Alteration of Glutathione and Antioxidant Status with Exercise in Unfed and Refed Rats\(^1,2\)

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ABSTRACT The influences of food deprivation and refeeding on glutathione (GSH) status, antioxidant enzyme activity and lipid peroxidation in response to an acute bout of exercise were investigated in the liver and skeletal muscles of male Sprague-Dawley rats randomly divided into three groups: starved for 48 h without refeeding; starved for 48 h and refed for 24 or 48 h. Half of each group of rats was exercised on a treadmill until exhaustion and killed immediately, whereas the other half group was killed at rest. Food-deprived rats had significantly lower liver GSH concentration and GSH:glutathione disulfide (GSSG) ratio. Malondialdehyde concentrations in the liver and skeletal muscle were both higher in the starved than in the refed rats \((P < 0.05)\). Refed rats had significantly greater liver GSH level, \(\gamma\)-glutamylcysteine synthetase and glucose 6-phosphate dehydrogenase activities and plasma insulin concentration than unfed rats. Exercised 24- and 48-h refed rats had 27% and 31% lower liver GSH \((P < 0.05)\), respectively, and a 21% lower GSH:GSSG ratio \((P < 0.05)\) than their rested counterparts. Plasma insulin concentrations were significantly lower, whereas glucagon concentrations were greater in the exercised than in the rested rats. Muscle GSH concentration was significantly lower in the food-deprived than in the refed rats \((P < 0.05)\) but was unaffected by exercise. Exercised 24-h refed rats had significantly elevated muscle GSSG concentration compared with rested rats, along with a higher GSH peroxidase and a lower \(\gamma\)-glutamyltranspeptidase activity \((P < 0.05)\). These data indicate that both food deprivation-refeeding and exhaustive exercise influence liver and skeletal muscle glutathione status and that these changes may be controlled by hepatic glutathione synthesis and release due to hormonal stimulation. J. Nutr. 126: 1833–1843, 1996.

INDEXING KEY WORDS:
- antioxidant enzymes
- exercise
- glutathione
- rats
- food deprivation-refeeding

Glutathione (GSH; \(\gamma\)-glutamylcysteinylglycine) plays a vital role in maintaining cells in a reduced environment and in protecting the organs and tissues from oxidative stress (Meister and Anderson 1983). This function of GSH is especially important during strenuous exercise when aerobic tissues are exposed to increased production of reactive oxygen species as a result of the increased oxygen consumption (Ji 1995). Several previous studies showed that in skeletal muscle and liver, oxidation of GSH exceeds the intracellular reducing capacity provided mainly by the NADPH-linked pathways, resulting in an increased glutathione disulfide (GSSG) content and a decreased GSH:GSSG ratio (Ji et al. 1993a, Ji and Fu 1992, Leeuwenburgh and Ji 1995, Lew et al. 1985, Pyke et al. 1986, Sen et al. 1992, Villa et al. 1990). Furthermore, there is strong evidence that hepatic GSH may be mobilized and released into the circulatory system during strenuous exercise, because GSH concentrations are decreased in the liver and elevated in the blood and skeletal muscle (Ji et al. 1993a, Leeuwenburgh and Ji 1995, Lew

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\(^5\) Abbreviations used: DVL, deep vastus lateralis muscle; GCS, \(\gamma\)-glutamylcysteine synthetase; GGT, \(\gamma\)-glutamyltranspeptidase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; MDA, malondialdehyde; PCA, perchloric acid, redox, oxidoreductive; Refed-24, starved for 48 h and refed for 24 h; Refed-48, starved for 48 h and refed for 48 h, SOD, superoxide dismutase.
et al. 1985]. The signal by which liver GSH efflux is stimulated during exercise is not clear; however, both in vitro and in vivo studies suggest that glucagon and vasopressin may play an important role [Ji et al. 1993b, Lu et al. 1990]. Insulin stimulates hepatic GSH synthesis by inducing γ-glutamylcysteine synthetase (GCS), the rate-limiting enzyme in the γ-glutamyl cycle, but inhibits GSH release into the plasma [Lu et al. 1992]. Plasma concentrations of these hormones undergo dramatic changes during strenuous exercise, which may therefore affect hepatic GSH turnover and whole body GSH homeostasis.

Manipulation of tissue and whole body GSH concentrations by pharmacological and nutritional interventions causes a significant disturbance to the cellular oxidoreductive (redox) status and oxidative stress in the healthy and diseased states [Bray and Taylor 1994, Martensson and Meister 1989]. Depletion of tissue GSH by buthionine sulfoximine, an irreversible inhibitor of GCS, was reported to interfere with GSH homeostasis and cellular antioxidant defense function during prolonged exercise in rodents [Leeuwenburgh and Ji 1995, Sen et al. 1994]. Food deprivation also has a profound effect on liver GSH content and whole body GSH homeostasis [Cho et al. 1981, Tateishi et al. 1974, Tateishi et al. 1977]. The GSH concentrations in the plasma, lung and skeletal muscle are particularly affected by starvation because of the low hepatic GSH reserve [Cho et al. 1981, Lauterburgh et al. 1984]. Disturbance of the GSH system caused by food deprivation appears to influence not only antioxidant status but also oxidative stress in these tissues [Godin and Wohlaeb 1988, Langley and Kelly 1992]. However, the impact of food deprivation on GSH content and redox status during exercise has not been investigated. Because starvation and strenuous exercise exert similar physiological, metabolic and hormonal effects on the body, animals deprived of food for an extended period of time and subsequently exposed to high intensity exercise are expected to develop exaggerated GSH deficit and to become more vulnerable to oxidative stress. It is also of great interest to know whether refeeding can reverse these changes induced by starvation and exercise.

