Exercise-induced oxidative stress: glutathione supplementation and deficiency

CHANDAN K. SEN, MUSTAFA ATALAY, AND OSMO HÄNNINEN
Department of Physiology, Faculty of Medicine, University of Kuopio, FIN-70211 Kuopio, Finland

Sen, Chandan K., Mustafa Atalay, and Osmo Hänninen. Exercise-induced oxidative stress: glutathione supplementation and deficiency. *J. Appl. Physiol.* 77(5): 2177–2187, 1994.—Glutathione (GSH) plays a central role in coordinating the synergism between different lipid- and aqueous-phase antioxidants. We documented *1*) how exogenous GSH and *N*-acetylcysteine (NAC) may affect exhaustive exercise-induced changes in tissue GSH status, lipid peroxides [thiobarbituric acid-reactive substances (TBARS)], and endurance and *2*) the relative role of endogenous GSH in the circumvention of exercise-induced oxidative stress by using GSH-deficient [L-buthionine-(*S,R*)-sulfoximine (BSO)-treated] rats. Intraperitoneal injection of GSH remarkably increased plasma GSH; exogenous GSH per se was an ineffective delivery agent of GSH to tissues. Repeated administration of GSH (1 time/day for 3 days) increased blood and kidney total GSH [TGSH; GSH + oxidized GSH (GSSG)]. Neither GSH nor NAC influenced endurance to exhaustion. NAC decreased exercise-induced GSH oxidation in the lung and blood. BSO decreased TGSH pools in the liver, lung, blood, and plasma by ~50% and in skeletal muscle and heart by 80–90%. Compared with control, resting GSH-deficient rats had lower GSSG in the liver, red gastrocnemius muscle, heart, and blood; similar GSSG/TGSH ratios in the liver, heart, lung, blood, and plasma; higher GSSG/TGSH ratios in the skeletal muscle; and more TBARS in skeletal muscle, heart, and plasma. In contrast to control, exhaustive exercise of GSH-deficient rats did not decrease TGSH in the liver, muscle, or heart or increase TGSH of plasma; GSSG of muscle, blood, or plasma; or TBARS of plasma or muscle. GSH-deficient rats had ~50% reduced endurance, which suggests a critical role of endogenous GSH in the circumvention of exercise-induced oxidative stress and as a determinant of exercise performance.

antioxidant; free radical; performance; endurance; *N*-acetylcysteine; lipid peroxidation; therapy; thiol; buthionine sulfoximine; chain; redox

---

STRENUOUS PHYSICAL EXERCISE is capable of inducing oxidative stress (15, 34), a state wherein the production of reactive oxygen species (ROS) in the body transcends the antioxidant defense capacity. Our previous study with humans revealed that even submaximal exercise for 30 min may elevate oxidative stress indexes, e.g., blood glutathione [GSH; (L-γ-glutamyl-L-cysteinyl)glycine] oxidation and plasma lipid peroxides (36). ROS are known to have a wide variety of pathophysiological implications (17). It has also been suggested that a high concentration of ROS may contribute to oxidative skeletal muscle fatigue (3). A large number of recent studies have been directed toward the management of exercise-induced oxidative stress. Antioxidant supplementation studies have revealed a beneficial trend (15, 34).

GSH plays a central role in coordinating the body's antioxidant defense processes. Antioxidants like vitamins E and C and GSH are known to act synergistically (7, 12, 13, 22, 26, 34, 41) in the form of an antioxidant chain reaction (36). Lipophilic vitamin E is a major lipid peroxidation chain-breaking antioxidant. However, this antioxidative reaction of α-tocopherol generates another weakly reactive chromanoxyl radical, tocopherol-O·, as a byproduct. The water-soluble antioxidants, ascorbate and GSH, may be involved in regenerating α-tocopherol from its radical byproduct (7, 12, 13, 22, 26, 41). GSH also may regenerate ascorbate from its oxidized byproduct that may be formed during the interaction of ascorbate with reactive metabolites. The primary components of the physiological antioxidative defense system are the superoxide dismutases, catalase, and the GSH system. In the presence of H^+, superoxide dismutases are known to rapidly dismutate superoxide anions to hydrogen peroxide. Although hydrogen peroxide is not a free radical, it has strong prooxidant properties and is therefore listed as an ROS. Decomposition of hydrogen peroxide is mainly carried out by either catalase- or GSH-dependent reactions. Although synthesis of GSH is cytosolic in nature, mitochondria are considerably rich in their GSH content. Catalase is not present in the mitochondria, a major site of oxygen-derived free radical generation. Therefore, scavenging of mitochondrial hydrogen peroxide is largely a GSH-dependent process.

Designing of antioxidant supplementation protocols should be guided by the requirements of the antioxidant chain reaction; such an approach will maintain a favorable redox status of each of the constituent antioxidants and avoid the accumulation of reactive oxidized antioxidant byproducts (e.g., chromanoxyl and ascorbyl radicals). Although antioxidant protection dependent on vitamins E and C can be enhanced by the simple use of oral supplements, increasing the reducing power of the thiol pool (primarily contributed by -SH of GSH) is far more challenging. GSH per se is not efficiently transported into most animal cells. However, two recent studies showed that exogenous GSH remarkably increased the endurance to physical exercise in mice (5, 29). Compared with placebo-treated control mice, 0.5, 0.75, and 1 g/kg ip doses of GSH increased endurance to swimming by 102.4, 120, and 140.7%, respectively (29). At a dose of 0.25 g/kg, GSH did not affect endurance when injected once but did significantly increase endurance when injected once a day for seven consecutive days. In another study, oral GSH at a dose of 0.25–1 g/kg caused a dose-dependent significant improvement in endurance to swimming (5). Both above-mentioned studies employed brief bouts (~1.5–5 min) of swimming as the exercise challenge. In a recent study with humans, we showed that *N*-acetylcysteine {NAC [(2-mercaptopropionyl)glycine]; a GSH-replenishing drug (32)} supplementation may spare exercise-associated blood GSH oxidation and the thiol redox status perturbation (36).

We were interested in revealing *1*) the possible fate of intraperitoneally administered GSH and *2*) the effects of GSH and NAC supplementation on exhaustive exercise-induced oxidative stress and endurance to such exercise. The effect of repeated GSH administration was also compared with that of a single injection. We realized that this biochemical investigation was necessary before any supposition regarding the role of the thiols in endurance enhancement, an area of obvious popular interest, could be formulated. Our hypothesis that endogenous tissue GSH status is crucially important in protecting against exercise-induced oxidative stress and thus in influencing endurance to exhaustive exercise was tested using GSH-deficient rats. This is the first time that such a model has been used for the study of exercise-induced oxidative stress. Almost all the evidence supporting the contention that a single bout of exercise may induce oxidative stress has been obtained from studies using exercise types that were long in duration and mostly running or cycling in nature (15, 16, 20, 21, 33, 36). Because we aimed to test the effectiveness of exogenous and endogenous GSH in controlling exercise-induced oxidative stress, the long-lasting (~2 h) treadmill run protocol was used in this study.

MATERIALS AND METHODS

L-buthionine-(S,R)-sulfoximine (BSO) administration, and exercise. To test the time-dependent distribution of intraperitoneally administered GSH (*experiment 1*), 34 male Han-Wistar rats (8 wk old, body wt 170–180 g) were randomly divided into two groups: GSH solution-administered rats (G rats; 1 g/kg of GSH in 0.8–1.0 ml of solution; pH was adjusted to 6.5–6.8 by cautious addition of NaOH just before use; $n = 22$) and placebo saline-injected control rats (P rats; 0.8–1.0 ml of saline; $n = 12$). The group of G rats was randomly subdivided into four subgroups of rats that were killed 0.5, 1.5, 4, and 24 h after GSH administration (G1–G4 rats, respectively; $n = 4$ each). In another subgroup (G5 rats; $n = 6$), the effect of repeated administration of GSH was tested. GSH, as above, was administered once every 24 h for three consecutive days. On the 3rd day, G5 rats were killed 0.5 h after the last injection so that the effect of repeated GSH administration could be revealed by comparing results from G5 and G1 rats. The P rats were randomly subdivided into two subgroups of rats that were killed 0.5 and 4 h after saline injection (C1 and C2 rats, respectively; $n = 6$ each). The deaths of C1 and C2 rats were time matched with those of G1 and G3 rats, respectively.

The effect of GSH and NAC supplementation in the circumvention of exercise-associated oxidative stress and endurance capacity was tested in *experiment 2*. Thirty-five rats were divided into five equal groups ($n = 7$ each): intraperitoneally injected P rats killed at rest 2.5 h after the injection (SR rats; duration matched with deaths of SE rats); intraperitoneally injected P rats killed immediately after exhaustive exercise (SE rats); NAC-injected rats (1 g/kg ip in 0.8–1.0 ml of solution; pH adjusted to 6.5–6.8 as described above) killed immediately after exhaustive exercise (NE rats); G rats (as used for *experiment 1*) killed at rest 2.5 h after injection (GR rats; duration matched with deaths of GE rats); and G rats killed immediately after exhaustive exercise (GE rats). Exercise was performed on a 10-lane rodent treadmill (10% uphill grade) 0.5 h after the intraperitoneal injection. During the first 10 min of treadmill running, the running speed was set at 1.2 km/h. After that period, the speed was increased to 1.8 km/h and was maintained at that level until the animal was exhausted. Exhaustion due to exercise was identified by the loss of righting reflex of the rats on being turned on their back.

