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LIPID PEROXIDATION AND ANTIOXIDANT ENZYME LEVELS OF INTESTINAL RENAL AND MUSCLE TISSUES AFTER A 60 MINUTES EXERCISE IN TRAINED MICE

ILGI SEMIN, BERKANT MUAMMER KAYATEKIN, SEVIL GÖNENÇ*, OSMAN AÇIKGÖZ, NAZAN UYSAL, YASEMIN DELEN** AND ATAMAN GÜRE

**Department of Physiology, Dokuz Eylül University School of Medicine, Izmir, Turkey*

and

***Department of Biochemistry, Ege University School of Medicine, Izmir, Turkey*

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Abstract : To investigate the effect of blood perfusion difference on oxidant status, mice were trained by a 7-week running program. Two days after the last training session, mice were exercised for 60 minutes at the same training intensity. Changes in the concentration of thiobarbituric acid reactive substance (TBARS), as an index of lipid peroxidation, in intestine, kidney and muscle, were studied in trained mice immediately (0 h), 3 h and 24 h after the running exercise and in unexercised control group. The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and xanthine oxidase (XO) were determined in these tissues. Tissue SOD activities were unaffected by the exercise. Muscle GPx activity increased after exercise (0 h and 3 h group, $P<0.01$) and returned to control levels at 24 h, but there was not any significant difference in intestinal and renal tissues. Renal tissue XO activity could not be determined. There was not any significant difference among groups in intestinal tissue XO activity. The activity of XO was decreased only in skeletal muscle at 0 h ($P<0.05$). TBARS levels of exercised groups were higher than control in muscle ($P<0.01$). Intestinal TBARS levels decreased at 0 h ($P<0.05$), than reached to control level. Renal TBARS levels of 0 h and 24 h group was higher than control ($P<0.01$, $P<0.01$ respectively). The results show that a long distance running exercise may cause lipid peroxidation damage in skeletal muscle and kidney.

Key words : free radicals, mice, superoxide dismutase, glutathione peroxidase, xanthine oxidase, thiobarbituric acid reactive substance, exercise

*Corresponding Author

INTRODUCTION

Strenuous exercise is characterized by increased oxygen consumption and the disturbance between intracellular pro-xidant and antioxidant homeostasis (1). Increased energy demand during physical exercise, especially of the aerobic type, necessitates a multifold increase in oxygen supply to active tissues. The rate of oxygen uptake by the body during exercise may increase by 10 to 15 fold. Oxygen flux in the active peripheral skeletal muscle tissue may, however, increase by ~ 100-fold with an ~ 30 fold increase in blood flow and 3-fold increase in arteriovenous oxygen difference (2). Therefore, during exercise, there is great blood perfusion disturbances among tissues. Skeletal muscle perfusion is increased, while splanchnic area perfusion is decreased and then they return to normal levels (3, 4). In other words, ischemia/ reperfusion may occur in splanchnic area.

Gastrointestinal disturbances like pain, diarrhea, melena (5, 6, 7, 8) and hematuria (9, 10) are reported in endurance sportsmen, especially 1–2 days after a marathon. Running-induced bleeding may be due to transient gut ischemia, because during vigorous exercise splanchnic perfusion decreases by as much 80% (3). Furthermore, diarrhea and other abdominal complaints common during or after long-distance running have been attributed to splanchnic ischemia (5, 6).

Strenuous physical exercise induces oxidative damage to lipids in various tissues (2). Free-radical-mediated events are believed to be involved in ischemia-reperfusion injury in skeletal muscle, heart, kidney, pancreas, small intestine, brain, and skin (11, 12). Xanthine oxidase (XO) is one of the possible sources for oxygen free radicals, mainly investigated under conditions of ischmia/reperfusion (13). Primary components of the physiological antioxidant defence are superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). SOD catalyzes the dismutation of superoxide to O_2 and H_2O_2, which catalase converts to water and O_2. GPx can reduce H_2O_2 to form glutathione disulfide and water (14, 15). In human, skeletal muscle antioxidant defence is poor, thus rendering this tissue highly susceptible to oxidative stress. It is suggested that, the increase in energy metabolism by aerobic performance enhance the intracellular concentration of oxygen free radicals, which in turn enhance the rate of the process of lipid peroxidation, inducing damage in muscular structures (2).