Thus, the primary purpose of the present study was to investigate the response of tissue GSH status to an acute bout of exhaustive exercise in the unfed and refed states. Liver, plasma and skeletal muscle were selected for the study because of their major roles in the synthesis, transport and consumption of GSH, respectively, during prolonged exercise. Tissue antioxidant enzyme activity and lipid peroxidation were measured to determine the levels of oxidative stress in rats. Selected enzymes in the γ-glutamyl cycle and plasma hormonal concentration were evaluated to provide some insight into the regulatory mechanisms of GSH homeostasis under the experimental conditions.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Ingredients of the semipurified diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td>Amount (g/100 g diet)</td>
</tr>
<tr>
<td>Casein [84% protein]</td>
<td>20.00</td>
</tr>
<tr>
<td>Cornstarch¹</td>
<td>66.13</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.00</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.20</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.30</td>
</tr>
<tr>
<td>Mineral mixture²</td>
<td>5.37</td>
</tr>
<tr>
<td>AIN-76A vitamin mixture³</td>
<td>1.00</td>
</tr>
<tr>
<td>Solka-Floc</td>
<td>2.00</td>
</tr>
</tbody>
</table>

¹ Purchased from A. E. Staley Manufacturing, Decatur, IL.
² Mineral mix provided per kilogram of diet: CaCO₃ 30.0 g, Ca₃(PO₄)₂ 28.0 g, K₂HPO₄ 9.0 g, NaCl 8.8 g, MgSO₄·7H₂O 0.65 g, folic acid, 0.5 g, ZnCO₃ 0.1 g, CuSO₄·5H₂O 0.30 g, H3BO3 9.0 g, NaMoO₄·2H₂O 9.0 g, KI 0.1 g, FeSO₄ 0.25 mg.
³ Vitamin mix provided per kilogram of diet: thiamin-HCl, 6 mg; riboflavin, 6 mg; pyridoxine-HCl, 7 mg; niacin, 30 mg; calcium pantothenate, 16 mg; folic acid, 2 mg; biotin, 0.2 mg; vitamin B-12, 10 μg; retinyl palmitate, 1.2 mg; cholecalciferol, 25 μg; DL-a-tocopheryl acetate, 50 mg; menadione sodium bisulfite, 50 μg.

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats [age 2 mo, body weight 280–300 g] were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Rats were housed individually in temperature controlled rooms [22°C] with a reverse 12-h dark and 12-h light cycle [0700–1900 h dark, 1900–0700 h light] at the animal facilities of the College of Medicine at the University of Illinois at Urbana-Champaign. Rats were monitored daily and body weight was recorded during the entire experimental periods. All experimental procedures were in compliance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the University of Illinois Laboratory Animal Care Advisory Committee.

**Dietary treatment.** Rats in this study were fed a semipurified diet that consisted of 66 g/100 g complex carbohydrate cornstarch, 20 g/100 g protein [casein], 5 g/100 g corn oil and adequate minerals, vitamins and other nutrients (Table 1). During the initial 3 wk of acclimation, rats had free access to the diet. After 3 wk, rats were randomly divided into three groups: the first group was deprived of food for 48 h, although tap water was available and not refed before being killed [Undef]; the second and third groups were deprived of food for 48 h and then refed the diet as described above for either 24 [Refed-24] or 48 h [Refed-48]. Food was removed at the designated time point between 1900 and 2200 h. After a 48-h starvation, refed groups of rats resumed access to food for 24 or 48 h before exercise or rest treatment (see below). Food consumption of each rat was recorded during the refeeding time. Each of the three dietary groups of rats was subsequently

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randomly divided into two subgroups: exercised until exhaustion or rested controls before being killed.

**Exercise.** Treadmill running was used as the exercise mode in the present study. During the initial 3-wk acclimatization period, all rats underwent a moderate treadmill running program on a Quinton rodent treadmill [Quinton, Seattle, WA] at 15 m/min, 0% grade for 10 min/d, 5 d/wk. This procedure ensured that the rats would be able to run effectively during the assigned exercise sessions while producing a minimal training effect on GSH content and antioxidant enzyme activities to be studied [Ji et al. 1993a]. On the day of the experiment, half of each dietary group of rats was randomly selected to run on a treadmill at 20 m/min, up a 5% grade until exhaustion. Exhaustion was defined as the loss of the righting reflex, i.e., the rat could not upright itself when placed on its back. Their weight-paired controls were rested for the same amount of time as their exercised counterparts ran on the treadmill before being killed. Food was not available for the rested animals to ensure equal food intake between the exercised and rested groups. The beginning of food deprivation, refeeding and exercise [or rest] for each rat was staggered so that the killing of the exercised rat and its weight-matched control could be performed sequentially within a 15-min time period. To avoid circadian variations of liver GSH, all rats were killed during the same time period of the day [1900–2200 h].

**Tissue preparation.** Rats were killed by decapitation followed by exsanguination immediately after being removed from the treadmill. Mixed arteriovenous blood was collected through a small funnel containing 100 μL of heparin (6 g/L). Blood was centrifuged with a clinical centrifuge at 500 × g for 15 min. An aliquot of 0.25 mL of obtained plasma was immediately mixed with 0.25 mL of perchloric acid [PCA, 140 g/L] and 2 mmol/L phenanthroline [pH < 2.0] for GSH analysis. The remaining plasma samples were frozen at −30°C for hormonal analysis. The abdominal cavity of the rat was opened, and a portion of the liver lobe from a consistent location was dissected and immediately submerged in liquid nitrogen. The deep portions of the vastus lateralis muscle [DVL] and the soleus muscle from both hindlimbs were removed quickly, divided into several portions and submerged in liquid nitrogen. Liver and muscle tissues were stored at −80°C until biochemical assays were performed.

**Biochemical analysis.** To prepare for GSH assay, a portion of the liver or muscle tissues was weighed and homogenized with a motor-driven Potter-Elvejhem glass homogenizer at 0°C in 70 g/L PCA and 2 mmol/ L phenanthroline [pH < 2.0], with a tissue weight to PCA volume ratio of 1:10, until a uniform suspension was obtained. The GSH, GSSG, cysteine and glutamate concentrations in the plasma and tissue samples were analyzed by HPLC [Reed et al. 1980] with slight modifications as previously described [Ji and Fu 1992]. Briefly, after the deproteinized homogenate was thawed and incubated on ice for 10 min, it was centrifuged with an Eppendorf desktop centrifuge at 1000 × g for 1 min. An aliquot of 250 uL of supernatant was transferred to an Eppendorf tube that contained 10 μL of 0.4 mmol/L iodoacetic acid and excess sodium bicarbonate. After incubating the homogenate at room temperature for 1 h, 2 μL of 2,4-dinitrofluorobenzene (Sanger's reagent, Sigma, St. Louis, MO) was added, and the samples were kept in the dark for at least 8 h before assay.

