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CHANGES IN THE BILIARY EXCRETION OF ORGANIC ANIONS FOLLOWING EXHAUSTIVE EXERCISE IN RATS

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Abstract—The effect of exhaustive exercise on the hepatobiliary transport of organic anions was investigated in rats. Animals were run on a rodent treadmill at 24 m/min up a 12% grade (152 ± 15 min). Exercise resulted in significant hypoglycaemia (−46%) and increased plasma levels of lactate (+12%), together with a marked reduction of glycogen concentration in the liver (−72%). When bromosulphthalein was administered i.v., its maximal biliary excretion (*Tm*) was significantly reduced (−30%), and plasma and liver concentrations of the dye were increased (+31% and +56%, respectively). The decrease corresponded both to the excretion of the conjugated and unconjugated dye (−30% and −33%, respectively). Cytosolic glutathione *S*-transferase activity in the liver was not affected by exercise, but there was a significant reduction in the hepatic concentration of glutathione (−50%). The *Tm* of dibromosulphthalein was also significantly reduced (−36%) and its plasma and liver concentrations increased (+67% and +33%, respectively) in exercised rats. The results suggest that, in addition to the direct effect of liver glutathione depletion, other factors must be involved in the impairment of the biliary excretion of organic anions caused by exercise.

In spite of the widespread idea that exercise is beneficial, different studies in the literature have demonstrated that increased levels of physical exercise can have deleterious effects and lead to tissue injury [1, 2]. Rats exercised to exhaustion have been reported to show a marked reduction in total liver glutathione concentration and an increased oxidized/reduced glutathione ratio [3, 4] in liver, plasma and skeletal muscle. Such results could be interpreted to indicate depletion of glutathione antioxidant systems during exhaustive exercise.

Glutathione is a molecule involved in a variety of important intracellular functions, not only including protection against oxidative stress, but also detoxification of a number of drugs and xenobiotics, that are conjugated with the tripeptide by means of glutathione *S*-transferases [5, 6]. Elimination of these molecules from the organism could, therefore, be altered as a consequence of glutathione depletion during strenuous exercise. Detoxification is also dependent on the transport through the hepatocytic membranes, including transfer from plasma to liver and canalicular secretion, and on bile flow [7]. These processes could be affected by the damage to cell constituents as a consequence of oxidative stress [8].

The purpose of this study was to investigate the effects of exercise to exhaustion on the different steps of the hepatobiliary transport of organic anions in rats. Two cholephilic dyes, bromosulphthalein (BSP) and dibromosulphthalein (DBSP), were studied. The fact that BSP is conjugated with glutathione in the liver while DBSP is excreted without prior biotransformation [9] would allow factors affecting hepatic transport from those interfering with conjugation to be distinguished.

MATERIALS AND METHODS

Chemicals. Amyloglucosidase, 5-5′-dithiobis-(2-nitrobenzoic acid), glucose-6-phosphate dehydrogenase, reduced glutathione, glutathione reductase, hexoquinase, lactic dehydrogenase, malic dehydrogenase, nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate and sulfobromophthalein were purchased from the Sigma Chemical Co. (St Louis, MO). Dibromosulphthalein was obtained from SERB (Paris, France). All other reagents were of the highest quality available. Distilled deionized water was used throughout.

Animals and experimental procedure. Male Wistar rats (Charles River, Barcelona, Spain), 12 weeks old, body wt 240–280 g, were housed in cages in a temperature controlled room (22°) with a 12 hr–12 hr dark/light cycle. They received a standard laboratory chow (Panlab, Barcelona, Spain) and tap water *ad lib*. Animals were randomly divided into two groups: runners and rested controls. Runners were exercised to exhaustion on a rodent treadmill (model LI8706 Letica, Barcelona, Spain) at a speed of 24 m/min and a 12% degree. Exercise was terminated when rats would no longer avoid the electrical grid system at the rear of the treadmill. The endurance run time was 152 ± 15 min.

