Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization

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SEN, CHANDAN K., EINE MARIN, MICHAEL KRETZSCHMAR, AND OSMO HÄNNINEN. *Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization.* J. Appl. Physiol. 73(4): 1265–1272, 1992.—Female beagle dogs were treadmill trained 40 km/day at 5.5–6.8 km/h, 15% upgrade, 5 days/wk for 55 wk. With training, hepatic and red gastrocnemius (RG) total glutathione increased, glutathione peroxidase (GPX) and glutathione reductase (GRD) increased in all the leg muscles studied, and hepatic glutathione *S*-transferase (GST) activity increased. Joint immobilization (11 wk) did not affect GPX, GRD, and GST of RG, but total glutathione decreased. Male Han Wistar rats were treadmill trained 2 h/day at 2.1 km/h, 5 days/wk for 8 wk. With training, hepatic total glutathione and leg muscle GPX increased but GRD of RG decreased, perhaps because of an increased muscle flavoprotein breakdown during exhaustive training. γ-Glutamyl transpeptidase was higher in the trained leg muscles. Exhaustive exercise decreased muscle γ-glutamyl transpeptidase of only control leg muscle, depleted muscle (lesser extent in trained rats) and liver total glutathione of both groups, decreased GRD only in untrained RG, and increased hepatic GST. Endurance training elevated the antioxidant and detoxicant status of muscle and liver, respectively.

glutathione peroxidase; glutathione reductase; glutathione *S*-transferase; γ-glutamyl transpeptidase; exercise and oxidative stress; antioxidant; detoxicant; dog; rat

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ENDURANCE EXERCISE may contribute to a two- to threefold increase in free radical concentrations of the muscle and liver (9), which may result in a considerable amount of histological disintegration. Such high concentration of reactive metabolites may contribute to oxidative skeletal muscle fatigue (4). Glutathione (L-γ-glutamyl-L-cysteinylglycine) is well established as being important in the circumvention of cellular oxidative stress (30). Glutathione peroxidase (GPX, EC 1.11.1.9) is specific for its hydrogen donor reduced glutathione (GSH) but may use a wide range of substrates extending from H_2O_2 to organic hydroperoxides. GSH is a major cellular electrophile conjugator as well. Glutathione *S*-transferases (GST, EC 2.5.1.18) catalyze the reaction between the -SH group of GSH and potential alkylating agents, thereby neutralizing their electrophilic sites and rendering them more water soluble. Synthesis of GSH is a two-step process; both reactions are ATP dependent. γ-Glutamyl cysteine synthase (GCS, EC 6.3.2.2) catalyzes the formation of the dipeptide γ-glutamyl cysteine, and subsequently the addition of glycine is catalyzed by glutathione synthase (GSHS, EC 6.3.2.3). Substrates for such synthesis are provided both by direct amino acid transport and by γ-glutamyl transpeptidase (GGT, EC 2.3.2.2), which couples the γ-glutamyl moiety to a suitable amino acid acceptor for transport into the cell. GSH is also generated intracellularly from its oxidized form glutathione disulfide (GSSG, produced as a by-product of GPX reaction) by glutathione reductase (GRD, EC 1.6.2.4) activity, which is coupled to a series of interrelated reactions. Thus GSH, GCS, GSHS, GPX, GRD, GST, and GGT are collectively the critical determinants of the glutathione homeostasis of an organ.

Endurance training and exercise-dependent alteration of certain aspects of glutathione metabolism in skeletal muscles and liver have been investigated in rats (15–17, 21–23, 28, 34). Lew et al. (22) reported that exhaustive exercise decreases both liver and muscle glutathione consistently. In a later report (28), they suggested the possibility of a far more severe hepatic GSH depletion after exhaustive exercise but observed no significant fall in skeletal muscle total glutathione content. The fact that the rats used were of the same strain and of very close age groups invites further clarification of those interesting findings. Recently, they reported the effect of endurance training and subsequent exercise on not only GSH and GSSG but also on the GPX-GRD cycle (23). Interestingly, endurance training not only augmented GPX and GRD activities but also decreased the severity of total glutathione depletion in skeletal muscles caused by a single bout of exercise. A similar increase in GPX activity after training was also observed by Laughlin et al. (21). They suggested a dependence of skeletal muscle GPX activity on oxidative fiber population. Ji et al. (16) observed a significant increase of skeletal muscle GPX on training and also subsequent to a single bout of exercise, the latter effect being more pronounced in untrained rats. However, no significant effect of a single bout of exercise was observed on hepatic GPX of sedentary rats (20). Thus there is a definite indication that endurance training and exercise have a profound influence on skeletal muscle and hepatic glutathione redox cycle. Any effect of training or an acute bout of exercise on hepatic or muscle GST could not be observed by Ji et al. (17). However, Vani et al. (34) reported a significant increase in hepatic GST activity subsequent to swimming training. A tendency of the hepatic GST to increase after a single bout of swimming exercise was also observed; however, the change was not significant (34). We

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were interested in a simultaneous study of the responses of GSH, GCS, GSHS, GPX, GRD, GST, and GGT to endurance training and exhaustive exercise because this could provide a better understanding of the response of skeletal muscle and liver GSH homeostasis as a whole. This approach was of particular interest in view of the wide array of implications of cellular glutathione (26). Unlike the response in the rat muscles (pilot study), both the synthesizing enzymes were observed to be active in the skeletal muscles of beagle dogs (unpublished observation). Because no relevant dog study has yet been reported, we decided to study the effect of training on hepatic and skeletal muscle glutathione homeostasis in dogs and rats. Skeletal muscle citrate synthase (CS, EC 4.1.3.7) activity was used as a metabolic marker of effective aerobic training. We have also studied the influence of a single bout of exhaustive exercise in the rat experiment. The effect of joint immobilization on the red gastrocnemius muscle of a second group of dogs was studied to determine whether skeletal muscle glutathione status is affected by long-term physical inactivity. This muscle was chosen because it has a high oxidative fiber population. With exercise, the oxygen uptake of the dog gastrocnemius muscle has been shown to increase 5- to 10-fold with a 2- to 4.5-fold increase in blood flow and a 4- to 6-fold increase in glucose uptake (10).