For tissue enzyme activity determination, a portion of the frozen muscle or liver sample was weighed and homogenized in a buffer medium containing 0.1 mol/L Tris-[hydroxymethyl] aminomethane hydrochloride [Tris-HCl, pH 7.4] at 0–4°C with a weight to volume ratio of 1:10. Activities of antioxidant enzymes were determined spectrophotometrically as previously referenced [Ji and Fu 1992]. The conditions of these enzyme assays were as follows: superoxide dismutase [SOD, EC 1.15.1.1] at 30°C; the amount of enzyme that inhibits the autooxidation of epinephrine by 50% is defined as one unit; glutathione peroxidase [GPX, EC 1.11.1.9] at 37°C with H2O2 as substrate; glutathione reductase [GR, EC 1.6.4.2.] at 30°C; glutathione S-transferase [GST, EC 2.5.1.18] at 25°C using 1-chloro-2,4-dinitrophenol as substrate. γ- Glutamyl-transpeptidase [GGT, EC 2.3.2.2.] activity was measured spectrophotometrically at 37°C according to Meister et al. [1981], with the following modifications. Muscle homogenate was preincubated at 25°C with 10 g/L deoxycholic acid [v/v ratio 15:1] for 20 min and then spun with a microcentrifuge at 500 × g for 30 s. The assay mixture contained 0.1 mol/L Tris-HCl, 9.6 mmol/L MgCl2, 0.1 mol/L glycylglycine, 5 mmol/L L-γ-glutamyl-p-nitroanilide [pH 8.0] and 1 g/L Triton-X100. After being preincubated at 37°C for 20 min, an aliquot of homogenate containing −10 mg of protein was added to initiate the reaction. p-Nitroanilide release was followed at 410 nm [ε = 8800 [mol/L]−1 cm−1] for 10 min. γ-Glutamylcysteine synthetase [GCS, EC 6.3.2.2.] activity was determined according to Seelig and Meister [1985]. The assay couples ADP formation from the GCS reaction with two enzymatic procedures in the presence of pyruvate kinase, lactate dehydrogenase, phosphoenolpyruvate and NADH. Rate of formation of ADP is followed by the decrease of NADH at 340 nm. Lipid peroxidation was determined by measuring malondialdehyde (MDA) content in muscle homogenate according to Uchiyama and Mihara [1978], with slight modification. Butylated hydroxytoluene [10 mmol/L] and 200 mmol/L ferrous sulfate were added to the assay mixture. Sealed tubes were incubated for 15 min at 99°C. The MDA content was calculated on the basis of a standard curve using 1,1,3,3-tetraethoxypropane as a standard. The concentration of plasma insulin and glucagon were determined with radioimmunological method kits [TKIN and KGNP] purchased from Diagnostic Products Corporation [Los Angeles, CA]. Antibody to human insulin
was used for insulin assay after it was verified that antibodies to human and rat insulin generated identical standard curves. The standard curve was validated with antibody to rat insulin every time when the radioimmunological assay was performed. The protein content in liver cytosol was measured by the Bradford (1976) method using bovine serum albumin as the standard.

**Statistics.** Two-way analysis of variance was used to determine the statistical significance among the various treatment groups using a computer software package (Systat Version 5.03, Wilkinson, Evanston, IL). The main treatment effects were feeding status (Unfed vs. Refed-24 vs. Refed-48) and exercise (exercise vs. rest). After an overall significant treatment effect was indicated, Fisher's least significant difference (LSD) post hoc test (Systat) was used to evaluate differences in various comparisons. The α level was set at 0.05.

**RESULTS**

**Body weight, food intake and exercise time.** Body weights of exercised and rested rats poststarvation, poststarvation and before killing are shown in Table 2. Poststarvation body weights were not significantly different among treatment groups. Starvation for 48 h decreased body weight significantly in all groups by 11% \(P < 0.05\). After refeeding, the body weights of the Refed-24 and Refed-48 rats recovered to 93% of their prestarvation weights and were no longer significantly different than those before starvation. Body weights before killing did not differ significantly between the unfed and refed rats. Exhaustive exercise had no significant effect on body weight in any of the groups.

Rats consumed similar amounts of food on the first and second day after the 48-h starvation period [Table 2]. Furthermore, food consumption adjusted by body weight was not different between the exercised and rested rats either after 24 or 48 h of refeeding.

Endurance time was significantly shorter in the Unfed rats compared with the Refed-24 and Refed-48 rats \(P < 0.05\), but no difference was found between the two refed groups [Table 2].

**Glutathione status.** Starvation for 48 h resulted in a significant decrease of liver GSH concentration compared liver GSH concentration in the unfed rats \(4.2 \pm 0.3 \mu\text{mol/g}\) [Table 3] and rats having free access to the same semipurified diet \(5.5 \pm 0.3 \mu\text{mol/g}\) (L., unpublished data). Liver GSH concentration in the unfed rats was approximately 30% lower than those in rats having free access to a commercial diet (Cho et al. 1981, Ji and Fu 1992). As shown in Table 3, refeeding for 24 h restored liver GSH concentration to 6.3 \mu\text{mol/g} and no additional increase was observed in rats refed for 48 h. Liver GSH:GSSG ratio was significantly lower in the starved rats compared with refed rats \(P < 0.05\). Liver GSSG concentrations in all groups were comparable with those in rats having free access to the same diet \(0.26 \pm 0.01 \mu\text{mol/g}\) (Ji, L., unpublished data).