The present study was designed to determine the effects of exercise on the antioxidant enzymatic system, OX, and lipid peroxidation in muscle, intestine and kidney, during the post-exercise period in trained mice.

METHODS

Male Swiss Albino mice (n = 31), weighting 29.8 ± 3.0 g, were used. Training and running exercises were performed on four small animal treadmills. All procedures were carried out between 9^{00} and 12^{00} a.m. 31 mice were randomly assigned to one four groups; unexercised control (n = 8), cervical dislocation immediately after exercise (0 h, n = 8), 3 hours after exercise (3 h, n = 8), and 24 hours after exercise (24 h, n = 7). Mice were ran for 20 m/min, 5 degrees slope, 5 min/session, 5 daily sessions/week for a week to adapt running. Then, the slope kept similar but the duration gradually increased; second week

15 min/session, 3rd and 4th weeks 20 min/session, 5th, 6th and 7th weeks 30 min/session. Control animals were ran for 20 m/min, 5 degrees slope, 5 min/session, 1 day/week to stress them.

Two days after the last training session, trained animals were ran on the treadmill for 60 min at 20 m/min, 5 degrees slope. The animals were killed by dislocation, immediately, 3 hours and 24 hours after the acute exercise. Untrained animals were killed without running exercise. Their gastrocnemius muscle, proximal small intestinal and renal tissues were quickly removed. Tissues were washed in cold homogenate medium and visible clots removed to minimise blood contamination. Tissue homognates were prepared as described by Carrillo et al. (16). An aliquot of the homogenate and supernatant was stored at –70°C until the determination of enzyme activities and thiobarbituric acid reactive substance (TBARS) levels which is lipid peroxidation marker.

SOD activity was determined using a Randox test combination (RANSOD). GPx was determined using a Randox test combination (RANSEL) (17). XO activity was determined according to the spectroscopic method of Majkic-Singh et al. (18). TBARS level was estimated according to the method of Rehncrona et al. (19). Protein contents of supernatant and homogenate were determined according to the method of Markwell et al. (20).

Results are presented as means ± S.E. Statistical analysis of the data was performed using Mann-Whitney U test.

RESULTS

Muscle GPx activity increased after exercise (0 h and 3 h groups $P<0.01$) and

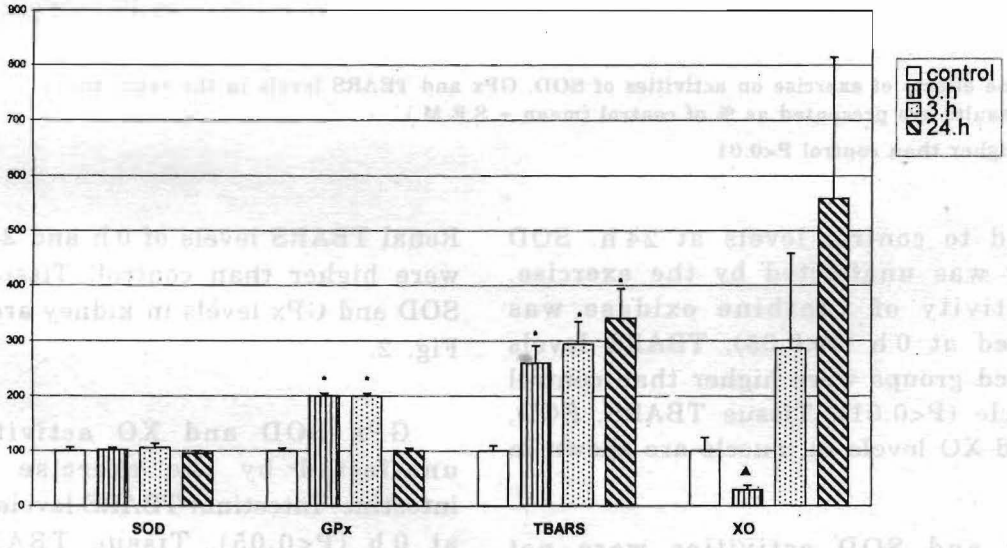

Fig. 1: The effects of exercise on activities of SOD, GPx, XO and TBARS levels in the skeletal muscle tissue. Results are presented as % of control (mean ± S.E.M.)
*higher than control $P<0.01$
▲lower than control $P<0.05$