MATERIALS AND METHODS

Animals, exercise, and immobilization. For the training experiment with dogs, 22 female beagle dogs (Shamrock, England; National Laboratory Animal Center, Kuopio), 15 wk old, were divided into two groups: trained (TR, n = 10) and untrained (UT, n = 12). The UT group was comprised of age-matched sisters of each runner. For the first 10 wk, the TR group was accustomed to running on a 10-track treadmill for dogs (University of Kuopio, Finland) at a speed of 0.5–4.0 km/h at 15% uphill grade. During the next 30 wk (5 days/wk), the running distance was linearly increased to 40 km/day at 5.5–6.8 km/h, 15% uphill grade. This work intensity was maintained for the subsequent 15 wk. The UT group did not participate in the treadmill-training program and was used as controls. For the immobilization experiment, 14 female beagle dogs (Marshall Farms), 29 wk old, were used. The right pelvic limb was immobilized for 11 wk in a light fiberglass cast that immobilized the knee and ankle joints. The limb was tied to the trunk with the knee in 90° flexion and the paw in dorsi flexion at the ankle joint. The gastrocnemius muscle was in a state of moderate tension to delay overall atrophy of the muscle (5). The use of light fiberglass cast just over the knee and ankle joints allowed the lower hindlimb muscles to have considerably more freedom of activity than during external fixation or even heavy plaster cast immobilization. This was done because we were interested in studying consequences of long-term physical inactivity rather than of overall muscular atrophy, such as during rigid immobilization. The left leg was used as the paired control. All the dogs were housed in individual stainless steel cages (floor 0.9 × 1.2 m, height 0.8 m) and maintained at 20 ± 2°C room temperature with 10:14-h dark-light cycle and 50–70% humidity. They were fed with commercial dog food (Hankkija, Kolppi, Finland) in portions determined on the basis of a weekly control (TR vs. UT) of weight gain. Tap water was given ad libitum.

For the experiment with rats, 44 male Han Wistar rats, 10 wk old, body weight 275–300 g, were randomly divided into two groups: trained (T, n = 20) and untrained (U, n = 24). The U group was again randomly subdivided into two equal subgroups: untrained rats to be killed at rest (UR) and untrained rats to be killed immediately after exhaustive exercise (UE). During the first 2 wk, the T group was accustomed to treadmill (for small animals) running. The training intensity was linearly increased such that by the beginning of the 3rd wk, the rats ran at a speed of 2.1 km/h, 2 h/day, 5 days/wk. Training at such a work intensity continued until the end of the 8th wk. During the 8th wk, the UE subgroup was also accustomed to treadmill running 1.0–1.2 km/h, 0.5 h/day, for 3 days. This regimen was used to ensure that the rats could run at the assigned work intensity in later experiments but had a minimum training effect.

Tissue collection. In the training experiment with dogs, all dogs were killed at the resting state when they were 70 wk old. TR dogs trained for the final time on the day preceding the day they were to be killed. They were anesthetized (thiopentone sodium; Hypnostan, Leiras, Finland) and subsequently exsanguinated through the vena jugularis externa. Portions of the red gastrocnemius muscle (RG), extensor carpi radialis muscle (ER), triceps muscle (TP), splenius muscle (SP), and liver were excised immediately, quickly cut into small pieces, and submerged in liquid nitrogen. Before being frozen, the skeletal muscles were freed from visible adipose and connective tissues. In DI, all dogs (aged 40 wk) were killed as described above. RG was collected from both legs as above. All rats were killed by decapitation and exsanguinated. The RG, mixed vastus lateralis muscle (MV), longissimus dorsi muscle (LD), liver, lung, heart, and kidney were quickly excised and collected in liquid nitrogen as described above. The T group was subdivided into two equal subgroups: T rats to be killed at rest (TO) and T rats to be killed immediately after the regular exhaustive exercise (TE). Rats of the TO and UR subgroups were killed at rest. Rats of the TO group were trained for the final time on the day preceding the day they were to be killed. Animals of the TE subgroup were killed immediately after the completion of their usual 2-h running program at 2.1 km/h, whereas those of UE subgroup were killed immediately after an exhaustive exercise at 1.2–1.4 km/h. Exhaustion due to exercise was identified by the loss of righting reflex of rats on being turned on their back. To eliminate diurnal effects, the killing of U and T animals was pair matched for time and all animals were in the fed state. To minimize the influence of age, all animals were killed in the same week.

