

Endurance Exercise Reduces Oxidative Stress in Mice

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Abstract. [Purpose] The purpose of this study was to investigate the effects of endurance exercise on glutathione in mice. [Subjects] Twelve mice were randomly assigned to one of two groups: the endurance exercise group (n=6), and the control group (n=6). [Methods] The exercise group ran on a motor driven treadmill 5 days per week for 30 minutes at a speed of 24 m·min⁻¹ for 4 weeks. The ratio of GSH to GSSG was measured as an indicator of cardiac oxidative stress. The independent t test was used for statistical analysis. [Results] The results show that the endurance exercise significantly increased the ratio of GSH to GSSG in the exercised mice compared to the sedentary mice. [Conclusion] The results indicate that the four weeks of the endurance exercise reduced oxidative stress levels in mice.

Key words: Oxidative Stress, Endurance Exercise, Glutathione

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INTRODUCTION

Glutathione (GSH) is one of the primary components of the physiological antioxidative defense system¹⁾ and nonenzymatic antioxidants in muscle fibers²⁾. GSH is a nonprotein thiol that is crucial for protecting the heart against hydrogen peroxide (H₂O₂) induced damage³⁾ because it scavenges hydroxyl radicals (OH·)⁴⁾. However, the source of cardiac GSH loss has not been completely elucidated. Oxidative stress can be defined as a measure of the steady state of reactive oxygen species (ROS), or oxygen radicals and indicates a failure of balance between the pro-oxidant and antioxidant systems^{1,5)}. Mitochondria are capable of producing ROS, including superoxide (O₂⁻) and OH·, as well as H₂O₂ when the rate of oxygen consumption is raised. ROS are known to have a wide variety of pathophysiological effects. These ROS promote lipid peroxidation resulting in disturbance of cation homeostasis and cellular damage⁶⁾. Lipid peroxidation of cell membranes results in decreased membrane fluidity, inability to maintain ionic gradients, cellular swelling, and tissue inflammation⁷⁾. Endurance exercise training is believed to play a key role in enhancing antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPX), reducing the oxidative stress of exercise. Starnes, Barnes, and Olesen examined whether endurance-trained rats' hearts would have decreased ROS production. They found that trained rats had a higher ability to prevent free radical generation than untrained rats⁸⁾. A similar study by

Venditti and Di Meo investigated the long-term effects of swimming training effects on antioxidant level as measured by GPX and GR. Their results showed that there was a significant difference between trained and untrained rats in the level of GPX and GR in the heart⁹⁾. Moreover, endurance training has been proposed to have beneficial effects on oxidative phosphorylation through increased mitochondrial biogenesis and reduced oxidative stress levels caused by decreased ROS¹⁰⁾. In that study, mice with mitochondrial myopathy, which is one of the most commonly inherited neurological disorders were used. The results indicated that adenosine triphosphate (ATP) levels were increased in exercised mice compared with sedentary myopathic animals, delayed the onset of myopathy and prolonged their lifespan.

Leeuwenburgh et al., investigated how endurance training altered antioxidant enzyme activity and GSH levels. They found that there was a decrease in GSH concentration with training, therefore a reduction of the GSH to GSSG ratio, indicating oxidative stress increased due to long-term exercise¹¹⁾. A similar study by Tonkonogi et al., examined the effects of endurance training and oxidative stress on the mitochondrial function in humans¹²⁾. The results of their study showed that there was a consistent level of skeletal muscle SOD and GPX activity, likely due to the short period of exercise training. In the present study we explored how the cardiac oxidative stress level of male mice was changed by short term endurance exercise training.

Table 1. GSH: GSSG Ratio of Exercised and Sedentary Mice

Variable	Exercise (n=6)	Sedentary (n=6)
GSH: GSSG (nmol/g)	0.606 ± 0.115*	0.235 ± 0.025
Run Time (min)	48.5 ± 1.455*	37.0 ± 1.897

Note. Values are mean ± S.E.M. * Significantly different from the sedentary (p<0.05).

