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Effect of acute exercise on uric acid serum levels in the experimental model

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Original scientific paper

Abstract
Increased oxygen consumption during aerobic exercise is one of the main cause of accompanied oxidative stress. Oxidative stress is a condition of a disturbed balance between reactive oxygen species (ROS), reactive nitrogen species (RNS) and antioxidative defense. Antioxidants are substances that reduce the damage caused by oxidative stress. Uric acid is one of the most important antioxidant. The aim of this study was to estimate the influence of acute exercise on uric acid serum levels in rats. Adults Wistar rats weighting between 280-330 g were divided into two groups: control group (n = 8) and exercise group (n = 8). Exercise group was exposed to acute bout of exercise (swimming), after short accommodation period. Control group was consisted of animals housed in the same condition without exercise sessions. Animals were sacrificed and blood sample was taken from the abdominal aorta to determine uric acid levels. Levels of uric acid in serum samples were determined by spectrophotometric method.
Acute exercise (swimming) significantly increased average concentration of uric acid in exercise group (246.5 ± 19.1 mmol / l) when compared to control group (226.11 ± 7.8 mmol / l) (p = 0.03).
Acute exercise increases the concentration of uric acid in serum probably as an antioxidant response to enhanced oxidative stress

Key words: **stress, swimming, uric acid**

Sažetak
Povećana potrošnja kisika u toku fizičkog opterećenja aerobnog tipa jedan je od glavnih uzroka povećanog oksidativnog stresa. Oksidativni stres je stanje poremećene ravnoteže između reaktivnih vrsta kiseonika (ROS) i reaktivnih vrsta azota (RNS) sa jedne i antioksidativne zaštite sa druge strane. Antioksidansi su tvari koje u organizmu pomažu u smanjenju oštećenja nastalih pri oksidativnom stresu. Jedan od najvažnijih antioksidansa je urična kiselina. Cilj istraživanja je bio da se ispita uticaj fizičke aktivnosti na koncentraciju urične kiseline u serumu štakora.
Odrasli Wistar štakori težine 280-330 g, bili su podijeljeni u dvije grupe: kontrolna grupa (n=8) i grupa štakora koji su vježbali (fizički aktivni) (n=8). Grupa fizički aktivnih štakora bila je izložena akutnoj sesiji vježbanja (plivanje), nakon kratkog perioda adatacije. Kontrolnu grupu su činile životinje koje nisu bile izložene fizičkoj aktivnosti, a držane su u istim uslovima. Nakon posljednje sesije vježbanja životinje su žrtvovane, a uzorak krvi za određivanje uричне kiseline uzet je iz abdominalne aorte. Koncentracija urične kiseline u serumu određena je spektrofotometrijskom metodom.
Fizička aktivnost je dovela do statistički značajnog porasta koncentracije urične kiseline u serumu, te je koncentracija urične kiseline u serumu u grupi fizički aktivnih štakora bila značajno veća u odnosu na kontrolnu grupu štakora (246,5 ± 19,1 mmol/l vs. 226,11±7,8 mmol/l; p = 0.03). Fizička aktivnost (plivanje) dovodi do porasta koncentracije urične kiseline u serumu, najvjerovatnije kao odgovor na povećan oksidativni stres.

Ključne riječi: **stres, plivanje, urična kiselina**

Introduction

Exercise is activity which affects practically every organ and tissue in our body, contributing to human health, while sedentary lifestyle is in relation with many chronic health problems. However, many studies have shown that acute aerobic exercise increased consumption of oxygen and leads to oxidative stress, which depends on type and intensity of exercise and general body status.

It has been shown that antioxidative status during acute exercise varies on type of the exercise (1). Two mechanisms connect acute aerobic exercise with oxidative stress: increased pro-oxidative activity and inadequate antioxidative defence (2). On the other hand, some studies have shown that daily training (30 min, 60%VO2max) increases resistance to lipid peroxidation caused by reactive oxygen species, decreases accumulation of oxidative proteins and decreases DNA damage, diminishing therefore the risk of many diseases, where oxidative stress is involved in their pathophysiological mechanism. It has been shown that sedentary subjects have less effective antioxidative system comparing to physical active subjects (3,4).

Oxidative stress is defined as damage in cells and tissues, caused by reaction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and impaired antioxidative defence mechanisms on the other side(5). Antioxidants are compounds, presented in all body fluids and tissues, which play role in protecting organism against endogenous produced free radicals, respectively oxidative stress.