Tissue preparation and assays. For the determination of total glutathione (GSH + GSSG), tissues were homogenized on ice in brief bursts by a Ultra-Turrax homogenizer (Janke and Kunkel, FRG) in a 1:4 (wt/vol) dilution of ice-cold 0.5 N perchloric acid. Homogenization times for liver and skeletal muscles were 50 and 100 s, respectively. Resultant homogenates were centrifuged at 10,000

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g for 15 min (2°C), and the supernatant was stored at 0–4°C. On the day of measurement, the supernatant was diluted with distilled water and total glutathione was estimated spectrophotometrically by the method of Tietze (33) with use of the reaction mixture as suggested by Adams et al. (1). The rate of change in absorbance at 412 nm was monitored, and tissue concentrations were estimated by linear regressions from the standard curve. For the assays of GPX, GRD, and GST, frozen tissues were crushed in liquid nitrogen and homogenized as above in ice-cold 0.25 M sucrose. The homogenate was centrifuged at 12,000 *g* (4°C) for 10 min. The supernatant was again centrifuged at 105,000 *g* (4°C) for 60 min and the postmicrosomal supernatant stored at −80°C. Activity of total GPX was assayed with cumene hydroperoxide as substrate (32). GRD activity was assayed according to Carlberg and Mannervik (7) with use of 10 mM potassium-*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer in the 1-ml reaction mixture. The dilution of the postmicrosomal supernatant was done in potassium phosphate buffer, pH 7. GST was assayed (12) with 1,2-dichloro-4-nitrobenzene as substrate. Activity of skeletal muscle CS was assayed spectrophotometrically (29). For the assay of GGT, tissues were homogenized in 1:19 (wt/vol) dilution of ice-cold 0.1 M tris(hydroxymethyl)aminomethane (Tris) · HCl, pH 8.0, and the homogenate was stored at −80°C. The enzyme activity was assayed (14) with L-γ-glutamyl-*p*-nitroanilide as substrate. The standard assay mixture contained (in mM) 4.4 substrate, 40 glycylglycine, 100 Tris · HCl, and 9.6 $MgCl_2$, pH 8.0, in a final volume of 1.3 ml (19). The reaction was initiated by the addition of protein (~1 mg/assay), and after incubation for 30 (for liver and lung), 60 (for heart and skeletal muscles), or 2 (for kidney) min at 37°C, the reaction was stopped by 1 ml of 20% trichloroacetic acid. The precipitated protein was removed by centrifugation at 3,000 *g* for 5 min. Absorbance of the supernatant was then measured at 407 nm against blanks to which trichloroacetic acid was added before incubation. Protein was determined by the method of Lowry et al. (24). GRD (type III), GSSG (grade III), GSH (free acid), β-NADPH (tetrasodium salt, type III), and 5,5'-dithio-bis(2-nitrobenzoic acid) were purchased from Sigma Chemical (St. Louis, MO). All other chemicals were of highest analytic grade. A Shimadzu UV-240 double-beam spectrophotometer or the Perkin-Elmer Lambda 2 UV/VIS spectrophotometer was used.

Statistical analysis. The SPSS/PC+ (SPSS, Chicago, IL) software was used. Significance of changes in parameters on training and during exhaustive exercise was tested with one-way analysis of variance. Subsequently, the location of significance was determined by Scheffé's test. The Student's *t* test and paired *t* test were used to compare group means. 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 ± SD.

RESULTS

Endurance training of dogs. Activity of skeletal muscle CS was significantly higher in the leg muscles (RG, ER, and TR) of the trained dogs than those of the untrained group (Fig. 1). Such an effect was most pronounced (60% increase) in the RG but could not be observed in the SP (Fig. 1). Exercise training increased total glutathione content of the RG as well as that of the liver (Table 1). Higher activities of GPX were observed in the leg muscles (RG, ER, and TP) of the trained dogs; such a response to exercise training is quite similar to reports from rat studies (21, 23) (Table 2). The effect was more pronounced (45–55% increase) in the lower hindlimb (Table 1). Activity of GRD was increased on training in all the studied leg muscles; however, the increase was statistically significant only in ER and TP. Skeletal muscle GST activity was not influenced significantly, but unlike reports from rat studies (17) (Table 2), hepatic GST activity was significantly increased by the training program (Table 1).

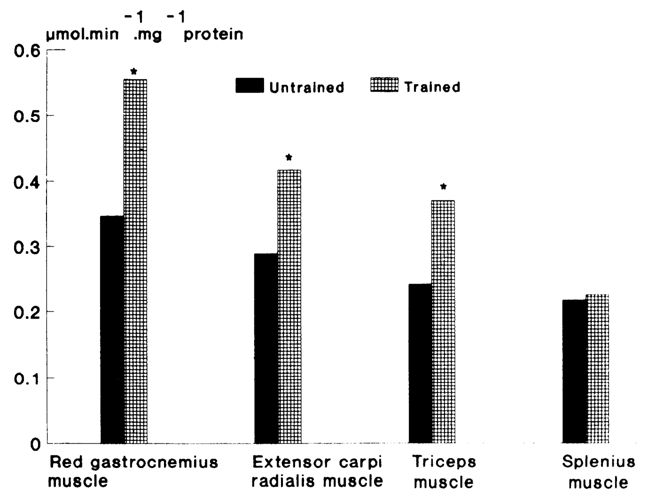

FIG. 1. Dog skeletal muscle citrate synthase activity in untrained and trained groups. Values are means ± SD. Training program significantly increased enzyme activity in all 3 leg muscles studied: * $P < 0.001$.

Changes in response to immobilization. Differences in the control values of the skeletal muscle and hepatic total glutathione content and activities of related enzymes between the dogs of the training (Table 1) and immobilized experiments (Fig. 2) might be because of the 30-wk age difference between the two sets of animals. No significant difference between the tissue protein concentrations (mg protein/g wet wt of RG) of the free and immobilized legs was observed (result not shown). We may therefore expect that the results obtained from this experiment are consequences of long-term physical inactivity and not just that of overall muscular atrophy due to the immobilization. GPX, GRD, and GST activities in dog RG remained unaffected (Fig. 2). There was, however, a significant decrease in the total glutathione content per unit wet weight of the muscle because of the physical inactivity (Fig. 2).