Exercise significantly decreased liver GSH concentration, with a 27% reduction in the Refed-24 rats and 31% reduction in the Refed-48 rats \(P < 0.05\) [Table 3]. Exercise did not change the liver GSSG concentration regardless of feeding status but did cause a significant decline in the GSH:GSSG ratio \(P < 0.05\) in both of the refed groups.

Unfed resting rats had significantly lower absolute liver weight than the refed rats \(P < 0.05\). Liver weight was significantly decreased by 20% after an acute bout of exercise in both Refed-24 and Refed-48 rats exercise \(P < 0.05\) [Table 3], whereas the unfed rats showed no significant liver weight loss. Postexercise liver weights did not differ among the various groups. The relative liver weights, adjusted for body weight, showed essen-

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**Table 2**

<table>
<thead>
<tr>
<th>Body weight, food intake and endurance time in rested (R) and exercised (E) rats that were unfed for 48 h or refed for 24 or 48 h</th>
<th>Body weight</th>
<th>Food intake</th>
<th>Endurance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Pretstarvation</td>
<td>Poststarvation</td>
</tr>
<tr>
<td>Unfed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>6</td>
<td>309 ± 10.1</td>
<td>271 ± 8.9*</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>307 ± 12.4</td>
<td>269 ± 10.7*</td>
</tr>
<tr>
<td>Refed-24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>7</td>
<td>290 ± 7.3</td>
<td>255 ± 6.1*</td>
</tr>
<tr>
<td>E</td>
<td>7</td>
<td>288 ± 4.8</td>
<td>255 ± 4.4*</td>
</tr>
<tr>
<td>Refed-48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>7</td>
<td>296 ± 3.9</td>
<td>261 ± 3.5*</td>
</tr>
<tr>
<td>E</td>
<td>7</td>
<td>295 ± 2.0</td>
<td>261 ± 1.9*</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. 
2 Refed-24 and Refed-48, rats deprived of food for 48 h and refed for 24 and 48 h, respectively. 
*\(P < 0.05\) vs. prestarvation. 
\(P < 0.05\) vs. unfed rats.
However, the concentration of GSSG was significantly lower compared to the unfed groups. The concentration of GSSG in the liver was not significantly different between the refed and unfed groups. However, the concentration of GSSG in the plasma was significantly lower in the refed groups compared to the unfed groups.

Plasma GSH concentrations at rest were significantly higher in both refed groups compared to the unfed groups. The concentration of GSH in the plasma was not significantly different between the refed and unfed groups. However, the concentration of GSH in the liver was significantly higher in the refed groups compared to the unfed groups.

The GSH concentration was significantly lower in the refed groups compared to the unfed groups. The concentration of GSH in the plasma was significantly lower in the refed groups compared to the unfed groups. However, the concentration of GSH in the liver was not significantly different between the refed and unfed groups.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>GSH</th>
<th>GSSG</th>
<th>GSH:GSSG</th>
<th>Liver weight</th>
<th>Liver weight</th>
<th>Total GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol·g wet wt⁻¹</td>
<td>μmol·μmol</td>
<td>g</td>
<td>g·kg body wt⁻¹</td>
<td>μmol·liver⁻¹</td>
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</tr>
<tr>
<td>Unfed</td>
<td>R</td>
<td>6</td>
<td>4.2 ± 0.3</td>
<td>0.24 ± 0.02</td>
<td>17.4 ± 1.1</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>6</td>
<td>3.6 ± 0.4</td>
<td>0.23 ± 0.03</td>
<td>17.0 ± 2.4</td>
<td>6.2 ± 0.4</td>
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<tr>
<td>Refed-24</td>
<td>R</td>
<td>7</td>
<td>6.3 ± 0.8*</td>
<td>0.24 ± 0.02</td>
<td>25.8 ± 2.5*</td>
<td>8.1 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>7</td>
<td>4.6 ± 0.6'</td>
<td>0.23 ± 0.03</td>
<td>20.4 ± 1.4'</td>
<td>6.6 ± 0.2'</td>
</tr>
<tr>
<td>Refed-48</td>
<td>R</td>
<td>7</td>
<td>5.9 ± 0.5*</td>
<td>0.22 ± 0.03</td>
<td>28.1 ± 1.9*</td>
<td>8.4 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>7</td>
<td>4.1 ± 0.6'</td>
<td>0.18 ± 0.03</td>
<td>22.2 ± 1.8'</td>
<td>6.7 ± 0.1'</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. 
*P < 0.05, Refed-24 or Refed-48 vs. Unfed rats. 
†P < 0.05, E vs. R.

