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### **Effects of Sprint Training on Glutathione Expression in Liver and Skeletal Muscle**

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##### ABSTRACT

**McCartney M, Keesee C, Fletcher L, Stover S**. Effects of Sprint Training on Glutathione Expression in Liver and Skeletal Muscle. **JEPonline**2011;14(5):66-74. Acute anaerobic exercise promotes oxidative stress. However, previous studies indicate that high intensity sprint training can attenuate the effects of oxidative stress. It was hypothesized that an upregulation of reduced glutathione (GSH) is responsible for the protection against oxidative stress associated with sprint training. Thirty-three mice were randomly divided into three groups: control, acute, and trained. Trained mice participated in a high intensity exercise program consisting of treadmill running two days a week for 12 weeks. At the end of the training period, trained and acute mice engaged in a single session of intense sprinting. Control mice did not exercise. Concentrations of GSH and oxidized glutathione (GSSG) were determined spectrophotometrically in the soleus and extensor digitorum longus (EDL) muscles, as well as the liver. Following acute exercise, GSH concentrations (mol/g tissue) in the soleus and EDL decreased significantly, relative to their respective controls. At the same time, GSSG concentrations (mol/g tissue) in both muscles increased significantly. After 12 weeks of sprint training, GSH concentrations in the soleus and EDL were not significantly different from their respective controls, even as GSSG concentrations in the muscles increased significantly. Results of the study failed to demonstrate any change in liver glutathione after acute sprinting or sprint training. Data indicate that control levels of GSH can be recovered by skeletal muscle as a result of high intensity sprint training. However, the upregulated GSH does not appear to be imported from the liver.

**Key Words**: Anaerobic exercise, Oxidative stress

**INTRODUCTION**

The generation of reactive oxygen species (ROS) such as singlet oxygen, superoxide radical, and hydroxyl radical occurs as a consequence of normal cellular metabolism (27). ROS-related molecular damage includes DNA strand breaks and single base modifications (11), oxidation of amino acid side chains and fragmentation of polypeptides (20), and the degradation of polyunsaturated fatty acids and phospholipids by lipid peroxidation (4). Processing of ROS is carried out by the body’s endogenous antioxidant defense system, which includes enzymatic activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR), in conjunction with exogenous antioxidants consumed through diet (27). Oxidative stress may be defined as a condition in which the cellular production of ROS exceeds the body’s physiological capacity to render it inactive (4).

The increase in oxygen uptake during aerobic exercise is accompanied by an elevation in ROS. Acute aerobic exercise generates ROS by creating a disturbance in electron transport that leads to excessive leakage of superoxide radicals (4). However, long-term endurance training effectively reduces the damage associated with increased oxygen uptake by enhancing the body’s antioxidant defenses. It has been demonstrated that GPx (30), GR (30), and SOD (7,19) activities increase in response to endurance training.

Acute anaerobic exercise can also promote oxidative stress. In rats, a single 1-min sprint at 45 m•min-1 elevates lipid hydroperoxides and thiobarbituric acid reactive substances in skeletal muscle, indicating significant lipid peroxidation (1). In mice, six 30-sec sprints at a pace of 30 m•min-1 significantly increase the concentration of malondialdehyde (MDA), a lipid peroxidation marker, in skeletal muscle (6). In humans, six 150 m sprints significantly increase plasma levels of MDA (22). Furthermore, previous studies indicate that sprint training can attenuate the effects of oxidative stress. In rats, GPx and GR activities increase significantly in cardiac and skeletal muscle following sprint training (3). In mice, sprint training reduces lipid peroxidation in skeletal muscle, as indicated by a decrease in MDA concentration (6). In humans, sprint training produces a decrease in plasma concentrations of MDA and protein carbonyls, both biomarkers of oxidative stress, when compared to untrained subjects (5).

