrane. Database. Syst. Rev*; 3: CD003793.
4. Blair SN, Cheng Y, Holder JS (2001). Is physical activity or physical fitness more important in defining health benefits? *Med. Sci. Sports. Exerc*; 33: S379–S399.
5. Boule NG, Haddad E, Kenny GP, Wells GA, Sigal, RJ (2001). Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *J.A.M.A.* 286: 1218–1227.
6. Jolliffe, JA, Rees K, Taylor RS, Thompson D, Oldridge N, Ebrahim, S (2001). Exercise-based rehabilitation for coronary heart disease. *Cochrane. Database. Syst. Rev*; (1): CD001800.
7. Urso Maria, Clarkson PM (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*. 189: 41-54.
Popovic LM, Mitic NR, Miric D, Bisevac B, Miric M, Popovic, B (2015). Influence of Vitamin C Supplementation on Oxidative Stress and Neutrophil Inflammatory Response in Acute and Regular Exercise. *Oxid. Med. Cell. Longev*; 2015: 295497. doi:10.1155/2015/295497. Epub 2015 Feb 23.
8. Powers SK, Talbert EE, Adhihetty PJ (2011). Reactive oxygen and nitrogen species as intracellular signals in skeletal muscle (SYMPOSIUM REVIEW). *J. Physiol*; 589 (Pt 9): 2129–2138.
9. Allen RG, Lamb GD, Westerblad H (2008). Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev*; 88: 287-332.
10. Tidball JG (2005). Inflammatory processes in muscle injury and repair. *The American Journal of Physiology — Regulatory Integrative and Comparative Physiology*; 288 (2): R345–R353.
11. Seo E, You Y, Yoon HG, Kim B, Kim K, Lee YH & all (2015). Rosa rugosa Aqueous Extract Alleviates Endurance Exercise-Induced Stress. *J.Med. Food*. 2015 Jun; 18(6):711-3. doi: 10.1089/jmf.2014.3290. Epub 2015 Feb 12.
12. Powers SK, Malcolm J (2008). Exercise induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol. Rev*; 88: 1243-1265.
13. Berndt C, Lilling CH, Holmgren A (2007). Thiol-based mechanisms of the thioredoxin and glutaredoxin systems: implication for diseases in the cardiovascular systems. *Am. J. Physiol. Heart. Circ. Physiol*; 292: 1227-1236.
14. Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA (2005). Uric acid and oxidative stress. *Curr. Pharm. Des*; 11: 4145–4151.
15. Combes JS, Rowell B, Dodd SL, Demirel HA, Naito H, Shanely RA, Powers, SK (2002). Effects of vitamin E deficiency on fatigue and muscle contractile properties. *J. App. Physio*; 87: 272-277.
16. Sies H (1985). *Oxidative stress*. London Academic Press. pp 1-8.
17. Jones DP (2006). Redefining oxidative stress. *Antioxid. Redox. Signal*; 8: 1865-1879.
18. Mankowski RT, Anton SD, Buford TW, Leeuwenburgh C (2015). Dietary Antioxidants as Modifiers of Physiologic Adaptations to Exercise. *Med. Sci. Sport. Exerc.* [Epub ahead of print].
19. Zhang H, Sun XQ, Cao JM, Zhou HT, Guo X, Wang ,Y (2014). Protective effect of epimedium combined with oligomeric proanthocyanidins on exercise-induced renal ischemia-reperfusion injury of rats. *Int. J. Clin. Exp*; 7 (12): 5730-6.
20. Lovatel GA, Bertoldi K, Elsnerb VR, Piazza FV, Basso CG, MoysesFdos S, Worm PV, Netto CA, Marcuzzo S, Siqueira, IR (2014). Long-term effects of pre and post-ischemic exercise following global cerebral ischemia on astrocyte and microglia functions in hippocampus from Wistar rats. *Brain Res*; 1587:119-26.
21. Otte JA, Oostveen E, Geelkerken RH, Groeneveld ABJ, Jeroen JK (2001). Exercise induces gastric ischemia in healthy volunteers: a tonometry study. *J. Appl. Physiol*; 91: 866–871.
22. Winterbourne CC (1979). Comparison of superoxide with other reducing agent in the biological production of, hydroxyl radicals. *J. Biochem*; 182: 625-628.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall, RJ (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem*; 193: 265-275.
24. Beers RF, Sizer IW (1952). A spectrophotometrical method for measuring the breakdown of H2O2 by catalase. *J. Biol. Che*; 195: 133-140.

25. Beutler E (1984). *Red cell metabolism: Manual of Biochemical Methods* 3rd, ed. New York, Grune and Stratton Inc.
26. Kathryn HM (2014). Polyphenol Supplementation: Benefits for Exercise Performance or Oxidative Stress? *Sports. Med*; 44 (Suppl 1): S57–S70.
27. Kohler M, Schanzer W, Thevis M (2015). Effects of exercise on the urinary proteome. *Adv. Exp. Med. Biol*; 845:121-31.
28. Morishita Y, Nagata D (2015). Strategies to improve physical activity by exercise training in patients with chronic kidney disease. *Int. J. Nephrol. Renovasc; Dis.* 9:19-24.
29. Kocer G, Senturk UK, Kuru O, Gunduz F (2008). Potential sources of oxidative stress that induce postexercise proteinuria in rats. *J. Appl. Physiol*; 104 (4), 1063-1068.

Corresponding author

Radu Marius- Daniel
Faculty of Natural and Agricultural Science
Ovidius University, Constanta, Romania
Phone: +40731682470
Email: drd_maryus@yahoo.com

Received: March 10, 2015

Accepted: May 25, 2015