For *experiment 3*, GSH-deficient (BSO-treated) rats ($n = 17$) were prepared by intraperitoneal injection of BSO (6 mmol/kg body wt, in 0.8–1.0 ml of solution, 2 times/day for 4 days; Sigma Chemical, St. Louis, MO). P rats were prepared by injecting 0.8–1.0 ml of saline. The BSO-treated rats were randomly divided into two subgroups: BSO-treated rats killed at rest 0.5 h after the last injection (BR rats; $n = 8$) and BSO-treated rats killed immediately after exhaustive exercise on a treadmill (BE rats; $n = 9$). P rats were randomly divided into two subgroups ($n = 7$ each): P rats killed at rest 0.5 h after the last injection (PR rats; duration matched with deaths of BR rats) and P rats killed immediately after exhaustive exercise on a treadmill (PE rats). The BE and PE rats were subjected to treadmill running 0.5 h after the last intraperitoneal injection of BSO and saline, respectively. The treadmill exercise protocol for *experiment 3* was the same as that described for *experiment 2*.

Before the commencement of *experiments 2* and *3*, all animals were acquainted with treadmill running (10% uphill grade, 1.0–1.2 km/h, 0.5 h/day for 4 days). Because body weight may affect endurance capacity, for *experiments 2* and *3* the test and corresponding control animals were pair matched for weight.

Tissue collection. All rats were killed by decapitation and were exsanguinated. Portions of the red gastrocnemius muscle, mixed vastus lateralis muscle, heart, lung, kidney, and liver were excised immediately, quickly cut into small pieces, and submerged in liquid nitrogen. Before being frozen, the skeletal muscle (red gastrocnemius and mixed vastus lateralis) was freed from visible adipose and connective tissues, rinsed in ice-cold saline, and blotted. Blood samples were collected from the pelvic limb vein with the use of capillary pipettes (Capilette, Boehringer Mannheim). To eliminate diurnal effects, the deaths of test and corresponding control animals were pair matched for time and all animals were in the fed state. The control and test animals had comparable body weight.

Tissue preparation. Immediately after sample collection, blood was added to 2 vol of ice-cold 0.5 N perchloric acid and 0.5 vol of ice-cold 10% 5-sulfosalicylic acid contained in separate Eppendorf tubes. The resultant mixtures were immediately vortexed. Eppendorf tubes containing the mixture were then briefly centrifuged at 10,000 *g* at 4°C. Immediately after the centrifugation, the clear protein-free supernatant obtained from 5-sulfosalicylic acid-treated blood was neutralized, reacted with 2-vinylpyridine to derivatize GSH as recommended by Griffith (10), and frozen at −80°C for the determination of oxidized GSH (GSSG). The protein-free supernatant obtained from the perchloric acid-treated blood was stored at −20°C for the determination of total GSH (TGSH). The plasma was processed for the determination of thiobarbituric acid-reactive substances (TBARS) according to the details described previously (36). For TGSH assay, tissues were homogenized in 0.5 N perchloric acid as described before (33). For the determination of GSSG, tissues were homogenized in 10 mM *N*-ethylmaleimide solution (10 mM *N*-ethylmaleimide in 100 mM potassium phosphate buffer containing 17.5 mM Na_2EDTA, pH 6.5), centrifuged, and extracted with C_{18} Sep-Pak cartridges (Waters, Millipore, Milford, MA) as recommended by Adams et al. (1). Tissue lipid peroxide was determined according to the method of Uchiyama and Mihara (39). To avoid ex vivo autoxidation of lipids, frozen tissue samples were homogenized in an acidic medium (pH 2.0) containing 1% H_3PO_4, 0.6% thiobarbituric acid (TBA), and 0.04% ethanolic butylated hydroxytoluene. TBARS, extracted in *n*-butanol, was assayed spectrophotometrically. Protein content of tissue extracts was determined according to the method of Bradford (4).

Biochemical analyses. TGSH in the acidified blood extract was determined by a GSSG reductase recycling method as described previously (33). GSSG, from the 2-vinylpyridine-treated blood extract, was determined according to the method of Griffith (10). GSSG reductase (type III), GSSG (grade III), reduced GSH (free acid), β-NADPH (tetrasodium salt, type III) and 5,5'-dithio-bis(2-nitrobenzoic acid) were purchased from Sigma Chemical. 2-Vinylpyridine was obtained from Aldrich-Chemie (Steinhein, Germany). All other chemicals were of the highest analytic grade. A Shimadzu ultraviolet-240 double-beam spectrophotometer or the Perkin-Elmer Lambda 2 ultraviolet/visible spectrophotometer was used. Determination of TBARS in the plasma was carried out spectrophotometrically. Collected blood was treated and frozen as described above. On the day of measurement, samples were thawed on ice and 1 vol of the sample was added to 1 vol of TBA solution. TBA solution was prepared by dissolving 1% (wt/vol) TBA in distilled deionized water initially passed through a Chelex membrane to remove traces of free metal contamination. Just before use, the TBA solution was deaerated by passing helium through it. The pH of the mixture of the sample and TBA solution was adjusted to 1.5 using HCl. The pH-adjusted mixture was placed in boiling water for 30 min. The reaction mixture was then cooled to room temperature, and absorbance was measured at 532 nm. For the standard curve, a 10 mM stock of 1,1,3,3-tetramethoxypropane (Sigma Chemical) was prepared by adding 1 mmol of the compound to 100 ml of 1% (vol/vol) sulfuric acid. The mixture was left at room temperature for 2 h to allow complete hydrolysis of the reference compound. Standard solution of different strengths (0.5–10 μM) of tetramethoxypropane was prepared to obtain the standard curve.

Statistical analysis. The SPSS/PC+ (SPSS, Chicago, IL) software was used. Significance of changes in means was tested with one-way analysis of variance. Subsequently, the location of significance was determined by Scheffé's test. Normality and homogeneity of distribution were estimated by Kolmogorov-Smirnov goodness of fit test and Bartlett-Box *F* test, respectively. Results are expressed as means.

RESULTS

Fate of intraperitoneally administered GSH. In *experiment 1*, data from the C1 and C2 rats were not different from each other (results not shown), i.e., no ultradian variation was observed in the 4-h interval. Therefore, results from C1 and C2 rats were pooled to obtain the control data [represented as the 0 h data (n = 12) in Figs. 1 and 2]. Injection of GSH solution resulted in a rapid appearance of GSH in the plasma. At 0.5 h postinjection, plasma TGSH increased by two orders of magnitude (Fig. 1). After this response, a rapid clearance of plasma TGSH was observed. At 24 h postinjection, plasma TGSH was restored to the preinjection control level. Figure 1, *inset*, illustrates that the excess GSH in the plasma after the injection was rapidly oxidized. The plasma GSSG/TGSH ratio increased steeply 0.5–4 h after the injection (Fig. 2). The single dose of GSH injection did not have any significant effect on any of the other tissues studied (Fig. 1). Although at 0.5 h postinjection blood, liver, heart, kidney, and red gastrocnemius muscle TGSH levels were marginally higher than the levels in P