Immediately after exercise was completed, animals were anaesthetized with sodium pentobarbitone (45 mg/kg body wt i.p.; Claudio Barcia, Madrid, Spain) and the left jugular vein catheterized to collect a 400 μL blood sample. After laparotomy, the common bile duct was cannulated with polyethylene tubing (PE-50). The entire surgical procedure took less than 10 min. Rectal temperature was monitored

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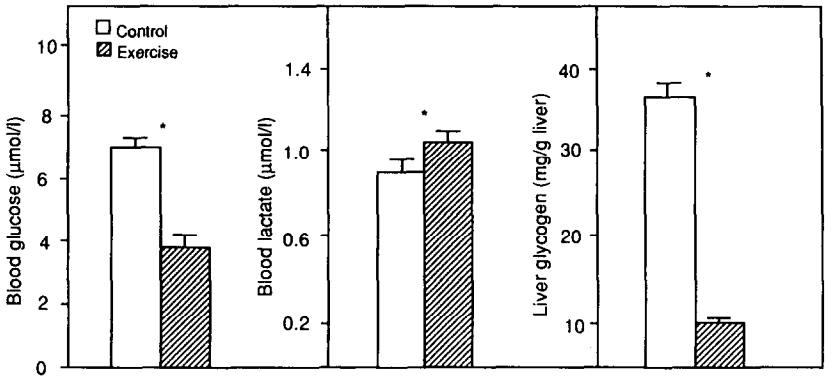

Fig. 1. Effect of exercise on blood glucose and lactate concentrations and on liver glycogen content. Blood values were obtained immediately after catheterization of the left jugular vein and liver values at the end of experiments. Values are means ± SE from twelve animals. *$P < 0.05$ significantly different from control value.

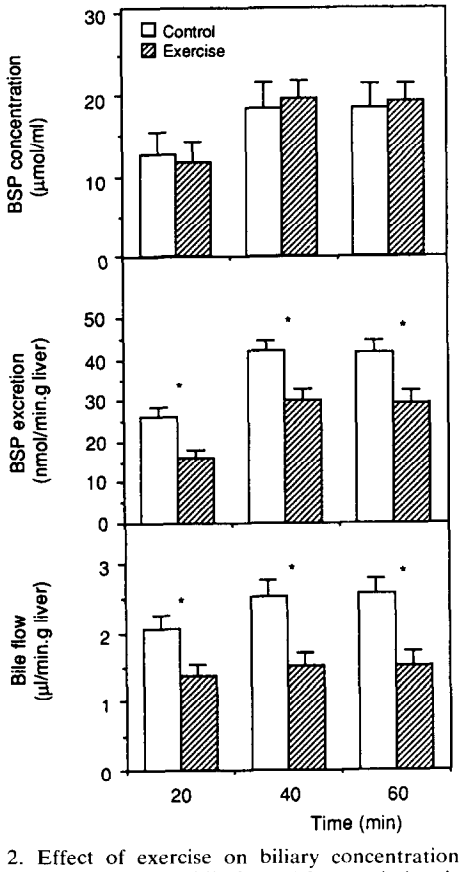

Fig. 2. Effect of exercise on biliary concentration and excretion of BSP and on bile flow. After a priming dose of 2.15 μmol/100 g body wt BSP was infused for 60 min at 215/min. 100 g body wt in control and exercised rats. Values are means ± SE from six animals. *$P < 0.05$ significantly differently from control value.

via a thermistor probe and maintained at 37° throughout the experiments.

Both control and runners were further divided into two subgroups, receiving either BSP or DBSP. The dyes (dissolved in 0.145 M NaCl) were administered as a i.v. injection of 2.15 μmol/100 g body wt followed by a 60 min i.v. infusion at 215 nmol/min 100 g body wt. Bile samples were collected under ice at 10 min intervals. At the end of the experiments the animals were killed by exsanguination and the livers washed with ice-cold 0.154 M NaCl, weighed and frozen at −80°.