Reduced glutathione (GSH) plays a prominent role in the cellular defense against oxidative stress by scavenging ROS, both directly and as a substrate for GPx (9). Previous research has demonstrated that strenuous physical exercise can affect GSH homeostasis by decreasing its tissue concentration, disturbing its cellular redox status, and interfering with its synthesis and transport (14,15). Furthermore, studies (13,21,26) suggest that endogenous cellular GSH is not sufficient to withstand the increased oxidation associated with vigorous exercise. It would therefore be beneficial to increase levels of cellular GSH to provide protection against exercise-induced oxidative stress.

The present study investigated the hypothesis that an upregulation of GSH is responsible for the protection against oxidative stress associated with sprint training. If the hypothesis is true, then GSH levels should decrease, while levels of oxidized glutathione (glutathione disulfide, or GSSG) increase, in skeletal muscle following a single oxidative stress-generating bout of high intensity sprinting. Furthermore, sufficient GSH should be recovered in skeletal muscle following long-term, high intensity sprint training to provide the protection against oxidative stress demonstrated by previous studies (3,5,6). Since the liver is the primary location of *de novo* glutathione synthesis (8), it is hypothesized that the liver will export significant amounts of GSH to the plasma and, eventually, skeletal muscles during acute exercise. Finally, post-training levels of GSH in the liver should recover as a result of increased *de novo* synthesis.

###### METHODS

**Animals**

The Institutional Animal Care and Use Committee of Davis & Elkins College approved this study. Thirty-three male albino ICR (CD-1®) mice (Harlan, Indianapolis, IN), 5 to 7 weeks old when the study began, were housed individually in ventilated cages (Maxi-Miser Positive Individual Ventilation System, Thoren Caging Systems, Inc., Hazelton, PA). The caging system was located in a room maintained at 16 to 22°C with 12:12 light-dark cycles. All mice had free access to water and food (Fortified Diet for Rats and Mice, Kaytee Products, Inc., Chilton, WI). The 33 mice were randomly divided into three groups: control (n = 9), acute (n = 11), and trained (n = 13).

**Sprint Training**

**Table 1. The mouse training schedule.**

|  |  |  |  |
| --- | --- | --- | --- |
| Training Week | Number of Sprints | Speed (m•min-1) | Incline (°) |
| 1-3 | 3 | 24 | 5 |
| 4 | 4 | 24 | 5 |
| 5-6 | 4 | 27 | 10 |
| 7-8 | 5 | 27 | 10 |
| 9 | 5 | 30 | 15 |
| 10-12 | 6 | 30 | 15 |

Trained mice participated in a high intensity exercise program consisting of treadmill running 2 d•wk-1 for 12 weeks. Each session included three to six 30-sec sprints at a pace of 24 to 30 m•min-1 (5 to 15° incline; refer to Table 1), with a 1-min recovery interval between each sprint. Control and acute mice were not involved in the training process. An electrified grid (0.1 mA) at the rear of treadmill was used sparingly as motivation to run. All exercise procedures were carried out between 8:00 a.m. and 9:00 a.m.

**Acute Sprinting**

At the end of the training period, trained and acute mice engaged in six consecutive 30-sec sprints on a rodent treadmill at a pace of 24 to 30 m•min-1 (15° incline), with a 1-min recovery interval between each sprint. Control mice did not exercise. An electrified grid (0.1 mA) at the rear of the treadmill was used sparingly as motivation to run. All exercise procedures were carried out between 8:00 a.m. and 9:00 a.m.