Endurance training and exercise of rats. The resting CS activity of the leg muscles (RG and MV) was significantly higher in the trained animals than in the untrained group (Fig. 3). In the UE group, a single bout of exhaustive exercise decreased CS activity of both leg muscles. A similar decrease in CS activity was also observed in the TE group, but the observation was statistically significant

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TABLE 1. *Influence of training on hepatic and skeletal muscle GPX, GRD, and GST activities and total glutathione content in dogs*

	Liver	RG	ER	Triceps	Splenius
			μmol/g wet wt		
TGSH					
UT	6.00±0.43	1.56±0.19	1.57±0.24	1.51±0.21	1.46±0.13
TR	6.46±0.62‡	2.22±0.24*	1.61±0.29	1.57±0.22	1.44±0.17
			$nmol \cdot min^{-1} \cdot mg\ protein^{-1}$		
GPX					
UT	225±23	21.13±2.31	14.92±1.50	13.64±1.56	13.62±1.52
TR	240±26	30.00±2.42*	21.57±2.03*	16.86±1.89*	13.78±2.35
GRD					
UT	55.90±9.90	9.55±1.58	8.39±0.88	6.21±0.60	4.46±0.91
TR	51.68±6.08	11.53±2.04	10.86±1.36*	6.90±0.72†	5.05±0.85
GST					
UT	1,105±93	88.2±13.2	99.9±12.2	81.9±14.1	81.3±14.2
TR	1,404±100*	91.1±13.2	106.1±10.7	88.6±11.9	79.7±12.8

Values are means ± SD. RG, red gastrocnemius; ER, extensor carpi radialis; TGSH, total glutathione; GPX, glutathione peroxidase; GRD, glutathione reductase; GST, glutathione *S*-transferase; UT, untrained; TR, trained. Difference on training: * $P < 0.001$; † $P < 0.05$; ‡ $P = 0.056$.

only in MV (Fig. 3). Any effect of training or exhaustive exercise on the LD of either the T or U rats could not be observed (Fig. 3). Table 2 shows that the training program used in the study resulted in a higher increase in hepatic total glutathione content in the rats than in the dogs (Table 1); however, skeletal muscles remained unaffected. GPX was increased in both the muscles of the leg (RG and MV), but it remained unchanged in the LD and liver. Contrary to results obtained from the dogs (Table 1), GRD activity was significantly decreased on training in rat RG. Activity of GRD in the other muscles and liver was unaltered (Table 2). A single bout of exhaustive exercise decreased total glutathione content of RG and MV of untrained animals by 30–35%, whereas the decrease in the trained group was only 10–20%, despite the fact that the untrained animals ran for a shorter duration at ~55–60% of the speed compared with their trained counterparts. LD was unaffected in all such cases. The fall in hepatic total glutathione content because of exhaustive exercise was slightly more in the trained group (43%) than in the untrained group (Table 2). Both skeletal muscle and hepatic GPX activities remained unchanged after a single bout of exhaustive exercise. In all the muscles studied and liver, GRD exhibited a tendency to decrease on exhaustive exercise, but the decrease was only significant in RG of U. In both the trained and untrained groups, hepatic GST activity was increased by ~40% after exhaustive exercise. A 15% increase was also seen only in the MV of the trained group. Table 3 illustrates the response of GGT to training and exercise in the rat study. A comparative account of

TABLE 2. *Influence of training and exercise on hepatic and skeletal muscle GPX, GRD, and GST activities and total glutathione content in rats*

	Liver	RG	MV	LD
		μmol/g wet wt		
TGSH				
UR	3.27±0.28	0.85±0.04	0.75±0.09	0.29±0.02
UE	2.32±0.26*	0.57±0.05*	0.50±0.05*	0.28±0.02
TO	3.96±0.32‡	0.86±0.03	0.73±0.09	0.28±0.02
TE	2.25±0.38*	0.75±0.11*	0.60±0.12†	0.28±0.02
		$nmol \cdot min^{-1} \cdot mg\ protein^{-1}$		
GPX				
UR	652±99	50.96±4.18	23.41±1.58	42.98±7.54
UE	640±108	50.77±6.61	23.98±2.29	43.85±6.82
TO	634±110	58.75±3.85‡	27.65±2.14‡	45.28±8.28
TE	615±106	57.27±3.64	25.71±2.22	45.70±8.34
GRD				
UR	72.59±11.18	12.24±1.19	7.45±0.90	8.36±1.58
UE	69.87±11.48	10.87±1.21†	7.00±0.82	8.00±1.00
TO	71.72±14.34	10.43±0.91‡	7.20±0.97	8.80±0.99
TE	72.36±11.86	10.25±0.78	7.15±0.79	8.18±1.56
GST				
UR	783±96	47.34±7.11	37.53±5.10	41.02±9.52
UE	1,100±169*	49.18±7.68	34.95±3.74	40.74±9.78
TO	841±103	48.62±5.19	40.23±5.44	39.58±10.18
TE	1,192±155*	49.28±5.48	46.22±2.80†	41.86±11.50

Values are means ± SD. MV, mixed vastus lateralis muscle; LD, longissimus dorsi muscle; UR, untrained (at rest); UE, untrained (after exhaustive exercise); TO, trained (at rest); TE, trained (after exercise). Differences due to a single bout of exercise: * $P < 0.01$; † $P < 0.05$. Differences due to training: ‡ $P < 0.01$.