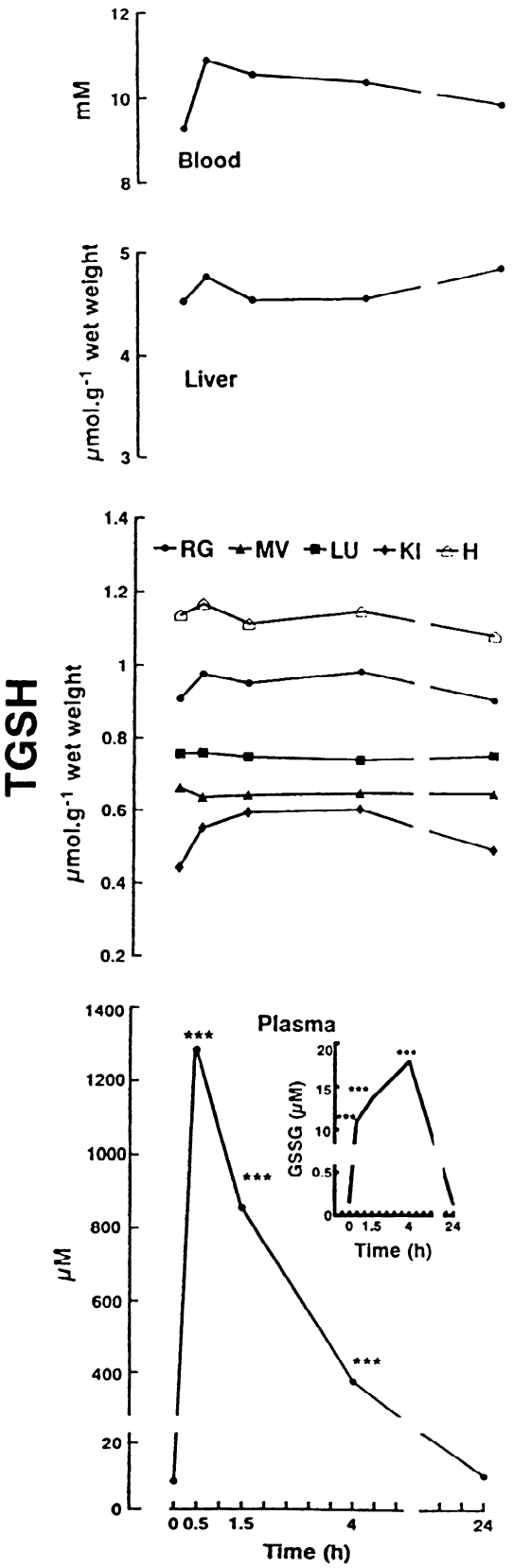

FIG. 1. Effect of intraperitoneal glutathione (GSH; 1 g/kg, 0.8–1.0 ml) injection on total glutathione [TGSH; GSH + oxidized GSH (GSSG), in GSH equivalents] levels at rest. 0 h, Placebo saline-injected control rats killed 0.5 or 4 h after injection (0.8–1.0 ml of placebo saline). GSH-administered rats were killed 0.5, 1.5, 4, or 24 h after single dose of injected GSH. *Inset*: plasma GSSG level in control and GSH-administered rats. Values are means. RG, red gastrocnemius muscle; MV, mixed vastus lateralis muscle; LU, lung; KI, kidney; H, heart. Significantly different from control (0 h) value: *** $P < 0.001$.

rats, none of the changes was significant (Fig. 1). However, after the repeated administration of GSH for three consecutive days (G5 rats), blood and kidney TGSH levels increased considerably. No effect of repeated GSH administration on the TGSH levels of liver, red gastrocnemius muscle, mixed vastus lateralis muscle, heart, or lung was observed (G5 rats; Fig. 3).

Effect of GSH and NAC supplementation. In P rats, exhaustive exercise resulted in a significant loss of hepatic TGSH (SE vs. SR; Fig. 4). After exercise, significant decreases in the TGSH level of the skeletal muscle (red gastrocnemius and mixed vastus lateralis) and heart

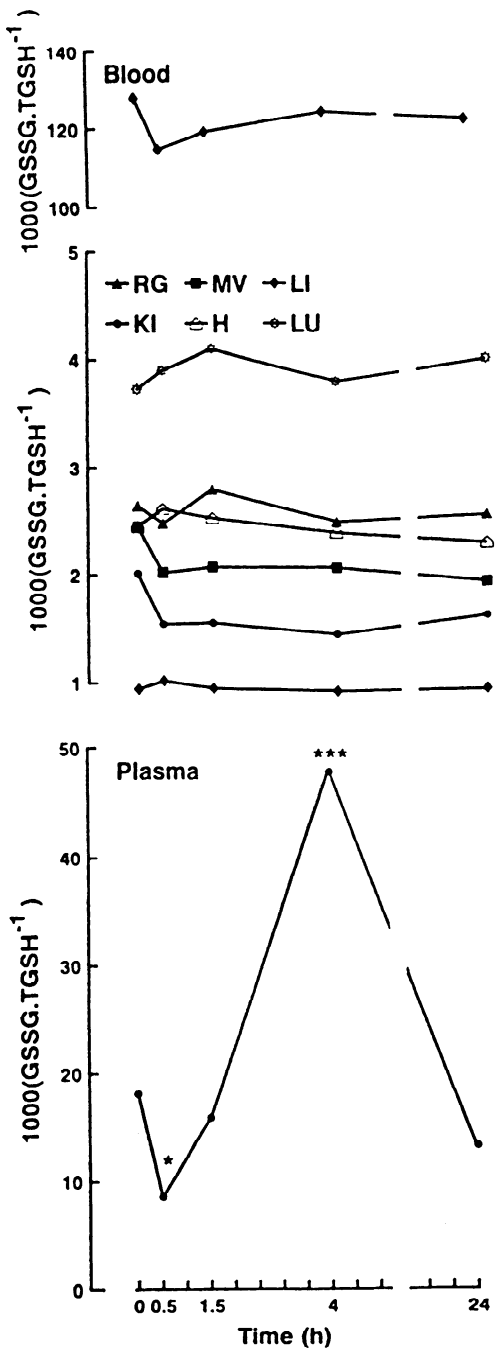

FIG. 2. Effect of intraperitoneal GSH (1 g/kg) injection on oxidation-reduction state of GSH. Values are means. LI, liver; GSSG, oxidized GSH or GSH disulfide. Significantly different from control (0 h) value: * $P < 0.05$; *** $P < 0.001$.

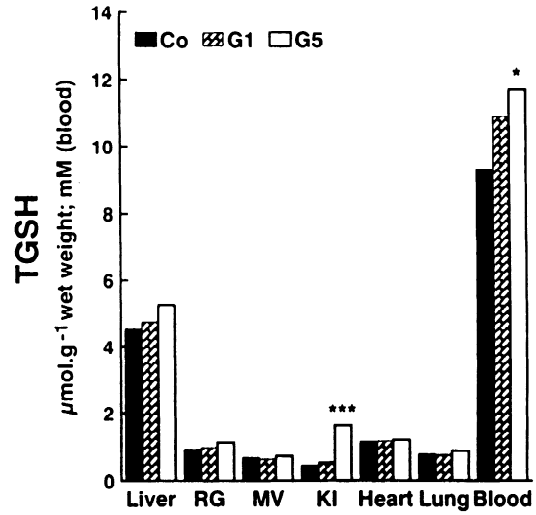

FIG. 3. TGSH levels. Effect of repeated (thrice, at 24-h intervals) intraperitoneal GSH (1 g/kg) injection compared with effect of single injection of GSH. Co, control rats (same as 0 h group of Figs. 1 and 2) killed after single intraperitoneal injection of placebo saline; G1, rats killed 0.5 h after single intraperitoneal injection of GSH (1 g/kg) (same rats as 0.5 h group of Figs. 1 and 2); G5, rats injected with GSH for 3 days, once every day; on 3rd day, G5 rats were killed 0.5 h after last GSH injection. Deaths of Co, G1, and G5 rats were matched for time. Values are means. Significantly different from Co and G1: * $P < 0.05$; *** $P < 0.001$.

were also observed. Compared with those of nonexercised rats, blood and plasma TGSH levels were significantly higher in exercised animals (Fig. 4). GSH or NAC supplementation did not have any additional remarkable effect on exercise-induced changes in TGSH levels in the liver, skeletal muscle, heart, or lung. Blood and plasma exercise TGSH levels were also not additionally affected by NAC supplementation. However, GSH supplementation resulted in a slight increase ($P < 0.055$) in resting blood TGSH level compared with control (GR vs. SR). The exercise-associated increase in blood TGSH level, as seen in the P rats, was not observed in the G rats. As observed in *experiment 1* (Fig. 1), GSH supplementation resulted in a dramatic increase in resting plasma TGSH level compared with control (SR vs. GR; Fig. 4). After a bout of exhaustive exercise, the plasma TGSH level of G rats was remarkably (>50%) decreased (GE vs. GR), indicating a rapid rate of clearance during treadmill running.

