

Does Long-term Exercise Modulate Oxidant/Antioxidant Status in Humans? Comparison between Lipid Peroxidation and Catalase Activity in Fresh and Stored Samples*

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RECEIVED NOVEMBER 26, 2004; REVISED MARCH 2, 2005; ACCEPTED MARCH 15, 2005

Physical activity may have beneficial effects on the balance between oxidant stress and the defense system. The purpose of this study was to evaluate the influence of exercise on oxidant/antioxidant status in both sexes during autumn and winter periods. Since all women were tested in a low estrogen phase, the possibility to test the same parameters in plasma stored at low temperature would be of great importance. Oxidant changes in plasma were determined by measuring the concentration of thiobarbituric acid reactive substances (TBARS). Antioxidant capacity was measured by catalase (CAT) activity in lysate of erythrocytes. The results showed significantly higher TBARS concentrations in untrained men compared to women. Antioxidant capacity was of the same value in both sexes. Exercise did not change the oxidant/antioxidant status of either sex. The results indicate that women were more protected from oxidant processes than men. This finding points to the role of lipid peroxidation in higher incidence of myocardial infarction in men during winter. Significant changes in the oxidant/antioxidant status in stored plasma allowed greater reliability of TBARS and CAT measurements only in fresh samples.

Keywords
lipid peroxidation
exercise
catalase
oxidant
antioxidant

INTRODUCTION

Free radicals are derived either from the normal essential metabolism in human body or are part of the pathomechanism followed by increased amounts of free radical damage products, particularly markers of lipid peroxidation in body fluids. On the other hand, the body maintains a pool of compounds with antioxidant activity but the main system of defense against damage by free radicals is the enzymatic system that opposes oxidation.¹ Imbalance between the production of free radicals capa-

ble of causing peroxidation of the lipid layer of cells and antioxidant defense of the body causes oxidant stress. Oxidant stress is possibly involved in exercise inflammatory conditions, multiple organ dysfunction, ischemic and reperfusion damage, myocardial infarction, stroke or other diseases (cancer, atherosclerosis, nephropathy, rheumatoid arthritis, *etc.*).^{2,3,4} Maxwell *et al.*⁵ pointed out that the intensity of exercise and training level of the subjects affects the lipid peroxide (LPO) value. In some studies, acute physical exercise has been reported as a

* Dedicated to Professor Željko Kućan on the occasion of his 70th birthday. Presented at the Congress of the Croatian Society of Biochemistry and Molecular Biology, HDBMB₂₀₀₄, Bjelolasica, Croatia, September 30 – October 2, 2004.

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stressor with a potential to increase LPO, primarily through mitochondrial superoxide and hydrogen peroxide generation, but also via other mechanisms such as ischemia-reperfusion, catecholamine and neutrophil activation.⁶ Regular physical exercise may influence the adaptive responses that have been shown to protect against oxidant stress induced by exercise. On the other hand, Rokitzky *et al.*⁷ found LPO amounts to decrease immediately after a marathon and Hübner *et al.*⁸ in long-distance skiers. A very inconsistent pattern of oxidant stress produced during exercise may, on the other hand, greatly influence antioxidant function. Toskulkao and Glinskon⁹ reported that trained runners had higher, Tessier *et al.*¹⁰ decreased and Duthie *et al.*¹¹ unchanged antioxidant capacity compared to untrained subjects.

Nowadays it is often speculated that factors such as species, sex, tissue, age, circadian or seasonal variations as well as environmental factors (cold, climatic factors) may influence the oxidant/antioxidant status in humans and mammalian species.^{12,13,14,15} Investigations of Sheth *et al.*¹⁶ and Boulayet *et al.*¹⁷ demonstrated a higher winter incidence of acute myocardial infarction, stroke and varical bleeding mortality. Results of Chang-Sheng *et al.*¹⁸ have showed that the warm climate of subtropical regions does not affect the frequency of myocardial infarction. Age and sex difference in skeletal muscle LPO, where men appeared to be more vulnerable to oxidant damage than women, was observed by Pansaransa *et al.*¹⁹ Recently, such questions have been extended to oxidant stress induced by various types of exercise. It appears that hormonal and exercise-induced oxidant/antioxidant status in both sexes of humans and animals are related in some way.^{20,21} These findings suggest that any study of oxidant stress and antioxidant capacity must take into account seasonal variations and hormonal status of the subjects.