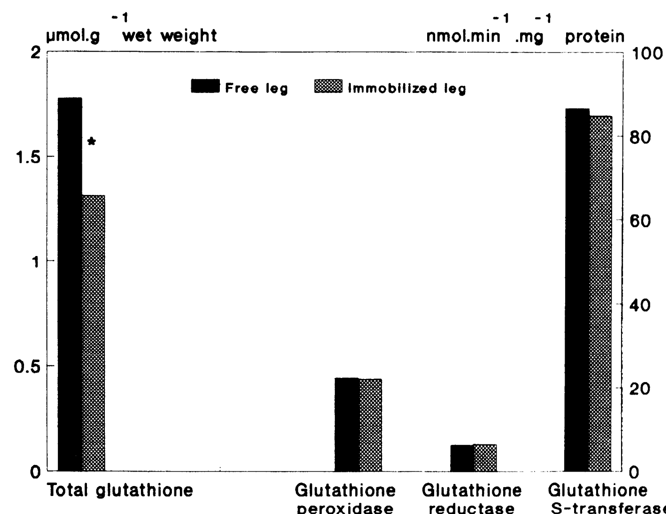

FIG. 2. Glutathione and its related enzymes in red gastrocnemius skeletal muscle of free and 11-wk joint-immobilized legs of the same dog. Values are means ± SD ($n = 14$). A significant lowering of total glutathione content was observed on immobilization: * $P < 0.001$. Activities of glutathione peroxidase, glutathione reductase, and glutathione *S*-transferase were not affected.

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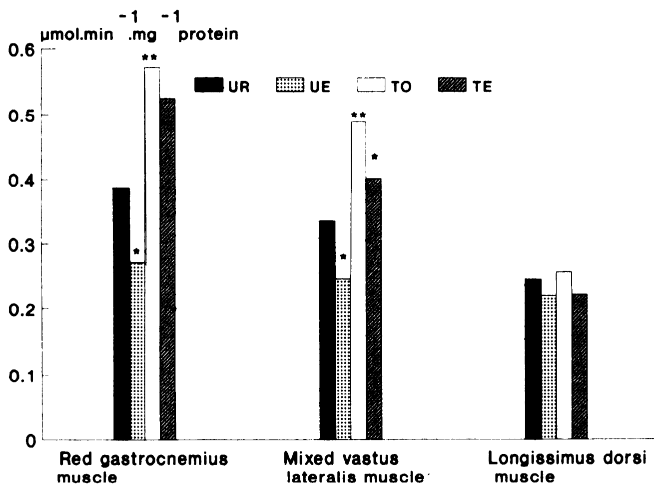

FIG. 3. Rat skeletal muscle citrate synthase activity in various groups. UR, untrained (at rest); UE, untrained (after exhaustive exercise); TO, trained (at rest); TE, trained (after exercise). Values are means ± SD. Resting activities of enzyme were significantly higher in leg muscles of trained rats: ** $P < 0.001$. This effect was not observed in longissimus dorsi muscle of neck and trunk region. Enzyme activity decreased significantly after exercise in both leg muscles of untrained group and mixed vastus lateralis muscle of trained group but not in red gastrocnemius muscle of trained group: * $P < 0.05$.

various organs is consistent with previous reports (2, 3) that GGT is exceptionally active in the kidney. Among other organs, GGT activity was considerably higher in the lung, liver, and skeletal muscles than in the heart. Comparison of GGT activity of RG with MV and LD showed that RG had ~50 and 150% higher activities in the untrained and trained groups, respectively (Table 3). In the RG, there was a 100% increase in GGT activity on training. GGT activity of the other leg muscle, MV, was also significantly increased on training. No such effect was observed in LD or any other organ studied (Table 3). In the untrained group, a single bout of exhaustive exercise decreased GGT activities of all the skeletal muscles studied, but there was no decrease in any other organ. This decrease could not be seen in the leg muscles of the trained animals. This adaptive effect of training was not seen in LD. Exercise training rendered the liver susceptible to a decrease in GGT activity in response to exhaustive exercise (Table 3).

DISCUSSION

Effects of exercise training of dogs. In most of the previous studies concerning the response of skeletal muscle and liver glutathione redox cycle to endurance training and exercise, the rat was used as the experimental animal. Beagle dogs possess a well-developed musculoskeletal system apparently suited for running and have been quite commonly used as a laboratory animal for exercise physiology studies. In view of the lack of data from canines, the dog was also used as an experimental subject in this study. Groups of skeletal muscle studied were RG, ER, TP, and SP. Activity of the oxidative enzyme CS was significantly higher in all the leg muscles of the trained dogs (Fig. 1). No significant difference in CS activity of SP was observed between the trained and untrained groups. SP belongs to the neck region and may have therefore remained unaffected by the treadmill running program. No effect of training could be seen in the glutathione system of SP as well (Table 1). RG, which is predominantly oxidative (10) (Fig. 1), was the most affected, followed by ER and TP of the lower and upper hindlimbs, respectively (Table 1). Exercise training resulted in a significant increase of the total glutathione pools of both RG and the liver at rest. The increase (42%) observed in RG was the highest (Table 1). GCS and GSHS were observed to be very active in all the skeletal muscles studied in dogs. In RG, MV, and ER, activities of both the enzymes, especially GSHS, were significantly enhanced on treadmill training (unpublished observation). This could provide an explanation for the increase in total GSH pool of the RG. In the rat study, we observed that the training program led to a 100% increase in GGT activity of RG (Table 3). Such an increase in GGT activity facilitates the availability of γ-glutamyl amino acids and cysteinylglycine to the activated GSH-synthesizing apparatus within the cell. In the trained dogs, GPX was substantially higher in all the leg muscles studied but not in SP or the liver (Table 1). The observation that skeletal muscle GPX activity increased on exercise training is in good agreement with previous reports (21, 23, 34). The training-induced increase in muscle GPX activity was highest (~50%) in the RG. At rest, the activity of GPX was also considerably higher in the RG than in other muscle groups studied. RG has a high oxidative capacity with a high at-exercise blood flow (10). This group of skeletal muscle may therefore be expected to produce a high concentration of reactive oxygen metabolites when exercising. The high antioxidant status of the skeletal muscle might be crucial in encountering such a stress caused by oxygen-derived radicals. The chronic intermittent increase in its substrate concentration, such as during regular treadmill running, might have caused the increase of GPX activity in the RG. A similar exposure to substrates, though perhaps of a lesser intensity, might have also occurred in the other exercising muscles of the leg, producing an increase in GPX activity as well. Glucose-6-phosphate dehydrogenase activity in the skeletal muscles has been reported to increase by ~74% on exercise training;