Analytical methods. Blood glucose was determined with hexoquinase and glucose-6P-dehydrogenase [10]. Perchloric acid extracts were analysed for blood lactate [11]. Liver glycogen content was determined by the method of Kepler and Decker [12]. Bile flow was measured gravimetrically, assuming a bile density of 1.0 g/mL. The concentration of BSP and DBSP in plasma and bile was estimated by absorbance at 580 nm after appropriate dilution of samples with 0.05 N NaOH. Liver BSP and DBSP concentrations were measured by the method of Whelan and Combes [13]. Free and conjugated BSP were separated by paper chromatography using as solvent system butan-1-ol/acetic acid/ethanol/water (120/1/20/40). The spots were cut, eluted with water and read at 580 nm after alkalinization. Bile acid concentration in bile was measured with 3-alpha hydroxysteroid dehydrogenase [14]. Determination of total glutathione content of liver homogenates prepared in cold 5% (w/v) trichloroacetic acid in 0.01 N HCl, was carried out as described by Tietze [15] with the modification of Griffith [16]. Liver cytosolic glutathione *S*-transferase activity was determined using BSP as substrate [17]. Liver glutamate oxalacetate transaminase (GOT) [18] and lactic dehydrogenase (LDH) [19] activities were measured in liver homogenates. Protein concentration was determined by the method of Lowry *et al.* [20].

Statistical methods. Results were expressed as means ± SE. Significance of the differences between means was evaluated by the Mann–Whitney U test. P values less than 0.05 were considered to be significant.

RESULTS

Blood glucose and lactate concentrations are

Table 1. Biliary excretion of unconjugated and conjugated BSP and liver glutathione and glutathione *S*-transferase activity in control and exercised rats

	Conjugated BSP (nmol/min.g liver)	Unconjugated BSP (nmol/min.g liver)	Glutathione (μmol/g liver)	Glutathione transferase (μmol/min.mg protein)
Control	36.1 ± 2.2	5.7 ± 0.8	3.2 ± 0.3	30.8 ± 3.6
Exercised	25.4 ± 2.9*	3.8 ± 0.5*	1.6 ± 0.2*	29.7 ± 2.0

Values are means ± SE from six animals. BSP was infused 60 min at 215 nmol/min. 100 g body wt after a priming dose of 2.15 μmol/100 g body wt. Biliary data correspond to 40–60 min after beginning of infusion. Plasma and liver data were obtained at the end of experiments. *$P < 0.05$ significantly different from control value.

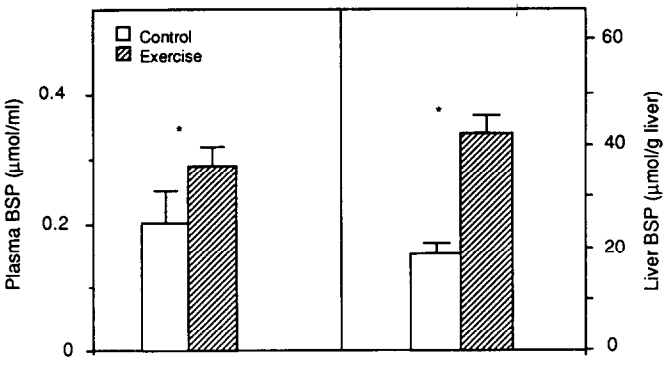

Fig. 3. Plasma and liver concentration of BSP in control and exercised rats. After a priming dose of 2.15 μmol/100 g body wt BSP was infused for 60 min at 215/min.100 g body wt. Data were obtained at the end of the infusion and are means ± SE from six animals. *$P < 0.05$ significantly different from control value.

shown in Fig. 1. Ten minutes after completion of exercise blood glucose levels were lower (−46%) and plasma lactate higher (+12%) in exercised than in non exercised rats. Exercised rats showed a significant decrease in hepatic glycogen concentration (−72%) (Fig. 1).

The maximal biliary BSP excretion *(Tm)* was significantly lowered by exercise (−30%), with no modification in its biliary concentration (Fig. 2). Cumulative excretion of BSP during this period was significantly reduced in exercised rats (14.8 ± 1.3 μmol vs 23.8 ± 2.6 μmol in the controls; $P < 0.05$). The decrease in the excretion of the dye corresponded both to a lowered excretion of unconjugated (−33%) and conjugated (−30%) BSP (Table 1). Plasma and liver BSP concentrations were significantly enhanced (+31% and +56%, respectively) at the end of experiments (Fig. 3). Exercised animals showed lowered bile flow values than the controls (−36%) (Fig. 1).