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>GSH</th>
<th>GSSG</th>
<th>GSH:GSSG</th>
<th>Liver weight</th>
<th>Liver weight</th>
<th>Total GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol·g wet wt⁻¹</td>
<td>μmol·μmol</td>
<td>g</td>
<td>g·kg body wt⁻¹</td>
<td>μmol·liver⁻¹</td>
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<tr>
<td>Plasma, μmol/L</td>
<td>R (n = 6)</td>
<td>E (n = 6)</td>
<td>R (n = 7)</td>
<td>E (n = 7)</td>
<td>R (n = 7)</td>
<td>E (n = 7)</td>
</tr>
<tr>
<td>GSH</td>
<td>7.7 ± 0.3</td>
<td>7.1 ± 0.6</td>
<td>8.7 ± 0.3*</td>
<td>10.3 ± 0.4'</td>
<td>8.6 ± 0.3*</td>
<td>8.9 ± 0.4</td>
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<td>GSSG</td>
<td>2.7 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>2.5 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>2.1 ± 0.2</td>
<td>2.2 ± 0.4</td>
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<tr>
<td>Soleus, μmol·g wet wt⁻¹</td>
<td>R (n = 7)</td>
<td>E (n = 7)</td>
<td>R (n = 7)</td>
<td>E (n = 7)</td>
<td>R (n = 7)</td>
<td>E (n = 7)</td>
</tr>
<tr>
<td>GSH</td>
<td>2.0 ± 0.10</td>
<td>2.1 ± 0.10</td>
<td>1.7 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.10*</td>
<td>1.6 ± 0.08</td>
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<tr>
<td>GSSG</td>
<td>0.13 ± 0.04</td>
<td>0.14 ± 0.04</td>
<td>0.25 ± 0.03</td>
<td>0.30 ± 0.02'</td>
<td>0.30 ± 0.01'</td>
<td>0.30 ± 0.02'</td>
</tr>
<tr>
<td>Deep vastus lateralis, μmol·g wet wt⁻¹</td>
<td>R (n = 7)</td>
<td>E (n = 7)</td>
<td>R (n = 7)</td>
<td>E (n = 7)</td>
<td>R (n = 7)</td>
<td>E (n = 7)</td>
</tr>
<tr>
<td>GSH</td>
<td>0.63 ± 0.03</td>
<td>0.52 ± 0.04</td>
<td>0.43 ± 0.07*</td>
<td>0.58 ± 0.08</td>
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<td>GSSG</td>
<td>0.09 ± 0.04</td>
<td>0.07 ± 0.02</td>
<td>0.10 ± 0.03</td>
<td>0.17 ± 0.03'</td>
<td>0.16 ± 0.02'</td>
<td>0.14 ± 0.02'</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. 
P < 0.05, Refed-24 or Refed-48 vs. Unfed rats. P < 0.05, E vs. R.
Liver glutamate concentration in the resting rats was not significantly different between the food deprived and refeed groups (Table 5). However, exercised Refed-24 and Refed-48 rats had 31 and 25% higher \( P < 0.05 \) glutamate concentration, respectively, than their rested counterparts. Glutamate concentration in DVL muscle also showed no significant differences between unfed and refeed rats. Exercise significantly increased muscle glutamate concentration only in the Refed-24 rats \( P < 0.05 \).

There was no significant difference in liver or muscle protein concentration among the various treatment groups (Table 5).

\textbf{γ-Glutamyl cycle enzyme activities.} Refeeding for 48 h significantly activated hepatic GCS (Table 6). The GCS activity in the liver was 83% higher \( P < 0.05 \) in Refed-48 rats compared with Unfed rats. Exercise did not affect liver GCS activity significantly.

The G6PDH was significantly greater in the refeed than unfed rats (Table 6). Refed-24 and Refed-48 rats had two and sixfold higher G6PDH activity, respectively, than the Unfed rats. Exercise had no effect on this enzyme activity regardless of feeding status.

There were no significant differences in the GGT activity between DVL muscles from the rested unfed and refeed rats (Table 6). An acute bout of exhaustive exercise caused a significant reduction of GGT activity in DVL muscle by 51 and 67%, respectively, in the Refed-24 and Refed-48 rats \( P < 0.05 \). The GGT activity in the liver and soleus muscle was not affected by refeeding or exercise (data not shown).

\textbf{Plasma insulin and glucagon.} Plasma insulin concentration was significantly higher in both of the refeed groups of rats compared with unfed rats \( P < 0.05 \) (Table 7). Exercise significantly decreased plasma insulin concentrations in both Refed-24 and Refed-48 rats \( P < 0.01 \) but not in the unfed animals. In contrast to insulin, plasma glucagon concentration was significantly lower in the Refed-24 rats compared with Unfed rats and Refed-48 rats \( P < 0.05 \) (Table 7). Exercise increased glucagon concentrations by 22 and 35%, respectively, in the Unfed and Refed-48 rats \( P < 0.05 \), but exerted no effect in the Refed-24 rats.

The plasma glucagon:insulin ratio was suppressed in the Refed-24 h rats compared with that in the Unfed rats \( P < 0.05 \). It was restored after 48 h refeeding because no difference was observed between Refed-48 and Unfed rats. The glucagon:insulin ratio was significantly greater in the exercised rats compared with the rested rats after 24 and 48-h refeeding \( P < 0.05 \) but not in unfed rats.

\textbf{Muscle antioxidant enzyme activity.} The GPX activity was significantly higher in the exercised Unfed (40%) and Refed-24 (38%) rats, compared with their rested counterparts \( P < 0.05 \) (Table 8). Exercise did not affect activity of GR or GST in the muscle. Further-

\begin{table}
\caption{Cysteine and glutamate concentrations in rested (R) and exercised (E) rats that were unfed for 48 h or refeed for 24 h or 48 h\textsuperscript{1}}
\begin{tabular}{|l|c|c|c|c|c|}
\hline
 & \multicolumn{2}{c|}{Unfed} & \multicolumn{2}{c|}{Refed-24} & \multicolumn{2}{c|}{Refed-48} \\
\hline
\text{Cysteine, nmol g wet wt\textsuperscript{-1}} & & & & & & \\
Liver & 134 ± 52 & 368 ± 36\textsuperscript{*} & 196 ± 29 & 263 ± 54 & 303 ± 92\textsuperscript{+} & 341 ± 57 \\
DVL & 65 ± 12 & 74 ± 9 & 81 ± 10 & 124 ± 33 & 64 ± 15 & 70 ± 13 \\
\text{Glutamate, μmol g wet wt\textsuperscript{-1}} & & & & & & \\
Liver & 4.18 ± 0.10 & 4.68 ± 0.10 & 3.71 ± 0.20 & 4.87 ± 0.10\textsuperscript{*} & 3.78 ± 0.10 & 4.73 ± 0.08\textsuperscript{*} \\
DVL & 1.74 ± 0.04 & 2.24 ± 0.04 & 1.59 ± 0.03 & 2.69 ± 0.02\textsuperscript{*} & 1.71 ± 0.01 & 2.14 ± 0.02 \\
\text{Protein, mg g wet wt\textsuperscript{-1}} & & & & & & \\
Liver & 179 ± 7 & 173 ± 5 & 170 ± 3 & 174 ± 4 & 183 ± 6 & 175 ± 5 \\
DVL & 91 ± 3 & 95 ± 5 & 94 ± 3 & 95 ± 2 & 93 ± 3 & 92 ± 2 \\
\hline
\end{tabular}
\textsuperscript{1} Values are means ± SEM. \textsuperscript{*}P < 0.05, E vs. R. \textsuperscript{+}P < 0.05, Refed-48 h vs. Unfed.