The purpose of the present study was to investigate the influence of long endurance exercise on the plasma concentration of TBARS (indicator of oxidative stress and free radical-mediated lipid peroxidation) and CAT (antioxidant enzyme activated in oxidant stress) in both sexes during autumn and winter periods. In women, each plasma sample (*i.e.*, low estrogen phase) has to be tested in the same hormonal period. Thus, the possibility to test the same parameters in plasma and lysate of erythrocytes stored at low temperatures would be of great help. The aim of our examination was to compare the oxidation process and antioxidant capacity in fresh and stored plasma.

EXPERIMENTAL

Chemicals

Trichloroacetic and hydrochloric acid were purchased from Merck, Darmstadt, Germany. Thiobarbituric acid, hydrogen

peroxide and other chemicals of analytical grade were obtained from Sigma, St. Louis, USA.

Experimental Design

The study involved professional hockey players (men, average age 28.0 ± 5.0) and professional soccer players (women, average age 20.6 ± 2.3), as well as age and sex matched untrained, non-sporting (control) volunteers (men, average age 24.0 ± 2.5 ; women, average age 22.6 ± 2.5). Female subjects were all in a low estrogen phase of their menstrual cycle. All subjects were thoroughly examined clinically for hematological, biochemical and vital parameters, which were found within normal limits. Blood was drawn from the cubital vein between 9 and 10 a.m. In highly-trained (endurance exercised) men and women, heparinized blood was taken prior to training. Heparinized blood samples were centrifuged and plasma was separated immediately ($3000 \text{ g} \times 5 \text{ min}$ at $+4 \text{ }^\circ\text{C}$). Part of the plasma was tested for oxidant/antioxidant capacity fresh while the other part was stored at $-20 \text{ }^\circ\text{C}$ and the same parameters were determined 7 days later. The study was approved by the local ethics committee and informed-consent documents were procured.

Biochemical Analyses

Oxidation of polyunsaturated lipids generates end products of peroxides, TBARS, mainly malonaldehyde. Measurement of such aldehydes provides a convenient index of general LPO. TBARS levels in plasma were determined spectrophotometrically by reaction with 2-thiobarbituric acid to give a red product absorbing at 535 nm, as described by Buege and Aostin.²² TBARS concentration were expressed in nmol per ml of plasma. CAT was determined spectrophotometrically as described by Aebi²³ by measuring absorbance changes in the reaction mixture using the final concentrations of $10 \text{ mol dm}^{-3} \text{ H}_2\text{O}_2$ and 50 mol dm^{-3} phosphate buffer ($\text{pH} = 7.0$) at 240 nm during a time interval of 30 s after sample addition. The CAT activity was expressed as the amount of hydrolysed H_2H_2 per minute ($\mu\text{mol min}^{-1}$) per mg of hemoglobine (Hb)

Statistical Analysis

Data were reported as means \pm SEM of n experiments, each representing an individual person. Student's t -test was applied to define the difference between groups. The level of significance was set at $p < 0.05$.

RESULTS

TBARS Concentration and CAT Activity in Plasma of Untrained and Exercised Humans of Both Sexes

TBARS concentration in untrained-control women was significantly lower ($p < 0.0001$) in comparison to untrained-control men (Figure 1). Exercise had not effect on the lipid peroxidation process either in men or women. Decreased TBARS concentration in exercised men and ex-