**GSH/GSSG Assessment**

The mice were sacrificed by cervical dislocation. Sacrifice of exercised animals was carried out immediately after the final 1-min recovery interval. For 22 mice (5 control, 8 acute, and 9 trained), the soleus and extensor digitorum longus (EDL) muscles were extracted, rinsed with cold distilled water (dH2O), homogenized in 20 mM Tris-HCl buffer (pH 7.4), and reacted with 5,5’-dithiobis-2-nitrobenzoic acid (DTNB) at room temperature. For the other 11 mice (4 control, 3 acute, and 4 trained), the liver was extracted and processed in the same fashion. DTNB combines with glutathione to generate a product with maximal absorbance at 412 nm (Cuvette Assay for GSH/GSSG, Oxford Biomedical Research, Oxford, MI). The assay employs a pyridine derivative as a thiol scavenger for GSSG samples. The scavenger reacts quickly to maintain glutathione in its oxidized state. Experimental samples and controls were analyzed spectrophotometrically at 412 nm. Total glutathione (GSHt) and GSSG concentrations were calculated from the absorbance values using the following formula: [GSHt/GSSG] = [(ΔA – ΔB) – *b*] ÷ *a* × *df*, where [GSHt/GSSG] is the μM concentration of either GSHt or GSSG in the sample, ΔA is the change in absorbance of the sample at 412 nm over 10 min, ΔB is the change in absorbance of the blank (dH2O) at 412 nm over 10 min, *a* is the slope of either the GSH or GSSG standard curve (standards were included in the assay kit), *b* is the intercept of the standard curve, and *df* is the dilution factor of the sample. Concentrations of reduced glutathione (GSH) were estimated by subtracting GSSG values from GSHt values.

**Statistical Analyses**

Data were analyzed with a one-way analysis of variance (ANOVA). Fisher’s least significant difference test was employed to compare specific groups in the ANOVA. An alpha level of P≤0.05 was regarded as statisticallysignificant. Data are expressed as mean ± standard deviation.

RESULTS

**Acute Sprinting**

Acute sprinting did not significantly alter GSH and GSSG concentrations (mol/g tissue) in the liver (Figure 1). Acute liver samples had mean GSH and GSSG concentrations of 3.17 ± 0.044 and 0.332 ± 0.009, respectively. Control samples had mean concentrations of 3.35 ± 0.091 and 0.32 ± 0.003, respectively. Following acute exercise, GSH concentrations (mol/g tissue) in the soleus (0.478 ± 0.059) and EDL (0.442 ± 0.125) decreased significantly, relative to their respective controls (0.619 ± 0.021 and 0.566 ± 0.02; Figure 2). At the same time, GSSG concentrations (mol/g tissue) in the soleus (0.077 ± 0.011) and EDL (0.084 ± 0.011) increased significantly, relative to their respective controls (0.055 ± 0.005 and 0.062 ± 0.003; Figure 3).

Figure 1. Effects of exercise on GSH and GSSG concentration in the liver.

Values represent mean ± standard deviation.

**Sprint Training**

Sprint training did not significantly alter GSH and GSSG concentrations (mol/g tissue) in the liver (Figure 1). Trained liver samples had mean GSH and GSSG concentrations of 3.213 ± 0.064 and 0.36 ± 0.009, respectively. Control samples had mean concentrations of 3.35 ± 0.091 and 0.32 ± 0.003, respectively. Following 12 weeks of sprint training, GSH concentrations (mol/g tissue) in the soleus (0.608 ± 0.048) and EDL (0.557 ± 0.035) were not significantly different from their respective controls (0.619 ± 0.021 and 0.566 ± 0.02; Figure 2). At the same time, GSSG concentrations (mol/g tissue) in the soleus (0.086 ± 0.017) and EDL (0.087 ± 0.008) increased significantly, relative to their respective controls (0.055 ± 0.005 and 0.062 ± 0.003; Figure 3).

Figure 2. Effects of exercise on GSH concentration in the soleus and EDL.

Values represent mean ± standard deviation; \*Significantly different from control

(P≤0.05).

Figure 3. Effects of exercise on GSSG concentration in the soleus and EDL.

Values represent mean ± standard deviation; \*Significantly different from control

(P≤0.05).

DISCUSSION

In the present study, trained and untrained mice were engaged in an acute anaerobic exercise session, consisting of 6 consecutive 30-sec sprints on a rodent treadmill at a pace of 24 to 30 m•min-1 (15° incline), with a 1-min recovery interval between each sprint. It has been previously demonstrated that this protocol effectively induces lipid peroxidation in fast-twitch skeletal muscle, as indicated by a significant increase in MDA concentration in the EDL (6). However, the mechanism by which acute anaerobic exercise leads to oxidative stress remains unclear. It has been suggested that glycolytic power peaks very early during high intensity exercise, and that it is followed by significant mitochondrial ATP synthesis (24,29). In addition to the contribution of aerobic metabolism, and the subsequent disturbance in electron transport, the elevation in ROS generated by sprint exercise may also be affected by ischemia/reperfusion. The sudden uptake of oxygen following a sprint may react with accumulated metabolic intermediates to generate elevated levels of ROS (4,12).