Figure 5 illustrates that the exercise bout resulted in a significant increase in the GSSG/TGSH ratio in the skeletal muscle, lung, blood, and plasma (SR vs. SE). Similar increases were also observed in the liver and heart; however, the changes were not significant. Such an observation in the skeletal muscle, lung, blood, and plasma indicates that the exercise challenge caused considerable oxidation of GSH in these tissues. Compared with P rats, the exercise-induced change in the GSSG/TGSH ratio was not different in G rats (GE vs. SE). NAC supplementation did not affect exercise-induced changes in the GSSG/TGSH ratio in the liver, skeletal muscle, heart, or plasma. However, the exercise-induced increase in the lung GSSG/TGSH ratio, as was evident in the P and G rats, was not observed in the NAC-supplemented group. Compared with the P rats, the exercise-induced increase

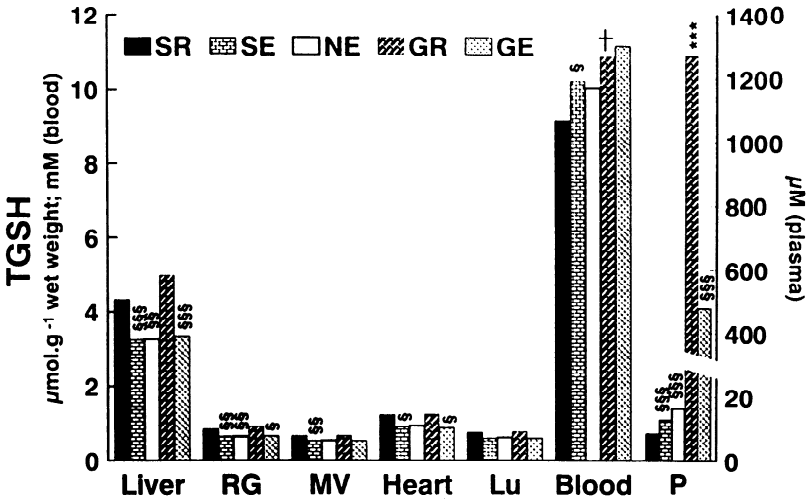

FIG. 4. Exhaustive exercise-induced changes in TGSH levels after GSH and *N*-acetylcysteine (NAC; 1 g/kg ip) supplementation. Values are means. SR, placebo saline-injected nonexercised rats; SE, placebo saline-injected rats killed after exhaustive exercise; NE, NAC-injected rats killed after exhaustive exercise; GR, GSH-injected nonexercised rats; GE, GSH-injected rats killed after exhaustive exercise. Exercise was commenced 0.5 h after injection. For further details see MATERIALS AND METHODS. P, plasma. Significantly different from corresponding nonexercised control value (SE vs. SR, NE vs. SR, GE vs. GR): § $P < 0.05$; §§ $P < 0.01$; §§§ $P < 0.001$. Significantly different due to GSH administration (GR vs. SR): † $P < 0.055$; *** $P < 0.001$.

in the blood GSSG/TGSH ratio was significantly less in NAC-supplemented animals (NE vs. SE).

Exhaustive exercise resulted in a significant increase in the concentration of lipid peroxides (expressed as TBARS) in the skeletal muscle and plasma (SE vs. SR, GE vs. GR); no such changes were seen in the heart (Fig. 6). Neither GSH nor NAC supplementation had any significant effect in reducing exercise-induced lipid peroxidation in the skeletal muscle or plasma.

Compared with that of the P rats, endurance to treadmill running of the G rats and NAC-supplemented rats was the same. All three groups of animals were exhausted in a little over 2 h (Fig. 7).

Effect of BSO treatment. Compared with the PR rats, the BSO administration protocol resulted in an ~50% decrease in the TGSH pools of the liver, lung, blood, and plasma and an 80–90% decrease in the TGSH pools of the skeletal muscle and heart (BR vs. PR; Fig. 8). Unlike the response in the corresponding P rats, exercise did not cause any significant decrease of TGSH levels in the liver, skeletal muscle, or heart of BSO-treated (GSH-deficient) rats. Although exhaustive exercise resulted in a significant increase in plasma TGSH of P rats (PE vs. PR), no such change was observed in the GSH-deficient animals.

Compared with the PR rats, BR rats had lower concentrations of GSSG in the liver, red gastrocnemius muscle, heart, and blood. Although a similar trend was observed in the lung, the difference was not significant (Fig. 9). Compared with the response in PR rats, GSSG concentration in the liver, skeletal muscle, blood, and plasma in PE rats was significantly higher. In contrast to the result obtained from the P rats, exercise to exhaustion of GSH-deficient rats did not cause any significant increase in GSSG concentration of the skeletal muscle, blood, or plasma. However, such an exercise-caused increase in GSSG concentration could be observed in the liver of GSH-deficient rats.

Figure 10 illustrates the observation that, although BSO treatment effectively decreased the TGSH pool in all the tissues studied, the treatment did not have any significant impact on the GSSG/TGSH ratio in the liver, heart, lung, blood, or plasma. However, the skeletal muscle GSSG/TGSH ratio was remarkably elevated after BSO treatment (BR vs. PR). Unlike the response in the control group, the exercise-caused increase in the GSSG/TGSH ratio was significant in the red gastrocnemius muscle and heart of the GSH-deficient rats (BE vs. BR). Such increases in the GSSG/TGSH ratio observed in the lung and blood of P rats (PE vs. PR) were, however, not observed in the BSO-treated rats. The exercise-associated increase in the GSSG/TGSH ratio of mixed vastus lateralis muscle and plasma was observed in P rats as well as BSO-treated rats.

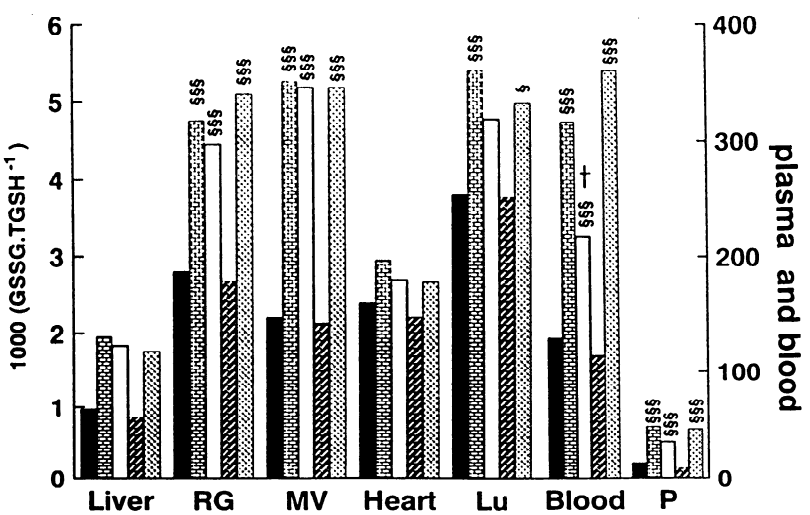

FIG. 5. Exhaustive exercise-induced changes in oxidation-reduction state of GSH after GSH and NAC supplementation (1 g/kg ip). Values are means. Details are same as in Fig. 4. Significantly different from corresponding nonexercised control value (SE vs. SR, NE vs. SR, GE vs. GR): § $P < 0.05$; §§§ $P < 0.001$. † Compared with SR rats, exercise-induced increase in blood was significantly lower in NE rats [i.e., (NE − SR) < (SE − SR), $P < 0.055$].

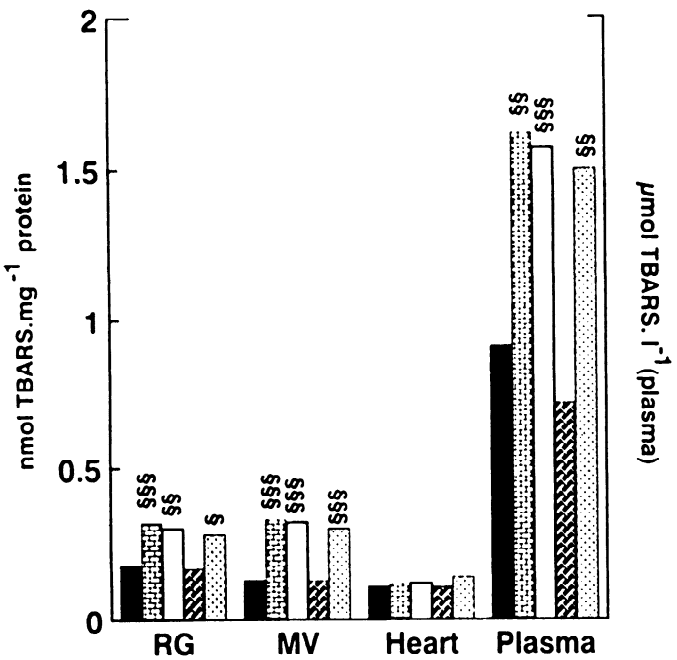

FIG. 6. Exhaustive exercise-induced changes in level of lipid peroxides [as assayed by thiobarbituric acid-reactive substances (TBARS)] in plasma and tissue of NAC- and GSH-supplemented rats. Values are means. Details are same as in Fig. 4. Significantly different from corresponding nonexercised control value (SE vs. SR, NE vs. SR, GE vs. GR): § $P < 0.05$; §§ $P < 0.01$; §§§ $P < 0.001$.

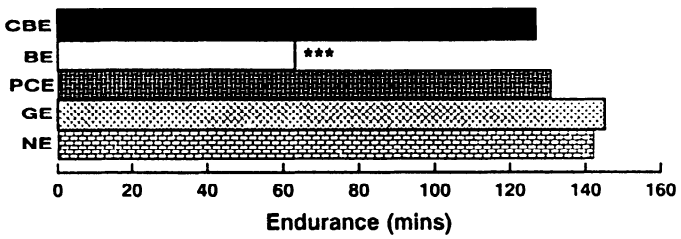

FIG. 7. Endurance to exhaustive treadmill running. Exhaustion due to exercise was identified by loss of righting reflex of rats on being turned on their back. Values are means. CBE, placebo saline-treated control rats [matched for L-buthionine-(*S,R*)-sulfoximine (BSO) treatment of *experiment 3*]; BE, GSH-deficient rats (produced by BSO treatment); PCE, placebo saline-treated control rats (matched for single-dose GSH and NAC injection of *experiment 2*); GE, GSH-administered rats (1 g/kg ip of GSH); NE, NAC-administered rats (1 g/kg ip of NAC). For further details of BSO, GSH, and NAC treatments and of preparation of corresponding placebo-treated control rats, see MATERIALS AND METHODS. Significantly different from all other groups: *** $P < 0.001$.