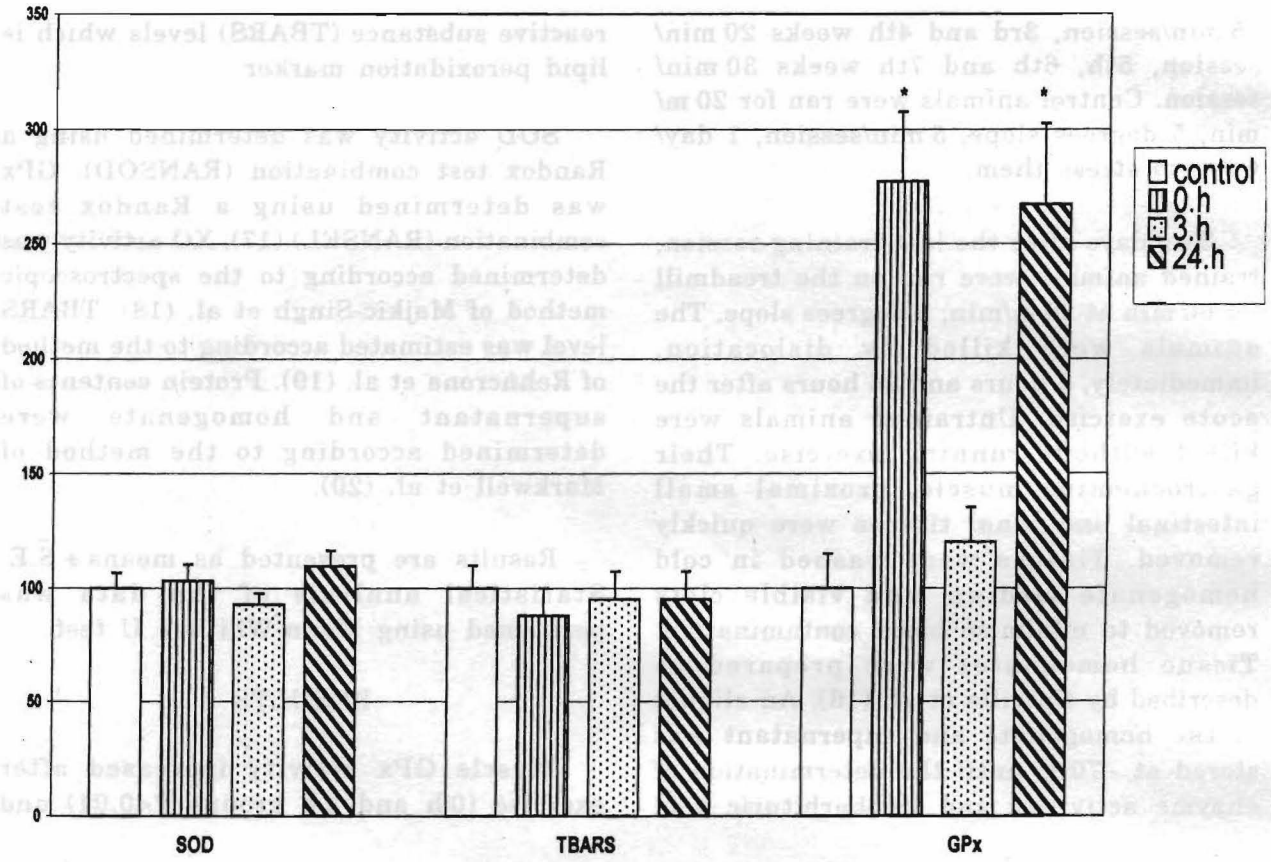

Fig. 2: The effects of exercise on activities of SOD, GPx and TBARS levels in the renal tissue. Results are presented as % of control (mean ± S.E.M.)

*Higher than control P<0.01

returned to control levels at 24 h. SOD activity was unaffected by the exercise. The activity of xanthine oxidase was decreased at 0 h (P<0.05). TBARS levels of trained groups were higher than control in muscle (P<0.01). Tissue TBARS, SOD, GPx and XO levels in muscle are shown in Fig. 1.

GPx and SOD activities were not changed by the exercise in kidney. Renal tissue XO activity could not be determined. Renal TBARS levels of 0 h and 24 h groups were higher than control. Tissue TBARS, SOD and GPx levels in kidney are shown in Fig. 2.

GPx SOD and XO activities were unaffected by the exercise in small intestine. Intestinal TBARS levels decreased at 0 h (P<0.05). Tissue TBARS, SOD, GPx and XO levels in intestine are shown in Fig. 3.