SUBJECTS AND METHODS

Twelve, 15-week old wild-type male mice (n=12) 129 SvJ/C57BL6 mice (LCAD+/+) were used for this study. The mice were divided into two experimental groups: 1) Exercised, n=6) and 2) Sedentary, n=6. The animals were housed six animals per cage and had access to laboratory rodent chow and water *ad libitum*. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Arkansas at Fayetteville.

The exercised group ran on a motor driven treadmill 5 days per week for 30 minutes at a speed of 24 m·min⁻¹ for 4 weeks. Each training session began and ended with a 5-minute warm-up and cool down, respectively, at 10 m/min. On the fifth day of each week, body weights were measured and recorded.

During the fifth week of this study, the mice were randomly selected for single bout of exhaustive exercise. When the mouse was unable to keep up with the treadmill, the exercise session was terminated. The sedentary experimental groups were left sedentary for the entire four weeks before undergoing the exhaustive bout of exercise. Twenty four hours after the exhaustive exercise, the mice were transported to the laboratory for data collection.

Using a Glutathione Colormetric Detection Kit (BioVision, Mountain View, CA, USA), cardiac mitochondrial GSH and GSSG were measured. Fifty milligrams of heart tissue were homogenized in 0.2 ml of glutathione reaction buffer containing 100 µl of 5% sulfosalicylic acid (SSA). Then, the sample was centrifuged at 8000 × g for 10 minutes. Following centrifugation, the supernatant was transferred to a new tube and used for the glutathione assay. Twenty microliters of NADPH generating mix, 20 microliters of glutathione reductase, and 120 microliters of glutathione reaction buffer were mixed. One hundred sixty microliters of the reaction mixture was added to each well and incubated at room temperature for 10 minutes to generate NADPH. Then 20 µl of either the GSH standard solutions or the sample solution was added before incubation at room temperature for 5 to 10 minutes. After 20 µl of substrate solution was added, the sample plate was incubated at room temperature for 5 to 10 minutes. The absorbance at 405 nm was read by a microplate reader.

Values are presented as mean ± S.E.M. The independent t test was used for statistical analysis. A p value of <0.05 was considered significant.

RESULTS

Table 1 demonstrates the ratio of GSH to GSSG and run time to exhaustion. The exercised mice had a higher mean ratio of GSH to GSSG than the sedentary mice (p<0.05). The exercised mice ran significantly longer than the sedentary mice (p<0.05).

DISCUSSION

Oxidative stress is a condition in which there is an elevation of oxidants compared to antioxidants in cells. GSH's best known role in cells is to scavenge free radicals. Higher levels of GSH in the cells indicate reduced oxidative stress levels¹³. These cells confer greater protection against attacking free radicals during physical activity and at rest. Cardiac tissue is vulnerable to attack by ROS, which are unstable oxygen-containing molecules, due to their ability to alter mitochondrial membranes¹⁴. In this study, the training affected the ratio of GSH to GSSG, meaning that the hearts of the exercised mice had a significantly higher the ratio of GSH to GSSG compared to the sedentary mice, which was expected. In other words, the sedentary mice had a higher level of GSSG. This higher level of GSSG in cells indicates lower antioxidants, meaning that cells with a higher GSSG level will be easily attacked by free radicals. As a result, the normal function of the cells might be damaged.

In this study, it should be noted that the exercised mice had higher GSH levels and a higher ratio of GSH to GSSG than the sedentary mice. This is consistent with the results of Leeuwenburgh and Ji¹⁵. They reported that GSH and the ratio of GSH to GSSG in skeletal muscle were increased. Sun et al., examined the effects of endurance training on oxidative stress, as measured GSH. They concluded that reduced GSH increased in the liver mitochondria after training¹⁶. Therefore, it is reasonable to conclude that the four weeks of treadmill running in the present study enhanced the oxidative capacity of the heart in the exercised mice.

It is imperative for working muscle to have efficient oxygen delivery to perform better in endurance types of exercise. As expected, the trained mice also ran longer than the sedentary mice. It should be noted that the endurance training enhanced fatty acids, oxidation as evidenced by increased mitochondrial oxygen consumption in the wild-type mice and improved their running performance on the treadmill.

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