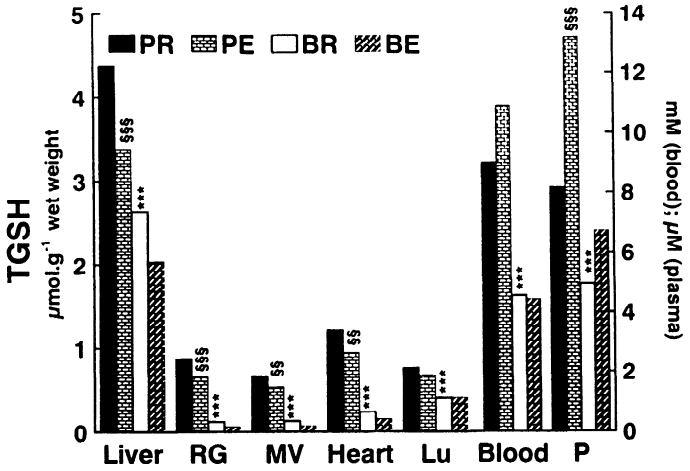

FIG. 8. Influence of BSO treatment (6 mmol/kg ip, 2 times/day, for 4 days) and exhaustive exercise on TGSH. Values are means. PR, placebo-treated nonexercised rats killed 0.5 h after last injection (duration matched with deaths of BR rats); PE, placebo-treated rats killed immediately after exhaustive exercise on treadmill; BR, BSO-treated nonexercised rats killed 0.5 h after last injection; BE, BSO-treated rats killed immediately after exhaustive exercise on treadmill. Significantly different from corresponding nonexercised control value (PE vs. PR): §§ $P < 0.01$; §§§ $P < 0.001$. Significantly different due to BSO treatment (BR vs. PR): *** $P < 0.001$.

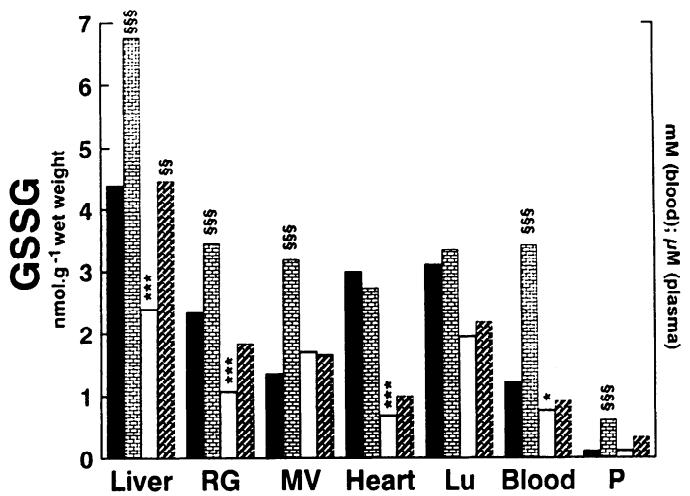

FIG. 9. Influence of BSO treatment and exhaustive exercise on GSSG (GSH disulfide). Values are means. Details are same as in Fig. 8. Significantly different from corresponding nonexercised control value (PE vs. PR, BE vs. BR): §§ $P < 0.01$; §§§ $P < 0.001$. Significantly different due to BSO treatment (BR vs. PR): * $P < 0.05$; *** $P < 0.001$.

Compared with the response in PR rats, the concentration of lipid peroxides (TBARS) in skeletal muscle, heart, and plasma was remarkably higher in BR rats (Fig. 11). The exhaustive exercise-dependent increase in TBARS of red gastrocnemius muscle, mixed vastus lateralis muscle, and plasma, which was evident in the P rats, was not observed in GSH-deficient animals.

BSO treatment resulted in an almost 50% reduction in endurance to uphill treadmill running (BE vs. PE; Fig. 7).

DISCUSSION

Intraperitoneal administration of GSH. The breakdown and uptake of extracellular GSH are catalyzed by the membrane-bound enzyme γ-glutamyl transpeptidase (GGT; EC 2.3.2.2). In the presence of GGT, cysteine may be translocated into the cell as γ-glutamylcysteine, the precursor of GSH. GGT is abundant in the kidney and accounts for the prevention of glutathionuria (11). However, GGT activity in most other tissues such as the skeletal muscle, heart, and lung is remarkably low (33). Therefore, GSH per se is poorly transported into most animal cells.

Previously it was observed that injection of GSH (5 mmol/kg, ~1.5 g/kg) into BSO-treated mice did not cause any remarkable increase in the GSH level of skeletal muscle and heart (24). Consistent with those findings, we observed no considerable change in skeletal muscle, heart, liver, lung, kidney, or blood TGSH levels after a single injection of GSH (Fig. 1). The GSSG/TGSH ratio

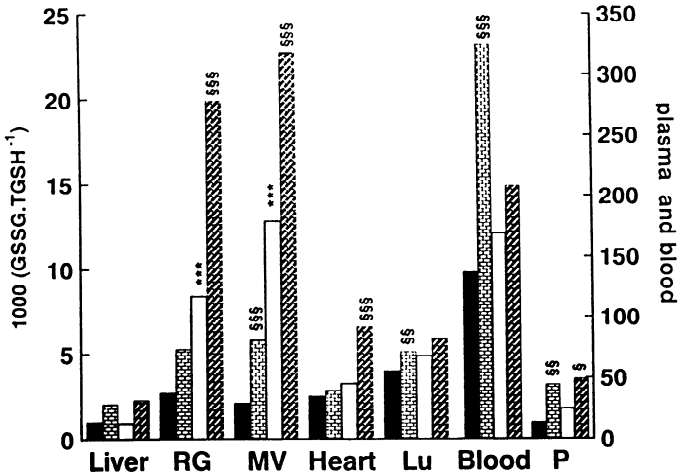

FIG. 10. Influence of BSO treatment and exhaustive exercise on oxidation-reduction state of GSH. Values are means. Details are same as in Fig. 8. Significantly different from corresponding nonexercised control value (PE vs. PR, BE vs. BR): § $P < 0.05$; §§ $P < 0.01$; §§§ $P < 0.001$. Significantly different due to BSO treatment (BR vs. PR): *** $P < 0.001$.

in the above-mentioned tissues also was not altered by the supplementation, which indicates that the single injection of GSH did not affect the GSH status of any of those tissues (Fig. 2). At 0.5 h after the injection, plasma TGSH increased by two orders of magnitude (Fig. 1). However, the oxidation-reduction potential of the injected GSH rapidly changed in the plasma, and consequently a remarkable increase in the plasma GSSG/TGSH ratio was observed (Figs. 1, *inset*, and 2). Therefore, it was evident that plasma failed to provide an environment suitable for maintaining the redox status of such a large TGSH pool. GSSG reductase (EC 1.6.2.4), the enzyme responsible for the reduction of GSSG to GSH, occurs only intracellularly, and there does not appear to be an extracellular mechanism for GSSG reduction. The elevated level of postinjection plasma TGSH rapidly subsided (Fig. 1). Compared with the plasma TGSH level after 0.5 h of GSH injection, the levels after 1.5 and 4 h of injection were ~65 and 30%, respectively. At 24 h after the GSH injection, the plasma TGSH level was restored to the control level. Such observations indicate that excess GSH in the plasma was rapidly cleared. It has been discussed that plasma GSH is rapidly cleared by the kidney and the lung and intestinal epithelia, resulting in a short half time of GSH in the plasma of ~20 min (18). We observed that repeated (thrice, at 24-h intervals) GSH injections increased the renal TGSH level by threefold (Fig. 3). However, such an increase in TGSH level of the kidney [an organ with very high GGT activity (33)] would account for only a small fraction of the total GSH administered. Thus, it is possible that only a minor fraction of the total GSH administered was taken up by cells possessing a powerful capacity for extracellular GSH uptake. The major portion of exogenously administered GSH is likely to have one of two fates: *1*) a small part of it may be split to its constituent amino acids by GGT activity in the serum and *2*) the remaining GSH may be lost through the urine after saturation of the renal capacity to reabsorb GSH. Recently it has been shown that renal GSH depletion is followed by increased urinary excretion of GGT and a decreased capacity of the organ to reabsorb GSH (9). Repeated administration of GSH also resulted in a significant increase in the blood TGSH level, an effect not observed at 0.5 h after a single injection (Fig. 3). GSH content in the erythrocytes is the primary component of blood TGSH. Erythrocytes possess an active machinery for GSH synthesis. Availability of cysteine, a preferred substrate, is a rate-limiting factor in GSH synthesis. It is likely that repeated administration of GSH resulted in a sustained increase in cysteine supply to the blood, which in turn facilitated GSH synthesis in the erythrocytes. In a recent ^{1}H-nuclear magnetic resonance spin-echo study of erythrocytes, it was also shown that although added extracellular GSH may not be directly available to the intracellular compartment of erythrocytes, such GSH can transduce its reducing power to the intracellular compartment by a thiol-disulfide exchange mechanism that sequentially involves sulfur-rich proteins spanning across the erythrocyte membrane (6).

