Changes in the glutathione status of plasma, liver and muscle following exhaustive exercise in rats

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Our results show that following physical exercise to exhaustion, the levels of reduced glutathione are increased in the plasma, but decreased in both the liver and skeletal muscle of rats Levels of glutathione disulfide are increased in all 3 tissues Our results are consistent with a mechanism in which the liver may be dumping reduced glutathione into the plasma in an attempt to deliver it to those tissues that need it the most in this case, skeletal muscle

Glutathione *Oxidative stress* *Physical exercise*

1 INTRODUCTION

Strenuous exercise has been shown to lead to damage in skeletal muscle and liver A single bout of exhaustive exercise causes focal necrosis, inflammation and increased lysosomal acid hydrolase activity in skeletal muscle [1,2]; it also leads to increased mechanical fragility of rat liver lysosomes, and protein loss mediated via the release of lysosomal enzymes [3,4]. Our work has shown that exhaustive exercise results in decreased mitochondrial respiratory control, loss of sarcoplasmic reticulum and endoplasmic reticulum integrity, increased levels of lipid peroxidation and an increase in free radical concentrations in rat skeletal muscle and liver [5]. Strenuous swimming results in the production of thiobarbituric acid reactive substances in rat skeletal muscle [6,7] We have also shown that vitamin E deficiency in rats leads to a reduction in endurance capacity and a corresponding faster rate of damage generation [5] in both liver and skeletal muscle.

Reduced glutathione (GSH) is, together with vitamin E, one of the most important antioxidants in cells. Mammalian cells contain reduced glutathione in the millimolar range [8], while the concentration of glutathione disulfide (GSSG) is 2 or 3 orders of magnitude lower in most cell systems. Under conditions of increased oxidative stress to cells, levels of GSH are usually reduced and levels of GSSG are increased [9,10].

Our work has mainly been concerned with the question of whether the damage associated with the liver and skeletal muscle cells during physical exercise might be oxidative in nature; if so, we would expect to see a corresponding lowering of GSH levels in these tissues following a bout of exhaustive exercise. We report on results that confirm our predictions.

2. MATERIALS AND METHODS

Fourteen female Wistar rats (Charles River, 65–70 days old) were divided into two equal groups designated as follows: (i) runners to be killed immediately after exercise to exhaustion, and (ii) sedentary controls. Both groups were fed an ad libitum diet of rodent laboratory chow (no.5001, Ralston Purina) and water. Runners were exercised on a Quinton rodent treadmill (model 42-15) at a speed of 24.1 m/min (0 9 mph) and a grade of 8.5° (15%). Point of exhaustion

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was determined by the runner's loss of righting reflex when turned on its back. All rats were matched for weight in each experiment and all experiments were performed within a single week to minimize the effect of age differences between the rats.

Each day, one control animal and one animal exercised to exhaustion (90–120 min running time) were decapitated with a guillotine and the blood was added directly to an equal volume of each of two solutions to minimize the artifactual autooxidation of GSH to GSSG and other mixed disulfides. These solutions were: (A) 17.5 mM EDTA, 10 mM bis(3-carboxy-4-nitrophenyl)disulfide (DTNB) in 100 mM potassium phosphate at pH 7.5, and (B) 17.5 mM EDTA, 10 mM *N*-ethylmaleimide (NEM) in 100 mM potassium phosphate at pH 6.5 [11]. No hemolysis was observed The blood mixtures were centrifuged at 2000 × *g* for 6 min and the supernatants used for the assays. Representative, weighed pieces of each liver lobe were quickly minced and then homogenized in either solution A or B for 60 s using a Polytron tissue homogenizer (Kinematica) at medium settings and at a 1:4 (g:ml) dilution. The total upper leg muscle from one leg was prepared in the same manner as the liver in solution A, while the other leg was homogenized in solution B. Homogenization time for the skeletal muscle was 120 s. The 4 homogenates were centrifuged at 13000 × *g* for 30 min and the supernatants used for the assays. Aliquots of freshly prepared GSSG at a known concentration (10 μM) were analyzed as described in [11] each day, to provide us with our standard curves. Aliquots of the supernatants of our tissue preparations were analyzed in the same manner within 2–3 h after preparation. Tissue concentrations of glutathione were estimated by linear regression from the standard curve. Our data are reported in terms of the absolute levels of both total glutathione and glutathione disulfide, as well as the percentage changes that occur with exhaustive exercise, as compared to control values (rested animals) for each day.

3. RESULTS

In table 1, the percentage change in glutathione levels due to exercise has been reported for both glutathione disulfide (GSSG) and total glutathione (GSH + GSSG) levels in all 3 tissues. Clearly, all 3 tissues are affected by exhaustive exercise.