Figure 4 shows the effect of exercise to exhaustion on biliary DBSP. Maximal biliary excretion of the dye and bile flow were also significantly reduced (−36% and −33%, respectively), while liver and plasma concentrations were significantly increased (+33% and +67%, respectively) (Fig. 5). Exercise induced a significant decrease in the biliary cumulative excretion of this organic anion (16.7 ± 1.9 μmol vs 29.1 ± 3.3 μmol in the controls; $P < 0.05$).

Mean bile acid secretion into bile corresponding to the 60 min experimental period was significantly reduced by 25% in the group of runners as compared to control animals (54.3 ± 4.1 nmol/min.g liver vs 72.3 ± 5.4 nmol/min.g liver in the controls; $N = 12$; $P < 0.05$). Liver glutathione concentration at the end of experiments was also significantly lowered in exercised animals (−50%) (Table 1). Cytosolic glutathione *S*-transferase activity remained, however, unchanged (Table 1). GOT and LDH activities in liver homogenates did not significantly differ between exercised and control animals (GOT: 148 ± 11 units/g liver vs 134 ± 19 units/g liver in the controls; LDH: 642 ± 24 units/g liver vs 586 ± 16 units/g liver in the controls).

DISCUSSION

Strenuous exercise is associated with metabolic alterations resulting from increased energy demands. In our experiments it was found, as reported by other authors [21, 22], that at the completion of exercise blood glucose levels were significantly reduced, reflecting the marked increase in glucose utilization by muscle [23]. The liver must prevent hypoglycaemia producing glucose primarily by glycogenolysis, which results in the reduced liver glycogen concentration found during exercise.

Following acute exercise in rats it has been shown that elevated levels of blood lactate return to normal within 15 min [21] and it has been suggested that its immediate fate would be the conversion to glucose

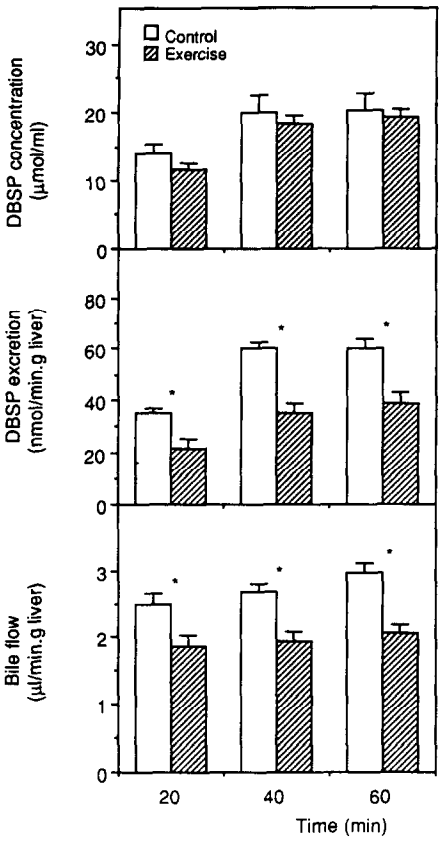

Fig. 4. Effect of exercise on biliary concentration and excretion of DBSP and on bile flow. After a priming dose of 2.15 μmol/100 g body wt DBSP was infused for 60 min at 215/min.100 g body wt in control and exercised rats. Values are means ± SE from six animals. *$P < 0.05$ significantly different from control value.

in the liver to compensate for the hypoglycaemia observed at exhaustion [21]. This would explain why blood lactate levels were only slightly increased when assayed in exercised rats.

Different investigations have demonstrated that exhaustive exercise results in oxidative stress as a consequence of an increased free radical production exceeding the antioxidant capacity of tissues [24]. As expected from processes that increase oxidative stress, following prolonged exercise large alterations of the glutathione system are found both in rats [3, 4] and humans [25]. This could modify the hepatobiliary transport of compounds that, as BSP, are conjugated with glutathione in the liver.