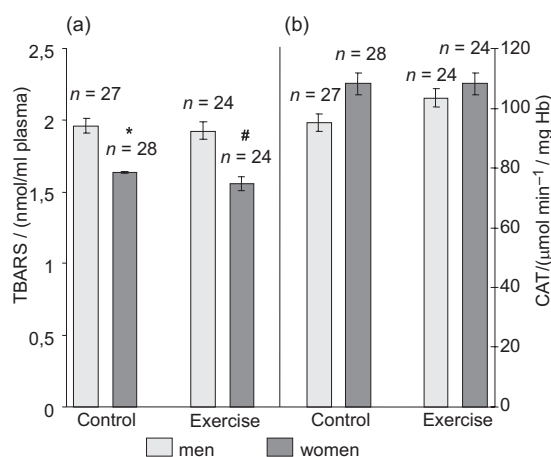


Figure 1. (a) Thiobarbituric acid reactive substances (TBARS) concentration and (b) catalase activity (CAT) in control and exercised men and women during autumn and winter. Data are presented as mean value ± SEM. n = number of subjects. *p < 0.0001, control women vs. control men; #p < 0.0001, exercised woman vs. exercised men.

exercised women remained at the same level as in the control group (about 18 %).

In contrast, CAT activity was of the same value in untrained groups of both sexes. No difference in CAT activity between untrained/exercised men and women was observed.

TBARS Concentration and CAT Activity in Plasma of Untrained and Exercised Humans of Both Sexes Stored at -20 °C

TBARS concentration in plasma during a storage period of 7 days at -20 °C remained unchanged in control-untrained men and women (Figure 2). In contrast, a signifi-

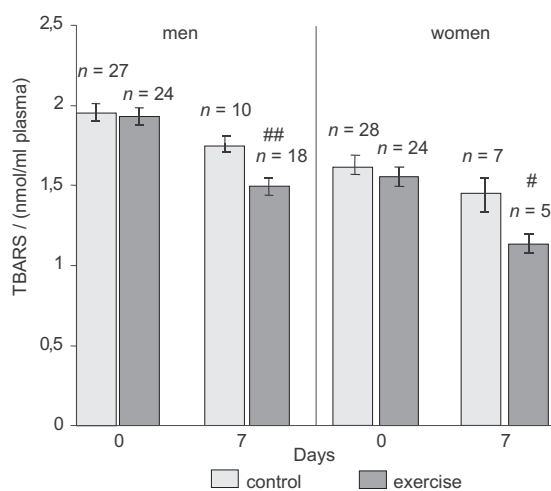


Figure 2. Thiobarbituric acid reactive substances (TBARS) concentration in fresh plasma and plasma stored at -20 °C for 7 days of control and exercised men and women. Data are presented as mean value ± SEM. n = number of subjects. #p < 0.002, fresh vs. stored plasma of exercised women; ##p < 0.0001, fresh vs. stored plasma of exercised men.

cant decrease in TBARS concentration occurred on day 7 after plasma storage at -20 °C in exercised men and women (p < 0.0001; p < 0.002, respectively).

Storage of samples for 7 days at -20 °C for men and women was associated with decreases in the CAT activity in both control (approx.: men 30 %; women 28 %) and exercised subjects (approx.: men 38 %; women 30 %) (Figure 3) (p < 0.00001).

DISCUSSION

Oxygen involved in the oxidation of substrates to produce energy in normal metabolic processes can produce oxygen radicals. Body oxygen uptake is greatly increased during intense exercise (100–200 times).²⁴ Most of this oxygen is transformed to water, but approximately 4 % is converted into superoxide within the electron transport system. However, the oxidant/antioxidant status of the body in normal physiological conditions and in exercise seems to depend on various factors, such as the type of exercise, intensity and duration of exercise, sex, age, etc.²⁵

Our results confirm such a finding. The group of control untrained as well as exercised men showed higher LPO levels compared to their women counterparts. In contrast, CAT activity remained unchanged in the two groups and sexes. Lower LPO levels were observed in untrained women compared to untrained men, perhaps partly due to the protective effect of female sexual hormones against LPO. Goodman *et al.*²⁶ found aged women more sensible to low density lipoprotein (LDL) oxidation and cardiovascular disease than aged men due to failure of the protective role of estrogens after the menopause. It is also known that incidence of myocardial infarction is lower in women than in men (except after the menopause) and men appear to be more susceptible to