TABLE 3. *Activity of GGT in response to training and exercise in rats*

	Untrained		Trained	
	At rest	After exercise	At rest	After exercise
	pmol · min⁻¹ · mg protein⁻¹			
Red gastrocnemius	210±53	142±32*	428±113‡	383±130
Mixed vastus lateralis	133±37	88±42†	173±49§	168±36
Longissimus dorsi	145±65	52±10*	164±24	75±30*
Lung	866±335	806±293	781±213	891±201
Liver	325±200	319±161	535±179	286±150*
Heart	20±6	19±7	18±7	19±6
	nmol · min⁻¹ · mg protein⁻¹			
Kidney	762.3±129.3	659.7±94.2	793.5±156.6	656.1±153.4

Values are means ± SD. GGT, γ-glutamyl transpeptidase. Differences due to a single bout of exercise: * $P < 0.01$; † $P < 0.05$. Differences due to training: ‡ $P < 0.01$; § $P < 0.05$.

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however, the effect was far less pronounced in the liver (23). This ensures a better availability of NADPH in the trained muscle, facilitating GRD activity. We observed an increase in leg muscle GRD on training; however, the finding was significant only in ER and TP. This increase in GRD might also be partly due to the increased ability of the lightly trained muscle to retain riboflavin (13). The above findings tend to explain the stabilization of the glutathione redox ratio of the trained skeletal muscle observed after a single bout of exhaustive exercise. Chasseaud (8) suggested that liver glutathione is primarily a detoxicant. It was observed that the training-mediated increase in the hepatic GSH pool was associated with a significant increase in hepatic GST activity. A somewhat similar response has also been observed in swim training of rats (34). Such an observation indicates that liver of the trained animal may have a higher detoxicant status. A training-dependent increase in activity of other drug biotransformation enzymes of rat liver microsome has also been reported (25). High activity of GST was observed in all the skeletal muscles; however, no effect of training could be observed (Table 1). It is possible that, in dogs, skeletal muscle GSH is not only an antioxidant but also a potent detoxicant.

Effects of one leg immobilization. There were no changes in GPX, GRD, or GST, indicating that resting levels of their activities are hardly dependent on long-term physical inactivity (Fig. 2). However, there was a significant decrease in the total glutathione content of RG. Long-term physical inactivity thus appeared to be a crucial factor in lowering the resting glutathione status of oxidative skeletal muscles. Such a decrease is expected to be physiologically significant in limiting the antioxidant and detoxicant status of the organ as determined by the glutathione pool. Thus regular physical activity appears to be critical in the maintenance of an elevated resting glutathione status of the oxidative skeletal muscles. Such a notion is supported by results of our training experiment with dogs (Table 1) as well.

Effect of exercise training and a single bout of exhaustive exercise in rats. The training program for the rats was chosen to be exhaustive to test the effect of training intensity by comparing the outcome with other reports involving more moderate protocols (15–17, 22, 23, 28, 34). The highest intensity of exercise at which the UE group ran without requiring any physical methods to compel them was chosen for their exhaustive exercise experiment. The intensity (1.2–1.4 km/h) was lower than the intensity of regular exercise of the trained group. Because exercising at an intensity lower than the regular level might not have produced a clear picture of exercise-induced changes in the TE group, the regular exercise intensity was chosen. Moreover, on an average, the UE group could run for a considerably shorter duration than the TE. Thus the exercise-induced effects seen in the T group (Table 2, Fig. 3) are perhaps consequences of exposure to a far higher concentration of exercise-generated oxygen radical than the U group.

Groups of muscle studied were RG, MV, and LD. Compared with the U group, CS activity was significantly higher in both the studied leg muscles of the T group (Fig. 3). This indicates that the training program was effective in enhancing the oxidative capacity of the skeletal muscles. Consequent to a single bout of exhaustive exercise, CS activity decreased significantly. This was observed in both the leg muscles of the untrained animals and in the MV of the trained group. Though the effect could be seen, it was not significant in the RG of the T group (Fig. 3). Exercise-induced downregulation of skeletal muscle CS activity was also observed previously and has been expected to be caused by an increased muscular proteolysis (16). In that study, although the downregulation was statistically significant only in the skeletal muscle of the U group, a considerable effect could also be seen in the T group (16). In our experiment, no effect of either training or exhaustive exercise could be seen in LD (Fig. 3, Table 2). LD is of the neck and trunk region, and therefore it might have remained unaffected by the treadmill-training program.

Exercise training increased hepatic total glutathione of the rats; this was also observed in our dog experiment. No such increase could be observed in the skeletal muscles studied (Table 2). The GPX activity in leg muscles of the T group was significantly higher than in those muscles of the U group. These data are consistent with our dog results (Table 1) and also with reports of other investigators (16, 21, 23). Ji et al. (15) reported a significant exercise-induced increase in skeletal muscle GPX activity in 4-mo-old untrained rats that was not observed in the present study. We observed a significant increase of leg muscle GRD activity on submaximal training of dogs (Table 1). Lew and Quintanilla (23) observed no significant change in either skeletal muscle or heart GRD activity on training. In contrast, Kihlström et al. (18) reported a decrease in myocardial GRD after training. A significant lowering of GRD activity was observed (Table 2) in RG subsequent to training. Such a response that goes against the maintenance of a favorable glutathione redox cycle in the RG at exercise might have been due to the intensive training program that may have increased flavoprotein turnover and breakdown in the muscle (6, 27). Observed values of muscle GST were considerably higher than those reported by Ji et al. (17). Such a difference might be strain and also age dependent (15). Hepatic GST did not respond to training; this is unlike our finding in the dog study in which a 55-wk-long submaximal training period was used. Training did not affect muscle GST as well. These observations are in good agreement with those of Ji et al. (17).