DVL, deep vastus lateralis muscle.
\end{table}

\begin{table}
\caption{Activities of liver γ-glutamylcysteine synthetase and glucose 6-phosphate dehydrogenase and muscle γ-glutamyl transpeptidase in rested (R) and exercised (E) rats that were unfed for 48 h or refeed for 24 or 48 h\textsuperscript{1}}
\begin{tabular}{|l|c|c|c|c|}
\hline
 & \multicolumn{2}{c|}{GCS} & \multicolumn{2}{c|}{G6PDH} & \multicolumn{2}{c|}{GOT} \\
 & \text{μmol min\textsuperscript{-1} g wet wt\textsuperscript{-1}} & \text{nmol min\textsuperscript{-1} g wet wt\textsuperscript{-1}} & \text{μmol min\textsuperscript{-1} g wet wt\textsuperscript{-1}} & \text{nmol min\textsuperscript{-1} g wet wt\textsuperscript{-1}} \\
\hline
\text{Unfed} & & & & & & \\
R & 6 & 2.3 ± 0.4 & 2.1 ± 0.3 & 69 ± 12 \\
E & 6 & 2.2 ± 0.4 & 1.9 ± 0.1 & 53 ± 14 \\
\text{Refed-24} & & & & & & \\
R & 7 & 2.9 ± 0.7 & 6.3 ± 0.8\textsuperscript{+} & 82 ± 8 \\
E & 7 & 3.4 ± 0.5 & 5.3 ± 1.0\textsuperscript{+} & 40 ± 10\textsuperscript{*} \\
\text{Refed-48} & & & & & & \\
R & 7 & 4.2 ± 0.4\textsuperscript{*} & 14.9 ± 1.1\textsuperscript{+} & 74 ± 6 \\
E & 7 & 3.1 ± 0.3 & 15.0 ± 1.8\textsuperscript{+} & 24 ± 4\textsuperscript{*} \\
\hline
\end{tabular}
\textsuperscript{1} Values are means ± SEM. GCS, γ-glutamylcysteine synthetase measured in the liver; G6PDH, glucose 6-phosphate dehydrogenase measured in the liver; GOT, γ-glutamyltranspeptidase measured in the deep vastus lateralis muscle. \textsuperscript{*}P < 0.05, E vs. R. \textsuperscript{+}P < 0.05, Refed-24 or Refed-48 vs. Unfed. \textsuperscript{1}P < 0.01, Refed-48 vs. Refed-24.
TABLE 7

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Insulin pmol·L⁻¹</th>
<th>Glucagon ng·L⁻¹</th>
<th>Glucagon:insulin ratio ng/pmol</th>
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</thead>
<tbody>
<tr>
<td>Unfed</td>
<td>R</td>
<td>6</td>
<td>70.3 ± 3.6</td>
<td>221 ± 7</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>6</td>
<td>75.3 ± 10.0</td>
<td>269 ± 12*</td>
</tr>
<tr>
<td>Refed-24</td>
<td>R</td>
<td>7</td>
<td>114 ± 13.6*</td>
<td>144 ± 31+</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>7</td>
<td>76.1 ± 1.4*</td>
<td>157 ± 17+</td>
</tr>
<tr>
<td>Refed-48</td>
<td>R</td>
<td>7</td>
<td>99.7 ± 3.6*</td>
<td>246 ± 33</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>7</td>
<td>73.9 ± 2.2*</td>
<td>316 ± 49*</td>
</tr>
</tbody>
</table>

*Values are means ± SEM. *P < 0.05, E vs. R. †P < 0.05, Refed-24 or Refed-48 vs. Unfed. +P < 0.05, Refed-24 vs. Unfed or Refed-48 h.

more, SOD activity was not altered with either feeding status or exercise in DVL muscle.

Lipid peroxidation. Liver MDA concentration was significantly higher in the Unfed compared with both of the refed groups (P < 0.05) [Fig. 1]. Exercise did not have a significant effect on liver MDA concentrations. The MDA concentration in DVL muscle was also significantly affected by feeding status. Unfed rats had about twice as much muscle MDA concentrations as the refed rats (P < 0.05). However, exercise did not significantly change MDA concentrations in the DVL muscle in any feeding group.

DISCUSSION

Glutathione and feeding status. Hepatic GSH provides a vital antioxidant reserve against reactive oxygen species and xenobiotic toxins. It also functions as a cysteine reservoir for the maintenance of interorgan homeostasis [Tateishi et al. 1977]. Liver GSH content per unit wet weight decreases between one-third and one-half during 48 h of starvation in rats [Hum et al. 1991, Langley and Kelly 1992, Lauterburg et al. 1984, Tateishi et al. 1974]. Taking into account the decrease of liver weight, the decline of total GSH reserve due to starvation is more dramatic. Consistent with these findings, the present study showed a 30% decrease of liver GSH concentration and almost a 50% decrease per whole liver in the rats deprived of food for 48 h compared with the refed animals, or compared with fed rats of the same sex and strain in previous studies [Cho et al. 1981, Ji and Fu 1992]. During starvation, hepatic consumption of GSH was negligible and no significant increase in bile flow of GSH occurred, indicating that the majority of the GSH was transported into the plasma [Lauterburg et al. 1984]. This scenario is consistent with our data showing that plasma GSH

FIGURE 1 Malondialdehyde (MDA) concentrations in the liver and deep vastus lateralis (DVL) muscle in rested (R) and exercised (E) rats that were unfed for 48 h or refed for 24 h or 48 h. Values are means ± SEM. *P < 0.05, Refed-24 or Refed-48 vs. Unfed in DVL. +P < 0.05, Refed-48 or Refed-24 vs. Unfed in the liver.