Uric acid is strong endogenous antioxidant in our body (6) and presents final product of purine bases (adenine and guanine). Its concentration in human blood depends on age, gender, body area and body weight, ethnic group and geographical position. It has also been considered as main salivary antioxidant, because it participates in approximately 70% of total antioxidative capacity of saliva (7,8).

Considering the fact that the main characteristic of human is their ability to protect themselves against excessive and uncontrolled oxidation, we believe that oxidative stress could activate defence systems and make them more resistant to possible impairments. It is possible that oxidative stress associated with exercise pres-

ents „trigger“ of adaptation mechanisms, such as synthesis of antioxidative and reparative enzymes, with development of antioxidative defence mechanisms as their final result. This is the way we could explain positive effects of exercise.

The aim of our study was to estimate the influence of swimming exercise on concentration of uric acid in the serum of rats.

EXPERIMENTAL ANIMALS AND METHODS

Experimental animals

Adults Wistar rats, weighting between 280-330 g, were divided into two groups: control group (n = 8) and exercise group (n = 8). All animals were kept under the same laboratory conditions of relative air moisture (45-55%) and room temperature. All the rats were given standard rat chow and tap water *ad libitum*.

All the experimental procedures were performed at Medical faculty Sarajevo, Department of Physiology and previously approved by the Ethic Committee of Medical Faculty Sarajevo.

Exercise protocol

Before we performed acute bout of exercise we exposed rats to daily swimming session aimed to the acclimatization to water conditions and swimming, as type of exercise. Exercise group was exposed to swimming exercise daily, between 10.00 AM to 11.00 AM, for 6 days. Duration of each swimming session progressively increased from 5 minutes on the first day to 30 minutes on the sixth day. The rats were swam in plastic tanks (width 90 cm, depth 120 cm), containing tap water (temperature of ≈25˚C). The depth of water was 40 cm. A maximum of two rats, same sex, were allowed to swim together.

Seventh day we performed acute bout of swimming exercise. Rats swam 40 minutes until exhaustion at the same conditions as in the period of adaptation. The body weight of each rat was measured before starting of accommodation period and after the last swimming session.

Determination of uric acid in the serum of rats

The blood sample is taken from the abdominal aorta and centrifuged 5 minutes at 3000 rpm. Serum was stored at -80°C until determination of uric acid levels .

Concentration of uric acid in the serum samples was determinated by spectrophotometric method using commercial kits (Bio-Systems S.A. Spain). The method is based on determination of colour intensity of the complex, resulting from uricase reaction.

Statistical analyses

Data are analysed with standard statistical methods using computer program Excel (Microsoft Office Excel 2003) and SPSS computer package 13.0 (SPSS-Statistical Package for Social Sciences) . Results are presented as mean (X) and standard error of the mean (SEM). Kolmogorov-Smirnov test was used to test the significance of differences in deviation of normal distribution. Data are analysed using parametric test, after determination of uniform distribution of variables. Value $p<0,05$ was considered significant.

Results

There were no statistically significant differences in body weight or food intake between control and exercise group at the beginning of the experiment. (Table 1).

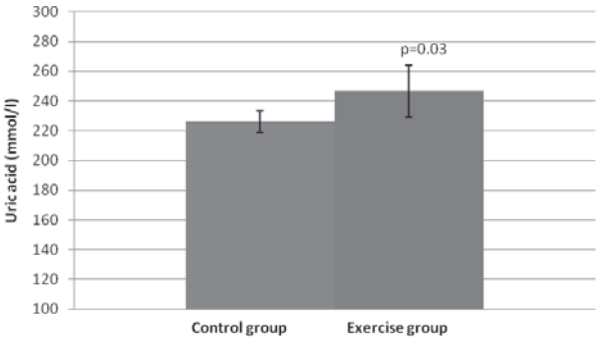
Table 1. Mean values of body weight, daily food intake and concentration of uric acid in control and exercise group before and after exercise

	Control group (n=8)	Exercise group (n=8)	
		Before	After
Body weight (g)	291,4±13,7	296,3±14,1	272,8±21,4
Food intake (g/d)	21,7 ± 2,3	22,4±1,4 (1. day)	21,4±2,3 (7. day)
Uric acid (mmol/l)	226,11±7,8		246,5±19,1

Results are presented as means ± SEM.

Swimming slightly decreased body weight in the exercise group of rats comparing to the values before the start of experiment (296,3 g vs. 272,8 g), but this difference was not statistically significant (Table 1). Exposure to daily exercise didn't affect daily food intake in stress group (Table 1).