The intensity of training does not seem to affect responses of GPX or GST but is perhaps a critical determinant of muscle GRD response. Hepatic GST of both trained and untrained animals increased significantly after exercise (Table 2). These results are in contrast to those of Ji et al. (17). In the U group, a similar increase, though not significant, was observed after a 30-min swimming exercise (34). Increase in hepatic GST caused by exhaustive exercise was accompanied by a considerable lowering of total glutathione content (Table 2). Thus the notion that a single bout of exhaustive exercise elevates the hepatic detoxicant status requires further investigation. After exhaustive exercise, the U group had a more severe depletion of the skeletal muscle glutathione pool; the depletion was considerably less yet significant in the T group (Table 2). However, the failure to maintain a better GSH redox status of the trained mus-

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cle (23) in this case may be attributed to the decrease in GRD activity. Hepatic GSH pool was almost equally depleted on exhaustive exercise in both groups. Hepatic efflux of GSH has been shown to be promoted by several hormones, such as vasopressin (31), which are also released in excess during exhaustive exercise. Additional GSH is thus made available to the peripheral tissues under need. This concept is consistent with the notion that during exercise there is a net shuttle of glutathione from liver to skeletal muscles. The trained liver, possessing a bigger resting GSH pool (Table 2), is thus more capable of catering to such protective needs.

GGT is an important component of the glutathione cycle in cells (2, 3). Maintenance of cellular GSH pool is chiefly dependent on the regeneration of GSH from GSSG by GRD and synthesis of the tripeptide by GCS and GSHS. GSH, as well as other γ-glutamyl-containing compounds, including GSSG and γ-glutamyl glutathione, reacts with GGT at the outer cell surface. The γ-glutamyl moiety is transferred to a suitable amino acid acceptor, and both the γ-glutamyl amino acid and cysteinylglycine are transported into the cell and reused for GSH generation. Thus GGT supplies substrates for the GCS-GSHS and GRD reactions to elevate cellular glutathione level. Training was observed to enhance GGT activities of both the leg muscles studied (Table 3). The effect was more profound (104%) in the RG. A single bout of exhaustive exercise decreased GGT activities of all the studied skeletal muscles of the untrained group. Such a tendency of exhaustive exercise-induced decrease of GGT activity was not found in the trained leg muscles. This may be an adaptive response of the trained muscles. Activated GGT of the trained muscle facilitates the import of substrates required for GSH generation. Furthermore it prevents the excretion of γ-glutamyl cysteine moieties (11). These are crucial in stabilizing the skeletal muscle glutathione redox cycle during exercise. At exercise, when hepatic efflux of GSH is accelerated, the trained muscle enjoys a greater ability to enrich its glutathione-dependent antioxidant and detoxicant status. Responses of GGT to training were not seen in LD (relatively passive during treadmill training; Fig. 3), lung, liver, heart, or kidney (Table 3). However, GGT activity of the trained liver decreased (by ~47%) after exhaustive exercise. This response might ensure that fewer γ-glutamyl compounds are retrapped in the liver from the circulation when the needs of the peripheral tissues are acute. However, because resting hepatic GGT activity is quite low (Table 3), its role in affecting the level of γ-glutamyl compounds in circulation might not be of remarkable physiological significance.

In summary, the study presents the first evidence regarding how *1*) exercise training affects the major aspects of the glutathione cycle in the skeletal muscle and liver of dogs, *2*) GGT of skeletal muscles, liver, kidney, lung, and heart responds to treadmill training and a single bout of exhaustive exercise, and *3*) long-term physical inactivity might lower the glutathione-dependent antioxidant and detoxicant status of oxidative skeletal muscles. Furthermore the possibility of oxidative muscle GRD activity being lowered on exhaustive training was evident. Long-term submaximal exercise training led to a favorable adaptation of the glutathione redox cycle of the leg muscles. The glutathione-dependent detoxicant status of the liver was not affected by the 8-wk exercise training of rats, but it was significantly elevated by the 55-wk aerobic training of dogs.