In our study maximal biliary excretion of BSP was significantly reduced following strenuous exercise. Elimination of this anion from plasma depends on several hepatic processes, including uptake by the hepatocytes, conjugation and transport into bile. The alteration of one or several of these steps could account for the change in the rate of BSP excretion.

The lowered BSP *Tm* caused by exercise cannot be explained only as the result of an inhibitory effect on the uptake of the organic anion, since in spite of the higher plasma BSP levels of the exercised rats compared with the controls, its concentration in liver was significantly enhanced.

An alternative explanation may be that of alterations in the hepatic biotransformation of the dye. Intrahepatic conjugation with glutathione plays an important role in the overall transcellular transport process of this anion, and a decreased BSP excretion has been reported after inhibition of glutathione *S*-transferase activity [26, 27] or glutathione depletion [28]. In the present study no significant changes in liver glutathione *S*-transferase activity were found. This coincides with previous data in that both glutathione peroxidase and glutathione *S*-transferase activities remained unchanged following prolonged exercise [29]. Liver glutathione concentration, however, was clearly reduced, which could account for the lowered BSP-glutathione excretion into bile due to the lower quantity of conjugated dye available for excretion.

Although reduced BSP conjugation could partly explain our results, the fact remains that the biliary excretion of the unconjugated dye is also significantly lowered by strenuous exercise. This would not be

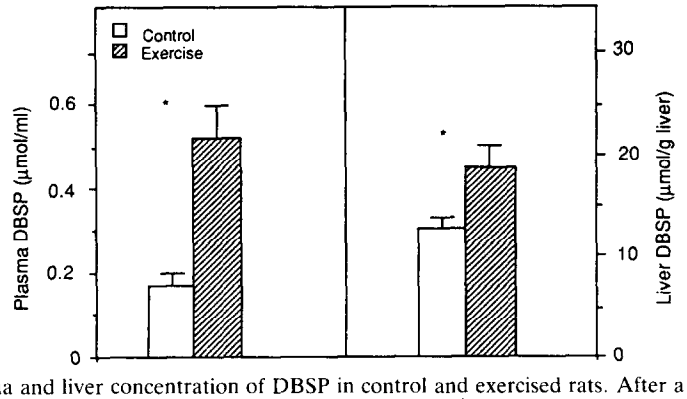

Fig. 5. Plasma and liver concentration of DBSP in control and exercised rats. After a priming dose of 2.15 μmol/100 g body wt DBSP was infused for 60 min at 215/min.100 g body wt. Data were obtained at the end of the infusion and are means ± SE from six animals. *$P < 0.05$ significantly different from control value.

directly related to the glutathione depletion, since the biliary excretion of DBSP, an organic anion that is not conjugated within the hepatocyte [9], was also significantly impaired.

Some explanations could be put forward to account for these effects. Cholephilic anions have been classified as bile acid dependent or independent according to whether their excretion is affected or not by modifications in bile acid secretion [30, 31]. Unconjugated BSP is included in the first group, whereas excretion of DBSP may or may not depend on bile acids [30, 31]. In our study, biliary secretion of bile acids and bile flow were significantly reduced and this could in turn partly affect the excretion of organic anions. Relationships are, however, complex and it cannot be ruled out that the decreased bile flow and secretion of bile acids are a consequence of the toxic effect caused by the liver accumulation of organic anions [32, 33].

Although our data apparently indicate that strenuous exercise does not cause a significant general hepatocyte damage, hepatocyte plasma membrane alterations cannot be totally excluded. It is well known that increased oxidative stress can be associated with lipid peroxidation of membranes, resulting in loss of structural integrity and selective permeability of membranes [34]. Although different authors have not found significant alterations of malondialdehyde levels (a commonly used index of lipid peroxidation) in either rat muscle or liver following strenuous exercise [35], in some cases it has been reported that an exhaustive bout of exercise can significantly increase its liver concentration [8]. It has also been shown that following exhaustive exercise there is a significant reduction of liver phospholipid content [36]. Changes in membrane composition or fluidity could therefore significantly interfere with the mechanisms of bile formation and the excretory process of the tested organic anions.