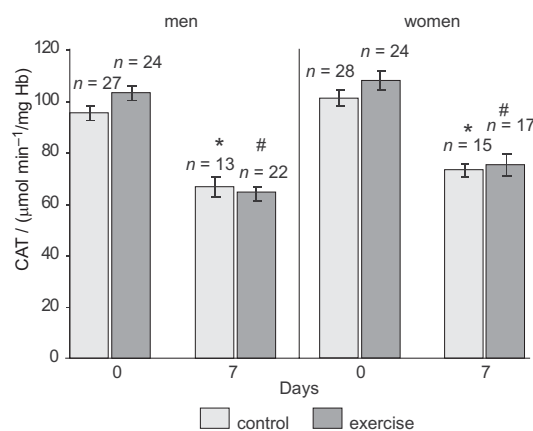


Figure 3. Catalase activity (CAT) in fresh plasma and plasma stored at -20 °C for 7 days of control and exercised men and women. Data are presented as mean value ± SEM. n = number of subjects. *p < 0.00001 fresh vs. stored plasma of control men and control women; #p < 0.00001, fresh vs. stored plasma of exercised men and exercised women.

oxidant stress than women.¹⁹ Viña *et al.*²⁷ demonstrated that female mitochondria produce approximately half the amount of lipide peroxide (LP) of those of males. Ovariectomy abrogates this protective effect. They maintain that estrogen activates the low density lipoprotein MAP kinase pathway. MAP kinase activates the nuclear factor kappa B (NF κ B) signaling pathway, which in turn activates antioxidant enzyme genes.

Protective role of estrogens as free radical scavengers in exercise-induced increase in LPO parameters in amenorrheic female athletes was demonstrated by Ayres *et al.*²⁸ as well. Also, estrogen and progesterone play an important role during exercise substrate utilization in explaining gender differences. Estrogen has been reported to decrease glucose utilization but may increase lipid availability by stimulating lipolysis.^{29,30} More energy is derived from fat oxidation and less from carbohydrate oxidation during exercise in women compared to men at the same relative intensity.³¹ However, the data of Schmitt *et al.*³² point to exercise-induced selective increase in mRNA levels of enzymes, which are involved in enhanced lipid utilization during moderate-intensity exercise in the muscles of endurance-trained male subjects. These results push forward the idea that during moderate intensity and regular physical exercise in endurance-trained males (such as our exercised men group), the substrate source could be lipids like in the exercised women group. However, it is important to note that estrogen may have an antioxidant role and is a naturally occurring antioxidant³³ while this is not the case with testosterone.³⁴ Moreover, there are some data on oxidant stress in men showing a prooxidant effect of testosterone.³⁵ This phenomenon may explain the high levels of LPO in exercised men as opposed to women, especially when we take into account that CAT activity was not changed either in untrained or exercised subjects of both sexes. This is supported by the observation that sex steroids may have indirect effects through interaction with other hormones, especially catecholamines. Exercise induced a significantly stronger catecholamine response in men than in women. One of the major effects of catecholamines is stimulation of lipolysis. Catecholamines and autooxidation enhance myocardial and skeletal muscle oxidant metabolism, thereby potentially increasing LPO production, which is associated with heart impairment.³⁶ In contrast, it appears that in women other pathways may be also involved in lowering the level of the oxidation process. One of them is the contribution of micronutrient depletion in exercised women.³⁷ Recent results from our laboratory (unpublished data) have pointed to the need to consider seasonal variations in the oxidant/antioxidant status as well. In this study, we chose to determine CAT activity because it is more likely to be activated in stress conditions than glutathione peroxidase (Gpx) or superoxide dismutase (SOD). Still, the question remains how

to explain the unchanged CAT activity in endurance-trained men and women. In fact, there are many conflicting reports concerning exercise-induced variation in blood antioxidant activity.^{38,39,40} Which antioxidant enzyme and under which conditions an enzyme can be activated is still a controversy. Namely, each of the antioxidant systems may have a different response to exercise, depending upon their biochemical and molecular mechanisms of regulation.⁴¹ However, many studies preceding our research failed to detect marked adaptive responses to chronic exercise, especially during moderate intensity exercise in endurance-trained subjects.^{11,42} Also, Bailey *et al.*⁴³ indicated that oxidant stress may be considered as a biological prerequisite for adaptation to physical stress in men. He suggests that controlled free radicals may prove to be an important component of the signal transduction sequence, which could adjust homeostasis and initiate protective adaptation. Such adaptive response against oxidant stress may constitute a beneficial effect of exercise induced by long endurance training.⁴⁴