GSH homeostasis [Tateishi et al. 1977]. Liver GSH content per unit wet weight decreases between one-third and one-half during 48 h of starvation in rats [Hum et al. 1991, Langley and Kelly 1992, Lauterburg et al. 1984, Tateishi et al. 1974]. Taking into account the decrease of liver weight, the decline of total GSH reserve due to starvation is more dramatic. Consistent with these findings, the present study showed a 30% decrease of liver GSH concentration and almost a 50% decrease per whole liver in the rats deprived of food for 48 h compared with the refed animals, or compared with fed rats of the same sex and strain in previous studies [Cho et al. 1981, Ji and Fu 1992]. During starvation, hepatic consumption of GSH was negligible and no significant increase in bile flow of GSH occurred, indicating that the majority of the GSH was transported into the plasma [Lauterburg et al. 1984]. This scenario is consistent with our data showing that plasma GSH

TABLE 8

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>GPX</th>
<th>GR</th>
<th>GST</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μmol·min⁻¹·g wet wt⁻¹</td>
<td>units·g wet wt⁻¹</td>
<td></td>
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</tr>
<tr>
<td>Unfed</td>
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<td>0.52 ± 0.07</td>
</tr>
<tr>
<td>Refed-24</td>
<td>R</td>
<td>7</td>
<td>1.8 ± 0.3</td>
<td>0.75 ± 0.15</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
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<td>E</td>
<td>7</td>
<td>2.5 ± 0.2*</td>
<td>0.58 ± 0.06</td>
<td>0.53 ± 0.03</td>
</tr>
<tr>
<td>Refed-48</td>
<td>R</td>
<td>7</td>
<td>1.5 ± 0.2</td>
<td>0.56 ± 0.03</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>7</td>
<td>1.7 ± 0.1</td>
<td>0.52 ± 0.04</td>
<td>0.46 ± 0.04</td>
</tr>
</tbody>
</table>

*Values are means ± SEM. *P < 0.05, E vs. R. GPX, glutathione peroxidase; GR, glutathione reductase; GST, glutathione S-transferase; SOD, superoxide dismutase.
concentrations were maintained relatively constant after 48 h of starvation despite a severe decrease in the liver GSH reserve (Table 4).

One important reason that explains the substantial decline of liver GSH content after prolonged starvation is a decreased amino acid availability, especially cysteine, the limiting factor for hepatic GSH synthesis. It has been shown that a 24-h starvation decreased liver cysteine concentration by ~30% and that the changes in cysteine availability for the large part accounted for the change in liver GSH concentration (Tateishi et al. 1974 and 1977). Liver cysteine concentration in the food-deprived rested rats (134 nmol · g⁻¹) (Table 5) was comparable with that reported in rats starved for 24 h (150 nmol · g⁻¹) (Tateishi et al. 1974). In contrast, glutamate concentration was not altered by starvation (Table 5) (Cho et al. 1981, Tateishi et al. 1974). Thus, liver GSH synthesis appears to depend primarily on the dietary source of cysteine (Bray and Taylor 1994).

Another possible reason may be related to an altered plasma hormonal milieu. After prolonged starvation, plasma insulin concentration was suppressed, whereas glucagon level was elevated (Table 7). Lu et al. (1992) demonstrated that insulin is required for the normal expression of GCS in the liver. Diabetes resulted in lower GSH levels associated with low GCS activity in rat liver, whereas insulin replacement prevented these abnormalities. In contrast to insulin, glucagon antagonizes hepatic GSH synthesis by inhibiting GCS (Lu et al. 1991). However, high levels of glucagon in rat liver have been shown to stimulate hepatic GSH efflux into the circulation (Lu et al. 1990). Thus, the high plasma glucagon:insulin ratio observed in the food-deprived rats resulted in two consequences: inhibiting hepatic GSH synthesis and promoting GSH efflux into the circulation, possibly for the maintenance of GSH homeostasis in other vital organs such as brain and lung that might be under oxidative stress (Godin and Wohabeib 1988).

Liver has a high rate of GSH turnover (~4.5 μmol · h⁻¹) (Griffiths and Meister 1979), therefore hepatic GSH is rapidly restored upon refeeding. We showed that after 24-h refeeding rats had essentially restored liver GSH content to normal concentrations and no additional increase was found after 48-h refeeding. Others have shown that GSH concentrations can recover to those found in fed rats within 10-h refeeding of 48-h starved rats, paralleled by an increase in liver cysteine concentration (Cho et al. 1981, Hum et al. 1991, Tateishe et al. 1974). Several factors may account for the restoration of liver GSH during refeeding. First, plasma hormonal responses might play an important role in reestablishing hepatic GSH status. In the Refed-24 rats plasma concentration of insulin was increased, whereas glucagon concentrations were decreased in response to refeeding, resulting in a suppressed glucagon:insulin ratio (Table 7). This hormonal milieu clearly favored resynthesis of GSH in the liver (Lu et al. 1990, 1991, 1992). Second, hepatic GCS activity was significantly increased during refeeding (Table 6). The GCS is the rate-limiting enzyme in the γ-glutamyl cycle and an up regulation of this enzyme was demonstrated to be the primary reason for an insulin-induced elevation of liver GSH (Lu et al. 1992). Therefore, it is tempting for us to conclude that the observed increase in GCS activity promoted the restoration of GSH content in the refed rats. However, Tateishi et al. (1974) showed that administration of cycloheximide or actinomycin D, inhibitors of protein synthesis, in refeeding animals suppressed the induction of GCS and G6PDH but did not affect the rise in GSH content. Furthermore, no change in total GSH synthesizing capacity (GCS + GSH synthetase activities) was observed in the liver after 2-18 h of refeeding. The present study showed that in Refed-24 rats, hepatic GCS activity increased little, whereas liver GSH was already restored to normal concentrations. These findings suggest that de novo synthesis of GCS may not be required for restoration of liver GSH. Instead, an increased amino acid availability upon refeeding, especially cysteine, has been proposed to be more important for GSH synthesis (Bray and Taylor 1994, Goss et al. 1994). This scenario is consistent with our finding that liver cysteine concentration increased strongly in the refed rats.