Another factor that could contribute to the results here reported would be the reduction of liver blood flow induced by exercise [37], that has been demonstrated to lower the hepatic detoxification of high clearance drugs [38]. In this respect, it has been proposed that liver glutathione depletion, such as that found in our experiments, can induce decreases in liver blood flow as a consequence of changes in prostaglandin metabolism [39].

In summary, we have shown that exhaustive exercise is associated in rats with an impaired biliary excretion of organic anions. Although this effect can be partly dependent on the reduction in liver glutathione content and the impairment in the process of hepatic biotransformation, other factors probably related to the oxidative stress induced by exercise must be involved. The exact mechanisms remain unclear and require additional study.

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REFERENCES

1. Kasperek GK, Dohm GL, Tapscott EB and Powell T, Effect of exercise on liver protein loss and lysosomal enzyme levels in fed and fasted rats. *Proc Soc Exp Biol Med* **164**: 430–434, 1980.
2. Friden J, Sjostrom M and Ekblom B, Myofibrillar damage following intense eccentric exercise in man. *Int J Sports Med* **4**: 170–175, 1983.
3. Pyke S, Lew H and Quintanilha A, Severe depletion in liver glutathione during physical exercise. *Biochem Biophys Res Commun* **139**: 926–931, 1986.
4. Lew H, Pyke S and Quintanilha A, Changes in the glutathione status of plasma, liver and muscle following exhaustive exercise in rats. *FEBS Lett* **185**: 262–265, 1985.
5. Meister A and Anderson ME, Glutathione. *Annu Rev Biochem* **52**: 711–760, 1983.
6. Ketterer B, Detoxication reactions of glutathione and glutathione transferases. *Xenobiotica* **16**: 957–973, 1986.
7. Klaassen CD and Watkins JB, Mechanisms of bile formation, hepatic uptake and biliary excretion. *Pharmacol Rev* **36**: 1–61, 1984.
8. Davies KJA, Quintanilha AT, Brooks GA and Packer L, Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* **107**: 1198–1205, 1982.
9. Vonk RJ, Danhof M, Coenraads T, Van Doorn ABD, Keulemans K, Scaf AHJ and Meijer DKF, Influence of bile salts on hepatic transport of dibromosulphthalein. *Am J Physiol* **237**: E524–E534, 1979.
10. Bergmeyer HU, Bernt E, Schmidt F and Stork H, Glucose. Determination with hexoquinase and glucose-6-phosphate dehydrogenase. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), pp. 1196–1201. Academic Press, New York, 1974.
11. Gutmann I and Wahlefeld AW, L-(+) Lactate. Determination with lactate dehydrogenase and NAD. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), pp. 1464–1468. Academic Press, New York, 1974.
12. Kepler D and Decker K, Glycogen. Determination with amyloglucosidase. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), pp. 1127–1131. Academic Press, New York, 1974.
13. Whelan G and Combes R, Competition by unconjugated and conjugated sulfobromophthalein sodium (BSP) for transport into bile. Evidence for a single excretory system. *J Lab Clin Med* **78**: 230–244, 1971.
14. Paumgartner G, Horak B, Probst P and Grabner B, Effect of phenobarbital on bile flow and bile salt excretion in the rat. *Naunyn Schmiedebergs Arch Pharmacol* **31**: 109–118, 1971.
15. Tietze R, Enzymatic method for quantitative determinations of nanogram amounts of total and oxidized glutathione. Applications to mammalian blood and other tissues. *Analyt Biochem* **27**: 502–55, 1969.
16. Griffith OW, Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vynylpyridine. *Analyt Biochem* **106**: 207–212, 1980.
17. Goldstein J and Combes B, Spectrophotometric assay of the liver enzyme that catalyzes sulfobromophthalein-glutathione conjugation. *J Lab Clin Med* **67**: 863–872, 1966.
18. Bergmeyer HU and Bernt E, Glutamate oxalacetate transaminase. UV assay. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), pp. 722–733. Academic Press, New York, 1974.
19. Bergmeyer HU and Bernt E, Lactic dehydrogenase. UV assay with pyruvate and NADPH. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), pp. 574–579. Academic Press, New York, 1974.
20. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–272, 1951.
21. Johnson JL and Bagby GJ, Gluconeogenic pathway in