GSH supplementation and exercise. As observed in *experiment 1*, a single injection of GSH had a negligible effect on the GSH status and lipid peroxidation in the tissues (Figs. 1, 2, and 6). Exercise-associated changes in GSH status and lipid peroxidation in the liver, skeletal muscle, heart, and lung of G rats were similar to those of the P rats (Figs. 4 and 6). Exhaustive exercise resulted in a significant mobilization of the hepatic GSH pool, an observation consistent with previous reports by Lew et al. (20) and our group (33). Previously we had hypothesized that during exercise GSH from the liver is released to meet the increased antioxidative need of the active peripheral tissues (33). Exhaustive exercise also caused a significant decrease in the TGSH pool of the skeletal muscle and heart. The observation with muscle is consis-

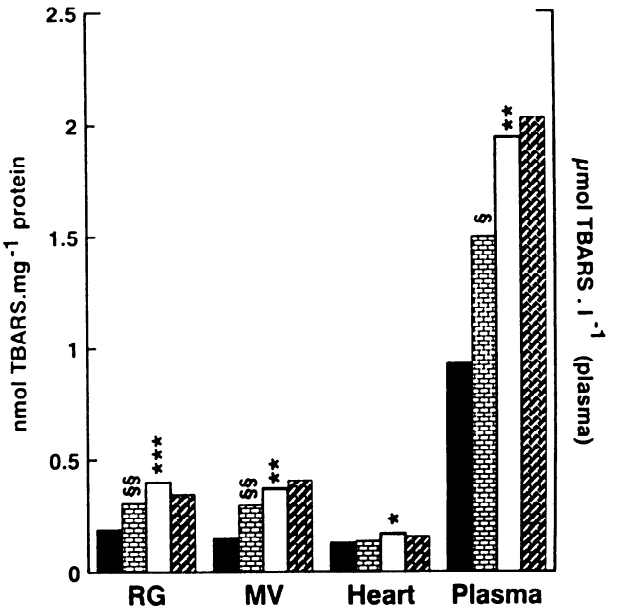

FIG. 11. Influence of BSO treatment and exhaustive exercise of lipid peroxides (as assayed by TBARS). Values are means. Details are same as in Fig. 8. Significantly different from corresponding nonexercised control value (PE vs. PR): § $P < 0.05$; §§ $P < 0.01$. Significantly different due to BSO treatment (BR vs. PR): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

tent with previous reports (20, 33). Skeletal muscle and heart are known to actively expel excess GSSG from the cell to the extracellular compartment (14, 35). It is likely that during exercise, when active tissues are subjected to a considerable extent of oxidative stress, much of the GSH in the skeletal muscle and heart is oxidized to GSSG. Such a contention is supported by our results presented in Fig. 5, which illustrates that the exhaustive exercise bout increased the GSSG/TGSH ratio in the skeletal muscle and heart. Increased GSSG in the skeletal muscle and heart may have been actively effluxed from these tissues, resulting in decreased tissue TGSH levels. Although the TGSH level of the lung remained unaffected by the exercise bout, the GSSG/TGSH ratio increased significantly because of an increased concentration of GSSG in the lung after exercise.

Our finding that the blood TGSH level was increased after exercise is consistent with recent observations of Ji et al. (16) and our group (36). However, the exercise-induced increase in blood TGSH was not significant in G rats. Because the resting blood TGSH level was already higher in GR rats than in SR rats, it is possible that the influence of exercise on blood TGSH was not distinctly revealed in the GR rats. The exhaustive exercise bout resulted in rapid blood GSH oxidation, an effect that has also been recorded in previous studies by our group and other groups (for discussion see Ref. 36). The increase in plasma TGSH after exhaustive exercise is a well-established phenomenon (Fig. 4; Ref. 20). It is suggested that the exercised-induced increase in plasma TGSH is contributed by mainly GSH released from liver and GSSG released from tissues active during exercise (20, 35). Although exhaustive exercise was followed by a significant increase in plasma TGSH, a simultaneous increase in the plasma GSSG/TGSH ratio was also observed (Fig. 5) because of a remarkable increase in the plasma GSSG concentration. The exercise-induced increase in plasma GSSG level is consistent with previous reports (20, 21). As observed in *experiment 1* (Fig. 1), the single injection of GSH led to a massive increase in plasma TGSH. The amount of TGSH cleared from the plasma during the exercise bout (mean duration ~2.3 h) was comparable to that cleared in 3.5 h in the P rats. Thus, we may infer that the exercise bout accelerated the rate of clearance of exogenously administered excess TGSH from the plasma. Because of the extremely high concentration of TGSH in the plasma, it is likely that the renal tubular capacity to reabsorb the thiol was saturated. Thus, the thiol may be expected to be excreted through the urine. During exercise, saturation of the tubular reabsorption process and enhanced glomerular permeability (30) may have contributed to accelerating the clearance of the thiol from plasma. Compared with that of SR rats, the plasma TBARS level of GR rats was slightly lower; however, the difference was not significant (Fig. 6). An extremely high concentration of GSH in the plasma after GSH injection may have contributed to maintaining a favorable redox status of vitamin E, the principal lipid peroxidation chain-breaking antioxidant, and may have decreased lipid peroxidation by GSH-dependent membrane-bound phospholipid hydroperoxide GSH peroxidase (38). The exercise-induced increase in plasma concentration of lipid peroxides in skeletal muscle and plasma observed in this study is in agreement with reports discussed previously (15). The ineffectiveness of administered GSH in controlling the exercise-induced increase in TBARS of skeletal muscle may be explained by the poor availability of the exogenous thiol to the tissues. Lipid peroxides, when formed in tissues, rapidly appear in the plasma and finally pass out through the urine (8). Thus, the exercise-induced increase in lipid peroxides in the plasma may be assumed to reflect lipid peroxidation in the tissues. GSH injection did not have any effect on the exercise-induced increase in tissue TBARS. Therefore, the exercise-induced increase in plasma TBARS was not affected by the supplementation of GSH either (Fig. 6).

NAC supplementation and exercise. NAC is known to have strong antioxidant and nucleophilic properties with little or no side effects in humans (2). Exogenous NAC may act to rescue GSH by promoting GSH synthesis in vivo by providing cysteine and/or directly scavenging ROS (2). However, Moldéus et al. (28) suggested that a rather high concentration of NAC is required for effective results. Thus, a 1 g/kg dose was used in the present study. Compared with the response in the P rats, the single injection of NAC resulted in a significant decrease in exercise-induced blood GSH oxidation. Although the exercise bout caused a significant increase in the blood GSSG/TGSH ratio, compared with the corresponding control values the magnitude of such an effect in the NE rats was significantly less [$(NE - SR) < (SE - SR)$, $P < 0.05$; Fig. 5]. Such an observation is in line with our previous study with humans wherein we observed that NAC supplementation spared maximal exercise-associated blood GSH oxidation (36). In the human study we could obtain a complete sparing of exercise-associated blood GSH oxidation; maximal exercise-associated blood GSH oxidation was not at all observed in NAC-supplemented subjects (36). However, in the present study, significant increases in the blood GSSG level and GSSG/TGSH ratio were observed in the NE rats. Such a difference in observation between the two studies may be primarily attributed to the remarkable difference in exercise duration. The maximal exercise bout in humans lasted for a mean duration of only ~14 min, whereas the exhaustive exercise of rats endured for >2 h (Fig. 7). Thus, it is likely that the exhaustive exercise-induced oxidative stress lasted for a duration long enough to exceed the capacity of exogenous NAC to spare blood GSH oxidation.

We have previously discussed that the GSH pool of the erythrocytes is the primary contributor to blood TGSH (36). Therefore, oxidation of GSH in the blood chiefly represents the transformation of GSH to GSSG in the erythrocytes. Udupi and Rice-Evans (40) have demonstrated that NAC is particularly effective in protecting erythrocytes exposed to oxidative stress. Such a property of NAC may serve as the principal factor underlying the capacity of the thiol compound to spare exercise-induced blood GSH oxidation (36). Comparable to its effect on the GSH status, exogenous NAC did not have any effect on exercise-induced changes in TBARS in the skeletal muscle or plasma (Fig. 6). Such an observation may be explained by the poor availability of NAC, a hydrophilic compound, to the tissue membrane lipid phase. Figure 5

illustrates that, compared with the SR rats, the lung GSSG/TGSH ratio was significantly higher in the SE rats. However, such an exercise-dependent increase in the lung GSSG/TGSH ratio was not significantly manifested in NE rats, indicating a tendency of NAC to protect against oxidative stress in the lung. Pharmacokinetic studies with exogenous [^{35}S]NAC demonstrated radioactivity in lung tissue biopsies, indicating that exogenous NAC is available to the lung tissue (31). NAC, available to the lung, is known to be particularly effective in protecting the tissue against oxidative stress by a number of possible mechanisms (28).