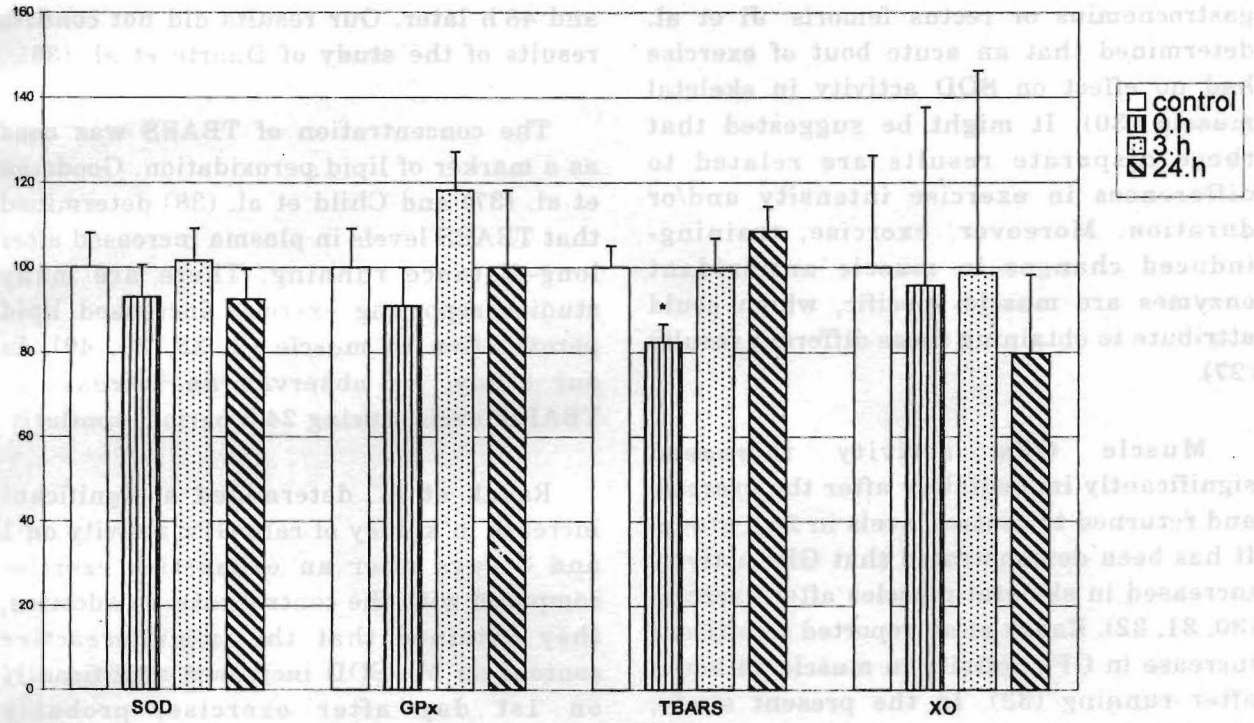

Fig. 3: The effects of exercise on activities of SOD, GPx, and TBARS levels in the intestinal tissue. Results are presented as % of control (mean ± S.E.M.)

*Lower than control P<0.01

DISCUSSION

It is generally accepted that exercise results in increased reactive oxygen species (ROS) production in skeletal muscle. These ROS generally have a toxic action on tissues. Given the potential role of these reactive species in mediating muscular dysfunction, it is not surprising that cells contain several naturally occurring defence mechanism to prevent oxidative injury. These protective mechanisms include the enzymes SOD, GPx and catalase (2, 21, 22).

The results from the present investigation demonstrated that muscle SOD activity was unaffected by exercise. Laughlin et al. showed that sixty minutes of ischemia followed by 60 min of reperfusion had no effect on SOD activities in any of the exercise trained rats skeletal muscles sampled (23). Studies by Ji et al. (24). Alessio and Goldfard (25) found no evidence of exercise training-induced upregulation of muscle SOD activity. Ji et al. determined that SOD activity in the vastus lateralis muscle was not altered significantly by training (26). In opposition, Powers et al. (27), Higuchi et al. (28) and Jenkins (22) have reported an increased SOD activity in skeletal muscle with training. Criswell et al. determined that SOD activity in the soleus was significantly higher in exercise trained groups compared with controls (29). However, SOD activity did not differ between groups for either

gastrocnemius or rectus femoris. Ji et al. determined that an acute bout of exercise had no effect on SOD activity in skeletal muscle (30). It might be suggested that these disparate results are related to differences in exercise intensity and/or duration. Moreover, exercise, training-induced changes in muscle antioxidant enzymes are muscle specific, which could attribute to obtaining these different results (27).