liver and muscle glycogen synthesis after exercise. *J Appl Physiol* **64**: 1591–1599, 1988.
22. Sellers T, Jaussi AW, Yang TH, Heninger R and Winder WW, Effect of the exercise-induced increase in glucocorticoids on endurance in the rat. *J Appl Physiol* **65**: 173–178, 1988.
23. Winder WW, Control of hepatic glucose production during exercise. *Med Sci Sports* **17**: 2–5, 1985.
24. Quintanilha A, Effects of exercise-induced oxidative stress on liver glutathione. In: *CRC Handbook of Free Radicals and Antioxidants in Biomedicine* (Eds Miquel J, Quintanilha AT and Weber H), Vol. II, pp. 133–143. CRC Press, Boca Raton, FL, 1989.
25. Gohil K, Viguie C, Stenby WC, Brooks GA and Packer L, Blood glutathione oxidation during human exercise. *J Appl Physiol* **64**: 115–119, 1988.
26. Fehring S and Ahokas JT, Effect of the glutathione *S*-transferase inhibitor, tienilic acid, on biliary excretion of sulfobromophthalein. *Chem Biol Interact* **69**: 23–32, 1989.
27. Mariscal MA, Muñoz ME, Collado PS, Esteller A and Gonzalez J, Impairment of sulfobromophthalein biliary excretion and inhibition of glutathione *S*-transferase activity induced by perhexiline maleate in rats. *Biochem Pharmacol* **33**: 2829–2834, 1988.
28. Aza MA, Gonzalez J and Esteller A, Effect of diethyl maleate pretreatment on biliary excretion and choleretic action of sulfobromophthalein in rats. *Arch Int Pharmacodyn Ther* **281**: 321–330, 1986.
29. Ji LL, Stratman FW and Lardy HA, Antioxidant enzyme systems in rat liver and skeletal muscle. Influences of selenium deficiency, chronic training and acute exercise. *Arch Biochem Biophys* **263**: 150–160, 1988.
30. Gregus Z, Fischer E and Varga F, Effect of cholestyramine-induced bile acid depletion on the hepatobiliary transport of cholephilic organic anions in rats. *Arch Int Pharmacodyn Ther* **245**: 311–322, 1980.
31. Gregus Z and Fischer E, Effect of sodium taurocholate on hepatic uptake and biliary excretion of organic anions in rats. *Arch Int Pharmacodyn Ther* **24**: 180–192, 1979.
32. Vonk RJ, Jekel P and Meijer DKF, Choleresis and hepatic transport mechanisms II. Influence of bile salt choleresis and biliary micelle binding on biliary excretion of various organic anions. *Naunyn Schmiedebergs Arch Pharmacol* **290**: 375–387, 1975.
33. Meijer DKF, Vonk RJ and Weitering JG, The influence of various bile salts and some cholephilic dyes on Na^+-K^+ and Mg^{2+} activated ATPase of rat liver in relation to cholestatic effects. *Toxicol Appl Pharmacol* **43**: 597–612, 1978.
34. Burr R, Schwenk M and Pfaff E, Interaction of sulfobromophthalein with mitochondrial membrane. Inhibition of respiration. *Biochem Pharmacol* **26**: 461–466, 1977.
35. Ji LL, Stratman FW and Lardy HA, Enzymatic down regulation with exercise in rat skeletal muscle. *Arch Biochem Biophys* **263**: 137–149, 1988.
36. Kasperek JG, Dohm GL, Barakat H, Strausbauch PH, Barnes DW and Snider D, The role of lysosomes in exercise-induced hepatic protein loss. *Biochem J* **202**: 279–288, 1982.
37. Sweeney GD, Drugs—some basic concepts. *Med Sci Sports* **13**: 247–251, 1981.
38. Dossing M, Effect of acute and chronic exercise on hepatic drug metabolism. *Clin Pharmacokin* **10**: 426–431, 1985.
39. Bottje WJ, Hassan AS and Holmes KR, Evidence for the association between total hepatic blood flow and hepatic glutathione content. *Biochem Pharmacol* **35**: 1629–1632, 1986.