The response of GSH to food deprivation and refeeding in skeletal muscle has hardly been studied. In one study, Cho et al. (1981) reported that GSH concentration in the abdominal muscle decreased with starvation, whereas refeeding for 24 or 48 h did not restore muscle GSH concentration to prestarvation concentrations. In the current study, GSH concentrations in the soleus and DVL muscles of the unfed rats were ~30 and 50% lower than the respective values reported in our previous study (Ji et al. 1993a). There was a further decline of GSH in both muscles during the refeeding period, along with an increased formation of GSSG after 48 h of refeeding. Associated with the decreased GSH concentration during refeeding were persistently low cysteine and glutamate concentrations in the DVL muscle. The ability of GSH uptake by muscle did not appear to be impaired because GGT activity was not affected in the refed rats. However, it cannot be excluded that GSH-synthesizing enzymes, such as GCS and glutathione synthetase, were down regulated for an extended period of time after refeeding. Activities of these two enzymes in skeletal muscle are very low, and the regulatory mechanism of muscle GSH turnover is poorly understood. Considering the large mass of skeletal muscle, a decreased GSH turnover can be advantageous to preserve blood GSH pool thereby increasing the supply of GSH to more vital organs and tissue for antioxidant protection.

Food deprivation results in oxidative stress to the liver and extrahepatic tissues, along with alterations of antioxidant enzyme status (Godin and Wohabeib 1988). In the present study, we studied antioxidant enzyme
responses to food deprivation-refeeding in the skeletal muscle and found no significant differences between the starved and refed rats. However, we did not measure catalase activity, which undergoes some dramatic changes with starvation (Lammi-Keefe et al. 1984). Nevertheless, the large increases in MDA concentration in the liver and skeletal muscle of unfed vs. refed rats shown in the present study were consistent with the concept that starvation and nutritional deficiency enhance tissue lipid peroxidation and overall oxidative stress (Godin and Wohaiab 1988, Sato et al. 1991). Asayama et al. (1989) showed that both insulin depletions and starvation could elicit peroxidative damage and alter antioxidant status in the kidney, heart and liver of rats, whereas insulin treatment and refeeding of diabetic rats restored these abnormalities. Although a uniform theory to explain the nature and patterns of the aforementioned alterations is still lacking, two related mechanisms may be worthy of considering. First, a marked decrease of dietary intake of antioxidant nutrients not only depletes GSH, as previously discussed, but also limits tissue glucose availability, which may affect production of reducing equivalents (Godin and Wohaiab 1988). This may be reflected in part by the dramatic down regulation of hepatic G6PDH activity shown in the current study (Table 6) and the decreased tissue GPX and GR activities reported by others (Langley and Kelly 1992). Second, starvation may activate oxidation of fatty acids both in the mitochondria and in the peroxisomes, in which hydrogen peroxide and other reactive oxygen species are byproducts (Godin and Wohaiab 1988).

Exercise and glutathione status. The significant decreases of liver GSH concentration after exhaustive exercise in the 24 and 48 h refed rats were consistent with our previous report and others in fed animals [Leeuwenburgh and Ji 1995, Lew et al. 1985, Pyke et al. 1986, Sen et al. 1992 and 1994, Villa et al. 1990]. However, different explanations for the observed changes have been offered. One possibility is that the oxidation of GSH to GSSG during exercise exceeds the reducing capacity of GR because of the increased oxygen-free radical production in the liver (Ji 1995). The formed GSSG may be released from the liver resulting in a deficit of total glutathione content [see discussion below]. A second possibility is that hepatic synthesis of GSH is impaired during exhaustive exercise because of the reduced availability of ATP and corresponding increases in ADP and AMP. These adenine nucleotide profiles are not favorable thermodynamically for the key steps of GSH synthesis catalyzed by GCS and glutathione synthetase, wherein ATP serves as a cosubstrate [Tateishi et al. 1974]. The diminution of plasma insulin concentrations during prolonged exercise may also play a role because refeeding-induced GCS requires insulin stimulation [Lu et al. 1991]. Finally, the decrease of liver GSH might be attributed to a hepatic efflux of GSH into the plasma stimulated by glucagon, vasopres-
tively exercised rats [Ji and Fu 1992, Ji et al. 1993a]. However, rats in the current study ran for a much longer time (~4 h) compared with those in the previous studies [40–60 min]. Although skeletal muscle is believed to be capable of taking up GSH from the plasma [Ji et al. 1993b, Lew et al. 1985], the uptake is limited by the enzyme activities of the γ-glutamyl cycle and is ATP dependent [Meister and Anderson 1983]. The decrease of GGT activity found in DVL, combined with a diminished energy supply at exhaustion, might both contribute to a compromised GSH uptake. In a previous study, we found that muscle GSH response to exercise was biphasic, with an initial increase followed by a decline after ~3.5 h of swimming to exhaustion [Leeuwenburgh and Ji 1995]. Nevertheless, the increased GSSG concentrations in both DVL and soleus and an increased GPX activity in DVL of Refed-24 rats agreed with our previous data and were reflective of an exercise-induced oxidative stress in the skeletal muscle [Ji and Fu 1992, Ji et al. 1993a].

In summary, food deprivation for 48 h depleted hepatic GSH content and exerted an oxidative stress to the liver and skeletal muscle in rats. Refeeding restored liver GSH content to adequate concentrations within 24 h, but GSH concentration in skeletal muscle was not recovered after 48 h. Plasma GSH concentration was kept constant during the food-deprivation-refeeding regimen. Restoration of GSH status was accompanied by increases in γ-glutamylcysteine synthetase and glucose 6-phosphosphate dehydrogenase activities in the liver and by an elevation of plasma insulin concentration. An acute bout of prolonged exhaustive exercise decreased liver GSH and the GSH:GSSG ratio in the fed state, whereas the GSSG concentration was unchanged. In skeletal muscle of 24 h refed rats, GSSG but not GSH concentration was significantly increased after exercise. These exercise-induced disturbances of GSH status in the liver and muscle showed no apparent exacerbation in the food-deprived state.

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LITERATURE CITED


Pyke, S., Lew, H. & Quintanilha, A. [1986] Severe depletion in
GLUTATHIONE IN EXERCISED UNFED AND REFED RATS