Beneficial effects of high intensity sprint training have also been established by previous research. Lipid peroxidation, as indicated by MDA concentration, is significantly decreased in the EDL after 12 weeks of sprint training (6). The mechanism behind the peroxidation reduction is unclear. It is possible that the beneficial effect is due to an increased capacity for oxidative phosphorylation. Previous studies have confirmed increased mitochondrial mass in response to conditions of oxidative stress (16,17). Furthermore, an increase in GSH may contribute to the training adaptation. Endurance training has been shown to increase GSH content in hind limb muscles of dogs (15) and rats (19). High intensity sprint training may produce a similar effect.

GSH plays a prominent role in the cellular defense against oxidative stress by scavenging ROS, both directly and as a substrate for GPx (9). Conversion of GSH to GSSG is catalyzed by GPx during the reductive detoxification of hydrogen peroxide (23). GSH is regenerated from GSSG by activity of GR (25). Although some epithelial cells have the capacity to take up intact GSH (10), most cells rely on *de novo* synthesis to maintain cellular stores. GSH is synthesized by a two-step process involving the enzymes -glutamate-cysteine ligase (GCL) and glutathione synthetase (GS). GCL catalyzes the intracellular reaction that results in attachment of a sulfhydryl-containing cysteine residue to a glutamate residue. GS activity adds a glycine residue to complete the tripeptide (9).

Results of the current study demonstrate a significant decrease in GSH concentration, accompanied by an increase in GSSG, in both the soleus and EDL of untrained mice after an acute sprinting session. Following 12 weeks of training, however, GSH concentration recovers to control levels in both muscles, even though GSSG concentrations remain significantly elevated compared to corresponding controls (Figures 2 and 3). These results suggest an increase in GSH synthesis as a response to the sprint training. An analysis of GCL activity, the limiting factor in GSH synthesis, will be necessary to confirm this hypothesis.

No significant difference was found between soleus and EDL muscles in any of the groups, despite the fact that the soleus is traditionally considered a slow-twitch, oxidative muscle, while the EDL is deemed to be fast-twitch, with more of a glycolytic metabolism. In rats, there is a clear distinction between slow- and fast-twitch muscles, in terms of fiber type composition. In the inbred Lewis strain, for example, soleus muscles are 96% type I oxidative fibers, whereas EDL muscles are roughly 76% type IIb glycolytic fibers, based on ATPase histochemistry (28). In mice, however, there is much more variation between strains, with more fiber type mixing. In the ICR strain, for example, ATPase histochemistry has demonstrated that soleus muscles are only 66% type I fibers, while EDL muscles are about 67% type IIb (2).

Liver is the primary location for *de novo* GSH synthesis (8). It has been demonstrated that liver GSH concentration decreases after an acute bout of endurance exercise (18,21). This decrease could be due to the fact that GSH is used by GPx as a substrate and is oxidized to GSSG by hydroperoxides, which are increased during exercise (13). Results of the current study failed to demonstrate any change in liver glutathione after acute sprinting or 12 weeks of sprint training (Figure 1). Since both the reduced and oxidized forms of glutathione remained unchanged in the liver, it is possible that the sprinting protocols failed to generate sufficient hydroperoxides to convert GSH to GSSG. Skeletal muscle may be synthesizing sufficient amounts of GSH itself to compensate for exercise-induced oxidative stress. It is also possible that the liver is exporting newly synthesized GSH to the muscles in concentrations that are too small, relative to total concentration, to detect with the current assay.

### **CONCLUSIONS**

The results of the present study demonstrate a significant decrease in GSH concentration in skeletal muscle following acute sprinting. Furthermore, the data indicate that control levels of GSH can be recovered as a result of high intensity sprint training. However, the upregulated GSH does not appear to be imported from the liver.

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