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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. ANDERSON, M. E., R. J. BRIDGES, AND A. MEISTER. Direct evidence for interorgan transport of glutathione and that the non-filtration renal mechanism for glutathione utilization involves gamma-glutamyl transpeptidase. *Biochem. Biophys. Res. Commun.* 96: 848–853, 1980.
3. ANDERSON, M. E., AND A. MEISTER. Transport and direct utilization of gamma-glutamylcyst(e)ine for glutathione synthesis. *Proc. Natl. Acad. Sci. USA.* 80: 707–711,1983.
4. BARCLAY, J. K., AND M. HANSEL. Free radicals may contribute to oxidative skeletal muscle fatigue. *Can. J. Physiol. Pharmacol.* 69: 279–284, 1991.
5. BOOTH, F. W. Time course of muscular atrophy during immobilization of hindlimbs in rats. *J. Appl. Physiol.* 43: 656–661, 1977.
6. BRO-RASMUSSEN, F. The riboflavin requirement of animals and man and associated metabolic relations. II. Relation of requirement to the metabolism of protein and energy. *Nutr. Abstr. Rev.* 28: 369–386, 1958.
7. CARLBERG, I., AND B. MANNERVIK. Glutathione reductase. In: *Methods in Enzymology,* edited by A. Meister. New York: Academic, 1985, vol. 113, p. 484–490.
8. CHASSEAUD, L. F. Conjugation with glutathione and mercapturic acid excretion. In: *Glutathione: Metabolism and Function,* edited by I. M. Arias and W. B. Jakoby. New York: Raven, 1976, p. 77–114.
9. DAVIES, K. J. A., A. T. QUINTANILHA, G. A. BROOKS, AND L. PACKER. Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* 107: 1198–1205, 1982.
10. DI PRAMPERO, P. E., P. CERRETELLI, AND J. PIIPER. O_2 consumption and metabolic balance in the dog gastrocnemius at rest and during exercise. *Pfluegers Arch.* 309: 38–47, 1969.
11. GRIFFITH, O. W., AND A. MEISTER. Excretion of cysteine and gamma-glutamylcysteine moieties in human and experimental animal gamma-glutamyltranspeptidase deficiency. *Proc. Natl. Acad. Sci. USA* 77: 3384–3387, 1980.
12. HABIG, W. H., M. J. PABST, AND W. B. JAKOBY. Glutathione *S*-transferases. *J. Biol. Chem.* 249: 7130–7139, 1974.
13. HUNTER, K. E. L., AND P. R. TURKKI. Effect of exercise on riboflavin status of rats. *J. Nutr.* 117: 298–304, 1987.
14. HUSEBY, N. E., AND J. H. STRÖMME. Practical points regarding routine determination of gamma-glutamyltransferase in serum with a kinetic method at 37°C. *Scand. J. Clin. Lab. Invest.* 34: 357–363, 1974.
15. JI, L. L., D. DILLON, AND E. WU. Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. *Am. J. Physiol.* 258 (*Regulatory Integrative Comp. Physiol.* 27): R918–R923, 1990.
16. JI, L. L., F. W. STRATMAN, AND H. A. LARDY. Enzymatic down regulation with exercise in rat skeletal muscle. *Arch. Biochem. Biophys.* 263: 137–149, 1988.
17. JI, L. L., F. W. STRATMAN, AND H. A. LARDY. Antioxidant enzyme systems in rat liver and skeletal muscle. *Arch. Biochem. Biophys.* 263: 150–160, 1988.
18. KIHLSTRÖM, M., J. OJALA, AND A. SALMINEN. Decreased level of cardiac antioxidants in endurance-trained rats. *Acta Physiol. Scand.* 135: 549–554, 1989.
19. KRETZSCHMAR, M., AND W. KLINGER. Gamma-glutamyltranspeptidase in liver homogenates of rats of different ages: enzyme kinetics and age course of K_m and V_{max}. *Z. Versuchstierkd.* 32: 41–47, 1989.
20. LANG, J. K., K. GOHIL, L. PACKER, AND R. F. BURK. Selenium

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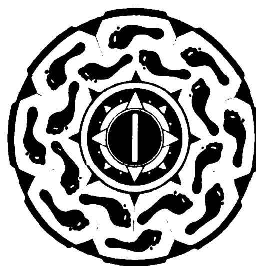
deficiency, endurance exercise capacity, and antioxidant status in rats. *J. Appl. Physiol.* 63: 2532–2535, 1987.
21. LAUGHLIN, M. H., T. SIMPSON, W. L. SEXTON, O. R. BROWN, J. K. SMITH, AND R. J. KORTHUIS. Skeletal muscle oxidative capacity, antioxidant enzymes, and exercise training. *J. Appl. Physiol.* 68: 2337–2343, 1990.
22. 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.
23. 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.
24. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193: 265–275, 1951.
25. MARIN, E., R. TUNNINEN, AND O. HÄNNINEN. Training increases drug metabolizing enzyme activities in rat liver. In: *Cytochrome P-450: Biochemistry and Biophysics,* edited by I. Schuster. London: Taylor & Francis, 1989, p. 887–890.
26. MEISTER, A., AND M. E. ANDERSON. Glutathione. *Annu. Rev. Biochem.* 52: 711–760, 1983.
27. POLLOCK, H., AND J. J. BOOKMAN. Riboflavin excretion as a function of protein metabolism in the normal, catabolic, and diabetic human being. *J. Lab. Clin. Med.* 38: 561–573, 1951.
28. PYKE, S., H. LEW, AND A. QUINTANILHA. Severe depletion in liver glutathione during physical exercise. *Biochem. Biophys. Res. Commun.* 139: 926–931, 1986.
29. SHEPHERD, D., AND P. B. GARLAND. Citrate synthase from rat liver. In: *Methods in Enzymology,* edited by J. M. Lowenstein. New York: Academic, 1969, vol. XIII, p. 11–16.
30. SIES, H., AND E. CADENAS. Biological basis of detoxication of oxygen free radicals. In: *Biological Basis of Detoxication,* edited by J. Caldwell and W. B. Jakoby. San Diego, CA: Academic, 1983, p. 181–211.
31. SIES, H., AND P. GRAF. Hepatic thiol and glutathione efflux under the influence of vasopressin, phenylephrine and adrenaline. *Biochem. J.* 226: 545–549, 1985.
32. TAPPEL, A. L. Glutathione peroxidase and hydroperoxides. In: *Methods in Enzymology,* edited by S. Fleischer and L. Packer. New York: Academic, 1978, vol. 52, p. 506–513.
33. TIETZE, F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal. Biochem.* 27: 502–522, 1969.
34. VANI, M., G. P. REDDY, G. R. REDDY, K. THYAGARAJU, AND P. REDDANNA. Glutathione *S*-transferase, superoxide dismutase, xanthine oxidase, catalase, glutathione peroxidase and lipid peroxidation in the liver of exercised rats. *Biochem. Int.* 21: 17–26, 1990.



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