In the plasma, the levels of total glutathione were consistently higher after exercise, and the levels of glutathione disulfide showed dramatic in-

Table 1

Tissue levels of glutathione disulfide (GSSG) and total glutathione (GSH + GSSG)

Experiment no	Type	Plasma[a]			Liver[b]			Muscle[b]		
		Control	Exercised	% change	Control	Exercised	% change	Control	Exercised	% change
1	GSSG	0 080	0.693	+766	0 0017	0 0031	+82	0 0014	0 0016	+14
	GSH+GSSG	6.00	8.80	+47	3 24	3 02	−7	0 44	0.26	−41
2	GSSG	0 147	0.413	+181	0.0053	0 0083	+57	0 0017	0.0030	+76
	GSH+GSSG	5 12	10 84	+112	3 09	2 78	−10	0 45	0 35	−22
3	GSSG	0 053	0 507	+857	0 0011	0 0019	+73	0 0021	0 0023	+10
	GSH+GSSG	6.08	10 24	+68	3 34	3 12	−7	0 46	0 30	−35
4	GSSG	0 133	0 467	+251	0 0069	0 0140	+103	0.0006	0 0012	+100
	GSH+GSSG	3 56	4.60	+29	3 15	2 58	−18	0.33	0.31	−6
5	GSSG	0 200	3 593	+1700	0.0029	0 0099	+241	0 0017	0.0036	+112
	GSH+GSSG	4 64	8 60	+85	2.23	2.18	−2	0 36	0 36	0

[a] Values expressed in μmol/l

[b] Values expressed in μmol/g wet tissue

creases. It is interesting to note that the levels of GSH always increased in the plasma after exercise.

In both liver and muscle, the levels of glutathione disulfide were consistently higher, and those of total glutathione, lower, after exhaustive exercise.

In table 2, the ratio of glutathione disulfide to total glutathione has been calculated for the 3 tissues in both control (rested) and exercised animals The percentage change in these ratios after exhaustive exercise (when compared to controls) is also reported. From these values, it is clear that exhaustive exercise increases the ratio of glutathione disulfide to total glutathione in all 3 tissues.

Of the 7 animals chosen to be exhaustively exercised, 5 ran well The two remaining animals ran intermittently, and it was never clear whether they were in fact ever exhausted, the results from these two animals were not significantly different from their two matched controls. We therefore report only on 10 animals (5 exercised and 5 matched sedentary controls).

4 DISCUSSION

Our control levels of GSSG and GSH + GSSG are in good agreement with published results [9,11,12]. Day to day variations in these levels are not surprising, since the experiments were not always performed at the same time of day, and pronounced circadian rhythms in the concentration of glutathione have been clearly demonstrated, at least in the liver [13].

Our results show that the levels of reduced glutathione are lower and that the levels of glutathione disulfide are higher in the liver and skeletal muscle of exhausted rats when compared to sedentary controls. We have previously demonstrated that animals exercised to exhaustion show higher levels of endogenous stable free radicals and peroxidized lipids in both liver and skeletal muscle when compared to controls [5] Since the protective action of GSH against oxidant injury is known to be due to its oxygen and other radical scavenging capacity [8,14,15], the depletion of GSH and the increase in GSSG in liver and skeletal muscle of exhaustively exercised rats is not surprising

On the other hand, while the increases in plasma GSSG might be explained simply by invoking well known cellular mechanisms that result in an efflux of GSSG from the cells when internal levels (of GSSG) increase [8,10–12], the increase in plasma

Table 2

Ratios of glutathione disulfide (GSSG) to total glutathione (GSH + GSSG)

Experiment no	Ratio	Plasma			Liver			Muscle		
		Control	Exercised	% change	Control	Exercised	% change	Control	Exercised	% change
1	$\frac{GSSG}{GSH+GSSG}$	0 0133	0 0788	+492	0 0005	0 0010	+100	0 0032	0 0062	+94
2	$\frac{GSSG}{GSH+GSSG}$	0 0287	0 0381	+33	0 0017	0 0030	+76	0 0038	0 0086	+126
3	$\frac{GSSG}{GSH+GSSG}$	0 0087	0 0495	+469	0 0003	0 0006	+100	0 0046	0 0077	+67
4	$\frac{GSSG}{GSH+GSSG}$	0.0374	0 1020	+173	0 0022	0 0054	+145	0 0018	0 0039	+117
5	$\frac{GSSG}{GSH+GSSG}$	0 0431	0 4180	+870	0 0013	0 0045	+246	0 0047	0 0100	+113

GSH is perhaps more intriguing.