The finding presented herein led us to conclude that homeostasis, which is essential for the prooxidant/antioxidant balance, is better regulated in control women as well as during prolonged physical exercise than in (untrained/trained) men. Regarding the oxidation process in fresh *vs.* stored plasma, only in fresh plasma of untrained men and women the TBARS concentration did not change during storage to day 7 at -20°C . In exercised subjects of both sexes, the TBARS concentration decreased significantly (approx. 30 %). Thus, our opinion is that plasma storage for 7 days at -20°C is useful only for LPO determination in untrained samples. Storage-associated alterations in CAT antioxidant activities were much more pronounced than in LPO concentration. According to our results, the degree of CAT activity depletion in stored plasma of untrained and exercised men and women is approximately 40 %. This means that storage of samples at -20°C for determination of CAT activity is definitely not applicable.

The results of oxidant/antioxidant processes in fresh plasma and plasma stored for 7 days at -20°C are very important because of the crucial role of hormones, which may play an essential role in the oxidant/antioxidant status of both sexes. In women, it is time consuming to test each plasma sample separately, since it has to be tested in the same hormone period. Unfortunately, the results of the present study suggest greater reliability of measurements only in fresh due to the significant variation of LPO level and CAT activity in samples stored at -20°C .

In conclusion, although some authors found that oxidant stress was induced in long-term exercise, this did not appear in the exercise regime employed in this study. Despite the absence of oxidant/antioxidant response in exercised subjects, there were evidences of significantly

higher oxidant stress in untrained and exercised men compared to women.

The results of this study, the first study of this kind to be conducted on young men and women during the autumn and winter periods, suggest that a relationship exists between oxidant stress and physiological factors such as sex. Seasonal variation or environmental factors (e.g., cold) have to be taken into consideration when the oxidant/antioxidant status in exercise is tested. Also, the data offer an opportunity to improve causative prevention to withstand supplementing physical activity with/without a diet rich in nutrients or vitamins.

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SAŽETAK

Modulira li dugotrajno vježbanje oksidacijski/antioksidacijski status ljudi? Usporedba između lipidne peroksidacije i aktivnosti katalaze u svježoj i pohranjenoj plazmi

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Tjelesna aktivnost može imati korisne učinke na ravnotežu između oksidativnoga stresa i obrambenoga sustava. Svrha ovog rada je bila procijeniti utjecaj vježbanja na oksidacijski/antioksidacijski status u oba spola tijekom jeseni i zime. Budući da su žene bile istraživane u niskoj estrogenskoj fazi od velike je važnosti mogućnost ispitivanja nekih pokazatelja u plazmi pohranjenoj na niskim temperaturama. Oksidacijske promjene u plazmi određene su mjerenjem koncentracije tiobarbiturno reaktivnih supstanci (TBARS). Antioksidativna sposobnost mjerena je aktivnošću katalaze (CAT). Rezultati su pokazali statistički značajno visoku koncentraciju TBARSa u netreniranih muškaraca u odnosu na žene. Nasuprot tome, antioksidacijska sposobnost bila je iste razine u oba spola. Vježbanje nije promjenilo oksidacijski/antioksidacijski status u oba spola. Rezultati pokazuju da su žene zaštićenije od oksidacijskih procesa od muškaraca. Taj nalaz ukazuje na ulogu lipidne peroksidacije u većoj pojavnosti srčanoga infarkta u muškaraca. Značajne promjene u oksidacijskom/antioksidacijskom statusu u pohranjenoj plazmi dopuštaju veću pouzdanost mjerenja TBARSa i CATa samo u svježoj plazmi.