GSH deficiency and exercise. Cellular GSH may be decreased by administering compounds that react with GSH to form conjugates or that oxidize GSH to GSSG; however, such approaches are limited by a lack of specificity of the reagents available and/or, because the effects obtained are transient, are associated with major perturbation of metabolism. The BSO treatment selectively inhibits γ-glutamylcysteine synthetase (EC 6.3.2.2) and turns off intracellular GSH synthesis. BSO does not react with GSH and is nontoxic at high (32 mmol/kg) doses (23, 27).

The BSO treatment employed in the present study resulted in an ~50% decrease in the TGSH pools of the liver, lung, blood, and plasma and an 80–90% decrease in the TGSH pools of the skeletal muscle and heart. Such observations suggest a rapid turnover of GSH in the skeletal muscle and heart. In a previous study with skeletal muscle-derived L6 myoblasts, we observed that supplementation of the growth medium with 1.75 mM BSO resulted in a 90% depletion of intracellular TGSH in 20 h (35). Previously, BSO (2 mmol/kg ip, 2 times/day, and 20 mM in drinking water) given to mice (28–32 g) for 4 days decreased the GSH level in the skeletal muscle and heart to ~20 and 15%, respectively, of the level in the corresponding control rats (24). In another study, BSO (2 mmol/kg ip, 2 times/day) given to 16-day-old rats for 9 days produced the following results (in %control value to which GSH level in that tissue was decreased): liver 36, skeletal muscle 5.7, heart 19, lung 39, and plasma 24 (23). Figure 8 illustrates that the organs actively required during exercise, e.g., the skeletal muscle and heart, were particularly affected by the BSO treatment. Thus, the GSH-deficient animals prepared for this study were well suited as a model for the study of the relative role of endogenous GSH in the circumvention of exercise-induced oxidative stress.

Although as observed for the corresponding P rats the hepatic TGSH level of BSO-treated rats decreased after exercise, the change was not significant (Fig. 8). It is possible that the capacity of the liver, a major organ of GSH synthesis and storage, to release GSH during exercise is influenced by the TGSH reserve of the organ. As discussed earlier, exercise challenge triggers the release of vasopressin, a hormone capable of promoting hepatic GSH efflux. Such a response was suggested to be a likely mechanism underlying hepatic GSH mobilization during exhaustive exercise (33). As is evident from Fig. 7, BSO-treated rats could run for less than one-half the duration of the corresponding P rats. Such a remarkable difference in the duration of exercise challenge may also be a crucial factor underlying the contrasting response of tissue GSH status to exercise in BSO-treated and P rats. The decrease in TGSH from the skeletal muscle after exercise is probably due to considerable oxidation of GSH in that tissue and subsequent export of excess GSSG to the general circulation (35). Unlike the response in the P rats, exhaustive exercise of BSO-treated rats did not result in a significant increase in GSSG concentration in the skeletal muscle or blood (Fig. 9). Although statistically nonsignificant, after exercise the muscle TGSH and GSSG levels slightly decreased and increased, respectively; such changes, in combination, were manifested as a significant increase in the GSSG/TGSH ratio after exercise (Fig. 10). A previous study has documented that BSO treatment does not affect GSSG reductase activity (37). It is plausible that, because the skeletal muscle and blood TGSH pools of BSO-treated rats were very small, GSSG reductase and NADPH stores in the skeletal muscle and erythrocytes were adequate to keep the exercise-induced increase in tissue GSSG level from being revealed. Effective maintenance of the GSH redox status during exercise decreases GSSG efflux from the skeletal muscle, an organ suggested to be crucial in influencing the plasma GSH status (18, 35). In contrast to the P rats, the exercise bout did not result in a significant increase in the plasma GSSG/TGSH ratio in BSO-treated rats (Fig. 10). Previously it has been suggested that the exercise-induced increase in plasma TGSH is contributed by mainly the release of GSH from the liver (16, 20, 33). Because the exhaustive exercise challenge of BSO-treated rats was not able to effectively mobilize the hepatic TGSH reserve (Fig. 8), the exercise-associated increase in plasma TGSH was not evident in the GSH-deficient rats. Compared with the response in the corresponding P rats, the level of lipid peroxides in the skeletal muscle, heart, and plasma of GSH-deficient rats was significantly higher (Fig. 11). GSH is well established as being important in the maintenance of a favorable redox milieu of the critical lipid peroxidation chain-breaking antioxidant vitamin E (12, 13, 22, 26, 41). Also, the protective action of phospholipid hydroperoxide-GSH peroxidase against membrane-damaging lipid peroxidation has been directly demonstrated (38). Thus, by a number of mechanisms, direct and indirect, GSH may control lipid peroxidation. GSH deficiency is also known to decrease tissue ascorbate levels (25), a key antioxidant compound also known to regenerate vitamin E from its oxidized byproduct, the chromanoxyl radical (7, 26, 41). Apparently, the decrease in TGSH reserve of the tissues of BSO-treated rats weakened the antioxidative defense capacity to such an extent that lipid peroxidative processes in the tissues could not be adequately controlled and that such an effect was reflected as high tissue and plasma TBARS. In contrast to the level observed in the P rats, the exercise bout did not result in a significant increase in tissue and plasma TBARS in GSH-deficient rats (Fig. 11). Such a difference is likely to be explained by the remarkable difference in endurance to treadmill running between the two groups. It may be expected that the duration of exercise was not long enough to obtain a significant increase in tissue TBARS. It is also possible that, because tissue TBARS levels of nonexercised GSH-

deficient rats were already so high, the exercise-induced changes in tissue TBARS were not distinctly manifested.

Endurance to exhaustive exercise. Previous studies have shown that exogenous GSH significantly increases endurance to swimming (5, 29). In the present study, neither GSH nor NAC administration before 0.5 h of the commencement of exercise had any considerable effect on endurance to uphill treadmill running (Fig. 7). Single intraperitoneal injection of the compounds barely had any effect on the GSH status of the tissues (Figs. 1, 2, 4, and 5). The level of TBARS in the tissues and plasma at rest and after exercise also was not affected by exogenously supplied GSH or NAC.

Previous studies that tested the effect of exogenous GSH on endurance to physical exercise employed an exercise protocol distinctly different from that used in the present study. The exercise challenge used in those studies was in the form of swimming, and the duration of the exercise was brief (<5 min) compared with the exercise duration of the present study (5, 29). Such a difference in the type of exercise challenge disallows a direct comparison of the results of those previous studies with the findings of this investigation. Compared with the P rats, endurance to exhaustion of BE rats was reduced by one-half (Fig. 7). Such an observation suggests a critical role of endogenous GSH in the circumvention of exercise-induced oxidative stress and as a determinant of exercise performance merit. Thus, effective approaches in increasing tissue GSH pools are likely to be important in controlling exercise-induced oxidative stress and perhaps also in enhancing physical performance.

Previous experimental studies have demonstrated that lipophilic GSH monoesters are more effective than GSH as a delivery agent of GSH to tissues (27). However, metal ion contamination of GSH monoesters remarkably decreased the capacity of the compound to serve as a GSH-delivering agent. Also, certain forms of esterified GSH such as the GSH dimethyl ester appeared to be toxic to mice (19). Very recently Levy et al. (19) reported that GSH diethyl ester may serve as the GSH-delivery agent of choice, especially for those species that lack GSH diester α-esterase in the plasma (e.g., humans but not rats or mice; Ref. 19). GSH diester was nontoxic to mice and hamsters. However, the diester is yet to be tested in in vivo studies with humans.

In summary, the study presents the first evidence regarding *1*) how a single dose of GSH and NAC administration may affect exercise-induced changes in tissue GSH status and lipid peroxidation and endurance to long distance running and *2*) the relative role of endogenous GSH in the circumvention of exercise-induced oxidative stress and in determining exercise performance. Intraperitoneally injected GSH per se was poorly available to tissues and did not influence exercise-induced oxidative stress or endurance to long-duration exhaustive treadmill run. NAC decreased exercise-induced GSH oxidation in the lung and blood. The GSH deficiency model used in this study overtly revealed that the endogenous GSH pool of tissues is critically important in the circumvention of exercise-induced oxidative stress and maintenance of physical performance.

This study was supported by research grants from the Section on Exercise Medicine, Finnish Ministry of Education (Dnro 236/722/92 and 28/722/93); Juho Vainio Foundation, Helsinki; and The University of Kuopio (590.22.94; 9307-5/HKP 213-4/va 8026-2) to C. K. Sen. M. Atalay is also affiliated with the Dept. of Physiology, Faculty of Medicine, University of Ankara, Turkey.