A trivial explanation might be that higher levels of GSH in the plasma could be due to tissue damage (cell lysis) and red blood cell hemolysis. It has been shown that creatine kinase and lactate dehydrogenase activities are elevated in the plasma immediately following strenuous exercise [16] and that these effects are probably due to cell damage We cannot at the present time exclude the possibility that part of the increase in plasma GSH might simply be due to a similar effect

On the other hand, and a far more interesting explanation for the increased levels of GSH in the plasma may be that it is the result of an exercise-induced stimulation of GSH efflux from the liver. Recent studies indicate that GSH efflux across the sinusoidal plasma membrane in an isolated perfused rat liver preparation is rapidly stimulated by increasing vasopressin levels in the perfusing fluid, within physiological concentrations [17] It is also well known that during physical exercise, plasma levels of vasopressin increase significantly [18,19]. Such a hormonally controlled mechanism of GSH efflux from the liver could be used by the animal to deliver reduced glutathione to skeletal muscle, where it is probably undergoing rapid oxidation during strenuous physical exercise activity. We believe that this may in fact be the mechanism whereby the body would try to protect skeletal muscle from oxidative damage under conditions of increased physical activity. Preliminary data (to be reported) suggest that in fact the endurance capacity of these animals depends strongly on the levels of GSH in the liver

It has occurred to us that the increased levels of GSSG in the liver and muscle of exercised animals could be due to blood contamination. This is unlikely, since a simple calculation of the amounts involved tells us that even if all the plasma were added to the liver or muscle homogenate, it would not be able to account for the observed increases in GSSG in these tissues.

Our observation that GSH is lower and GSSG is higher in the liver after exhaustive exercise also provides an elucidation to the curious observation of liver oxidative damage following exhaustive exercise, for which we previously had no clear explanation [5].

That glutathione is important in exercise is also supported by studies that show that endurance training increases the total GSH concentrations in muscle and that this is associated with an increase in resistance of skeletal muscle to peroxidative damage [2,20]. It will be very interesting to find out what endurance training will do to liver glutathione levels and to the mechanisms of efflux of GSH from the liver.

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REFERENCES

[1] Vihko, V , Rantamaki, J and Salminen, A (1978) Histochemistry 57, 237–249
[2] Vihko, V , Rantamaki, J and Salminen, A (1979) J Applied Physiol Respirat Environ Exercise Physiol. 47, 43–50
[3] Kasperek, G J , Dohm, G L , Tapscott, E P and Powell, T. (1980) Proc Soc Exp. Biol Med. 164, 430–434
[4] Kasperek, G J , Dohm, G L , Barakat, H A , Strausbach, P H , Barnes, O W and Snider, R D (1982) Biochem J 202, 281–288
[5] Davies, K J A , Quintanilha, A T , Brooks, G A and Packer, L (1982) Biochem Biophys Res. Commun 107, 1198–1205
[6] Brady, P S , Brady, L J and Ullrey, D E (1979) J Nutr 109, 1103–1109
[7] Jenkins, R R , Martin, D and Goldberg, E (1983) Med. Sci. Sports 15, 93
[8] Kosower, N S and Kosower, E M (1978) Int Rev Cytol 54, 109–160
[9] Akerboom, T P M and Sies, H (1981) Methods Enzymol. 77, 373–382
[10] Orrenius, S , Ormstad, K., Thor, H and Jewell, S A (1983) Fed. Proc 42, 3177–3188
[11] Adams, J D , Lauterburg, B H and Mitchell, J R (1984) J Pharmacol Exp Ther 221, 749–754
[12] Griffith, O W and Meister, A. (1979) Proc Natl Acad Sci USA 76, 5606–5610
[13] Isaacs, J and Binkley, F (1977) Biochim Biophys Acta 497, 192–204
[14] Kosower, N S and Kosower, E M (1976) in Free Radicals in Biology (Pryor, W A ed) vol II, pp 55–85, Academic Press, New York
[15] Burk, R F (1983) Biochim Biophys Acta 757, 21–28

[16] Armstrong, R.B , Ogilvie, R W and Schwane, J A (1983) J Appl. Physiol Respirat Environ Exercise Physiol 54, 80–93
[17] Sies, H and Graf, P (1985) Biochem J 226, 545–549
[18] Galbo, H (1983) Hormonal and Metabolic Adaptation to Exercise, Thiene-Stratton, New York
[19] Convertino, V A , Keil, L C , Bernauer, E M and Greenleaf, J E (1981) J Appl Physiol 50, 123–128.
[20] Salminen, A and Vihko, V (1983) Acta Physiol Scand 117, 109–113