Chart 1. Uric acid levels in control group and in exercise group after exercise



In the control group average concentration of uric acid in the serum was 226,11±7,8 mmol/l. Average values of uric acid in the exercise group after acute bout of exercise was 246,5±19,1 mmol/l.

Statistically significant difference was determined in concentration of uric acid in the serum between control and exercise group (p=0,03) (Chart 1).

Discussion and Conclusion

Oxidative stress induced tissue damages may be the cause of many disorders which occur after exposed to the stress situations. Today, there are more than fifty diseases whose pathophysiological mechanisms are associated with production of free radicals and oxidative stress, also including the two most expanded chronic diseases - atherosclerosis and cancer, as well as a whole variety of chronic degenerative diseases, such as arthritis, vasculitis, autoimmune diseases, early aging, etc. Reactive oxygen species can be involved in formation of so-called clastogenic factors (factors who ravage the hormons), appearance which is lately connected to process of cancerogenesis (9).

Several studies have shown that exercise causes oxidative stress accompanied by oxidative cell damage, whose range prior depends on type and intensity of exercise and general body conditions.

However, the appearance of free radicals doesn't mean the appearance of the disease at the same time, because there are protective defence mechanisms in the body, so-called antioxidative system. Antioxidants are compounds, presented in all body fluids and tissues, who play role in protecting organism against endogenous produced free radicals. Therefore the main role of antioxidants is the prevention or reduction of oxidative damages in biological systems, without interfering with physiological functions.

Antioxidants, endogenous (present in our diet) and exogenous (made in our body), include several enzymes (superoxid dismutase, glutation peroxidase), proteins, vitamins C and E, β-carotene, uric acid, bilirubin and albumin. Thus uric acid presents one of the the most important endogenous antioxidant of the body.

Evaluation of different antioxidants during stress is important for identification of mechanisms involved in oxidative stress.

The aim of our study was to determinate influence of exercise on the uric acid serum levels as an serum antioxidant.

Results of our study have shown significant differences in concentration of uric acid in the serum of rats in control and exercise group after acute bout of exercise (swimming). Our results are consistent with the results of previous studies, which explain this increase in concentration of uric acid by activation of antioxidative protection, as a reaction to incurred oxidative stress.

The increase of uric acid serum levels during exercise was confirmed also by Groussard et al. (10), who noticed that short-term exercises, among other things, stimulate purine catabolism and lead subsequently to increase of uric acid concentration. In addition Aguilo et al. (11) demonstrated in their research how exhaustive exercise (mountain bicycling) causes the increase of uric acid concentration, with the increase of glutation reduxtase activity and decrease of glutation peroxidase activity. Based on biochemical findings, it is considered that uric acid removes reactive oxygen species and thereby reduces ROS mediated damages in the body (12).

Many studies have shown that high intensity exercise can increase the production of free radicals and cause oxidative tissue damages (13,14). The impact of acute exercise on production of free radicals and antioxidative system is being explored intensively (15, 16). F2- isoprostane concentration (marker of oxidative stress) is significantly higher after moderate exercise (38%), exhaustion (45%) and 1 hour after recovery (31%) (17). Mastaloudis et al. (18) also established that the F2 isoprostane level significantly increased during 50 km long ultramarathon, returning to initial levels after the race.

Leeuwenburgh et al. (19) in their study showed that acute exercise causes higher levels of oxidants and oxidative stress in untrained animals. However, long-term exercise can cause opposite effect, increasing the activity of antioxidative enzymes and decreasing the production of oxidants. In fact, they showed that animals, after long-term exercise, had lower levels of oxidative stress markers in muscles and in urine.

McIntosh and Sapolsky (20) believe that increased level of reactive oxygen species in stress situations, like acute exercise, could be conditioned by increased concentration of glucocorticoids. In their research, mentioned hormones lead to increased production of reactive oxygen species in the body.

In exercise group, we measured levels of uric acid only in blood samples taken after animal sacrifice, assuming that taking the blood sample before exercise would cause additional stress which could affect tested values. Besides, we believe that, in that case, levels of uric acid taken from rat's tail vein would differ from levels measured in abdominal aorta. In fact, taking sample from rat's tail vein, part of extracellular fluid is retroceded, which attenuates the sample and decreases the concentration of examined supstances.

Our study shows that acute bout of 40-minutes swimming exercise leads to increase uric acid serum levels in rats, as possible antioxidative reponse to increased oxidative stress.

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Submitted: November 21, 2011.
Accepted: November 28, 2011.

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