Address for reprint requests: C. K. Sen, Snellmania no. 3016, Dept. of Physiology, Univ. of Kuopio, FIN-70211, Kuopio, Finland.

Received 25 January 1994; accepted in final form 27 June 1994.

REFERENCES

1. **Adams, J. D., B. H. Lauterburg, and J. R. Mitchell.** Plasma glutathione and glutathione disulfide in the rat: regulation and response to oxidative stress. *J. Pharmacol. Exp. Ther.* 227: 749–754, 1983.
2. **Aruoma, O. I., B. Halliwell, B. M. Hoey, and J. Butler.** The antioxidant action of *N*-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radical Biol. Med.* 6: 593–597, 1989.
3. **Barclay, J. K., and M. Hansel.** Free radicals may contribute to oxidative skeletal muscle fatigue. *Can. J. Physiol. Pharmacol.* 69: 279–284, 1991.
4. **Bradford, M.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254, 1976.
5. **Cazzulani, P., M. Cassin, and R. Ceserani.** Increased endurance to physical exercise in mice given oral reduced glutathione (GSH). *Med. Sci. Res.* 19: 543–544, 1991.
6. **Ciriolo, M. R., M. Paci, M. Sette, A. De Martino, A. Bozzi, and G. Rotilio.** Transduction of reducing power accross the plasma membrane by reduced glutathione. A ^{1}H-NMR spin-echo study of intact human erythrocytes. *Eur. J. Biochem.* 215: 711–718, 1993.
7. **Constantinescu, A., D. Han, and L. Packer.** Vitamin E recycling in human erythrocyte membrane. *J. Biol. Chem.* 268: 10906–10913, 1993.
8. **Draper, H. H., L. Polensek, M. Hadley, and L. G. McGirr.** Urinary malonaldehyde as an indicator of lipid peroxidation in the diet and in the tissues. *Lipids* 19: 836–843, 1984.
9. **Gemba, M., T. Yamaguchi, K. Kambara, A. Suzuki, and Y. Kawai.** Increases in urinary excretion in rats depleted of glutathione inhibited by scavenger of oxygen free radicals. *J. Pharmacobio-Dyn.* 15: 513–518, 1992.
10. **Griffith, O. W.** Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.* 106: 207–212, 1980.
11. **Griffith, O. W., and A. Meister.** Excretion of cysteine and gamma-glutamylcysteine moieties in human and experimental animal gamma-glutamyl transpeptidase deficiency. *Proc. Natl. Acad. Sci. USA* 77: 3384–3387, 1980.
12. **Haenen, G. R. M. M., and A. Bast.** Protection against lipid peroxidation by a microsomal glutathione-dependent labile factor. *FEBS Lett.* 159: 24–28, 1983.
13. **Haenen, G. R. M. M., J. N. L. Tai Tin Tsoi, N. P. E. Vermeulen, H. Timmerman, and A. Bast.** 4-Hydroxy-2,3-*trans*-nonenal stimulates microsomal lipid peroxidation by reducing the glutathione-dependent protection. *Arch. Biochem. Biophys.* 259: 449–456, 1987.
14. **Ishikawa, T., M. Zimmer, and H. Sies.** Energy-linked cardiac transport system for glutathione disulfide. *FEBS Lett.* 200: 128–132, 1986.
15. **Jenkins, R. R.** Symposium: oxidant stress, aging, and exercise. *Med. Sci. Sports Exercise* 25: 210–236, 1993.
16. **Ji, L. L., A. Katz, M. Griffiths, and M. Spencer.** Blood glutathione status during exercise: effect of carbohydrate supplementation. *J. Appl. Physiol.* 74: 788–792, 1993.
17. **Kehrer, J. P.** Free radicals as mediators of tissue injury and disease. *Crit. Rev. Toxicol.* 23: 21–48, 1993.
18. **Kretzschmar, M., U. Pfeifer, G. Machnik, and W. Klinger.** Glutathione homeostasis and turnover in the totally hepatectomized rat: evidence for a high glutathione export capacity by extrahepatic tissues. *Exp. Toxicol. Pathol.* 44: 273–281, 1992.
19. **Levy, E. J., M. E. Anderson, and A. Meister.** Transport of glutathione diethyl ester into human cells. *Proc. Natl. Acad. Sci. USA* 90: 9171–9175, 1993.
20. **Lew, H., S. Pyke, and A. Quintanilha.** Changes in glutathione

status of plasma, liver and muscle following exhaustive exercise in rats. *FEBS Lett.* 185: 262–266, 1985.

21. **Lew, H., and A. Quintanilha.** Effects of endurance training and exercise on tissue antioxidative capacity and acetaminophen detoxification. *Eur. J. Drug Metab. Pharmacokinet.* 16: 59–68, 1991.
22. **Marcus, S. R., M. V. Chandrakala, and H. A. Nadiger.** Interaction between vitamin E and glutathione in rat brain: effect of acute alcohol administration. *J. Nutr. Biochem.* 4: 336–340, 1993.
23. **Mårtensson, J., A. Jain, E. Stole, W. Frayer, P. A. M. Auld, and A. Meister.** Inhibition of glutathione synthesis in the newborn rat: a model for endogenously produced oxidative stress. *Proc. Natl. Acad. Sci. USA* 88: 9360–9364, 1991.
24. **Mårtensson, J., and A. Meister.** Mitochondrial damage in muscle occurs after marked depletion of glutathione and is prevented by giving glutathione monoester. *Proc. Natl. Acad. Sci. USA* 86: 471–475, 1989.
25. **Mårtensson, J., and A. Meister.** Glutathione deficiency decreases tissue ascorbate levels in newborn rats: ascorbate spares glutathione and protects. *Proc. Natl. Acad. Sci. USA* 88: 4656–4660, 1991.
26. **McCay, P. B.** Vitamin E: interactions with free radicals and ascorbate. *Annu. Rev. Nutr.* 5: 323–340, 1985.
27. **Meister, A.** Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. *Pharmacol. Ther.* 51: 155–194, 1991.
28. **Moldéus, P., I. A. Cotgreave, and M. Berggren.** Lung protection by a thiol containing antioxidant: *N*-acetylcysteine. *Respiration* 50, *Suppl.* 1: 31–42, 1986.
29. **Novelli, G. P., S. Falsini, and G. Bracciotti.** Exogenous glutathione increases endurance to muscle effort in mice. *Pharmacol. Res.* 23: 149–155, 1991.
30. **Poortmans, J. R., M.-F. Engels, M. Sellier, and R. Leclercq.** Urine protein excretion and swimming events. *Med. Sci. Sports Exercise* 23: 831–835, 1991.
31. **Rodenstein, D., A. Decoster, and A. Gazzangia.** Pharmacokinetics of oral acetylcysteine: absorption, binding and metabolism in patients with respiratory disorders. *Eur. J. Respir. Dis.* 61, *Suppl.* 111: 383–387, 1980.
32. **Ruffmann, R., and A. Wendel.** GSH rescue by *N*-acetylcysteine. *Klin. Wochenschr.* 69: 857–862, 1991.
33. **Sen, C. K., E. Marin, M. Kretzschmar, and O. Hänninen.** Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization. *J. Appl. Physiol.* 73: 1265–1272, 1992.
34. **Sen, C. K., L. Packer, and O. Hänninen (Editors).** *Exercise and Oxygen Toxicity.* Amsterdam: Elsevier Science. In press.
35. **Sen, C. K., P. Rahkila, and O. Hänninen.** Glutathione metabolism in skeletal muscle derived cells of the L6 line. *Acta Physiol. Scand.* 148: 21–26, 1993.
36. **Sen, C. K., T. Rankinen, S. Väisänen, and R. Rauramaa.** Oxidative stress after human exercise: effect of *N*-acetylcysteine supplementation. *J. Appl. Physiol.* 76: 2570–2577, 1994.
37. **Sun, J. D., S. S. Ragsdale, J. M. Benson, and R. F. Henderson.** Effects of the long term depletion of reduced glutathione in mice administered L-buthionine-S,R-sulfoximine. *Fundam. Appl. Toxicol.* 5: 913–919, 1985.
38. **Thomas, J. P., M. Maiorino, F. Ursini, and A. W. Girotti.** Protective action of phospholipid hydroperoxide glutathione peroxidase against membrane-damaging lipid peroxidation. *J. Biol. Chem.* 265: 454–461, 1990.
39. **Uchiyama, M., and M. Mihara.** Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal. Biochem.* 86: 271–278, 1978.
40. **Udupi, V., and C. Rice-Evans.** Thiol compounds as protective agents in erythrocytes under oxidative stress. *Free Radical Res. Commun.* 16: 315–323, 1992.
41. **Wefers, H., and H. Sies.** The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. *Eur. J. Biochem.* 174: 353–357, 1988.