Muscle GPx activity increased significantly immediately after the exercise and returned to control levels in 24 h group. It has been demonstrated that GPx activity increased in skeletal muscles after exercise (30, 31, 32). Radak et al. reported significant increase in GPx activity in muscle 24 hours after running (33). In the present study, the exercise intensity and duration are could be different than other studies. Therefore, the time course to return to normal levels might be shorter.

It has been suggested that the enzyme XO may be an important source of reactive oxygen metabolities during reperfusion (12). The fact that XO appears to b localized predominantly in the microvascular endothelial cells of skeletal muscle might allow this enzyme to play a role in ischemia/ reperfusion-induced microvascular injury, despite the low activity measured in whole muscle homogenated (34, 35). We have observed that the activity of XO was decreased in skeletal muscle at 0 h ($P<0.05$). Durate et al. suggested that endothelium-derived oxidative stress (probably XO) may contribute to exercise-induced muscle damage, which was most pronounced immediately after a single bout of exercise and 48 h later. Our results did not confirm results of the study of Duarte et al. (36).

The concentration of TBARS was used as a marker of lipid peroxidation. Goodman et al. (37) and Child et al. (38) determined that TBARS levels in plasma increased after long-distance running. There are many studies reporting exercise increased lipid peroxidation in muscle (2, 33, 39, 40). In our study, we observed an increase in TBARS levels, during 24 h period, similarly.

Radak et al. determined a significant increase in kidney of rats GPx activity on 1 and 3 days after an exhaustive exercise compared with the control rats. In addition, they obtained that the immunoreactive content on Mn-SOD increased significantly on 1st day after exercise, probably indicating an increase in mitochondrial superoxide formation. However, the immunreactive Cu, Zn-SOD content of renal tissue was unchanged throughout the experiment. They observed that there was no meaningful change in XO of the renal tissue throughout the period of the experiment. They determined that the exercise induced a significant increase in TBARS concentration on 3rd day after exercise (41). In the present study, we could not find any significant difference in kidney antioxidant enzyme activities, possible because exhaustive exercise was not applied. However, TBARS levels of renal tissue in 0 h and 24 h groups were higher than control. Exercise has been shown to induce a several fold increase in plasma XO (33) and this circulating XO could induce oxidative stress to the filtrating renal tissue and probably cause an increase in the TBARS level at 0 h. TBARS increase found

at 24 h in our study, can be explained by filtrating of increased XO in another tissue from renal tissue. In present study, XO activity gradually increased at 24 h but this increase was not significant.

Formation of toxic oxygen metabolites has been suggested to play an important role in the development of damage during ischemia/reperfusion injury (42). Malondialdehyde (MDA) is the end product of lipid peroxidation and is a well-known parameter for determining the increased free radical formation in intestinal tissue (43). It was reported that increased lipid peroxidation after reoxygenation of ischemic intestinal tissue (44, 45, 46, 47).

We could not observe any significant difference in antioxidant enzyme activities and XO activities of intestinal tissue which is inactive during exercise. TBARS level of the intestinal tissue decreased first, and then it reached to control level. Decrease in TBARS level right after exercise may be due to the markedly decrease in intestinal blood flow. TBARS level's increase to control level in post-exercise period can be explained by intestinal blood flow's return to normal. In this study, the reasons for not observing lipid peroxidation in intestine may be due to the less intensity of ischemia/reperfusion produced by exercise than the intensity produced by means of clamping mesenteric arteria which is a common method used in most studies.

As a conclusion, after a 60-minute exercise with trained mice, lipid peroxidation was observed in skeletal muscle and kidney tissues throughout 24 hours. However, lipid peroxidation was not observed in proximal small intestine tissue. This indicates, while muscle and kidney tissues whose perfusion changes during exercise are at risk of oxidative damage, in proximal small intestine whose perfusion also changes during exercise, no oxidative influence was observed. These findings show that, exercise-induced oxidative damage in renal tissue may play a role in the ethiology of hematuria seen after running in endurance sports.

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