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CARBOHYDRATE HOMEOSTASIS AND POST-EXERCISE KETOSIS IN TRAINED AND UNTRAINED RATS

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SUMMARY

1. Experiments were carried out to establish what relationship there is between the concentration of ketone bodies in the blood and the concentrations of glycogen in muscle and liver of thirty-six trained and thirty-six untrained rats exercised at the same absolute load. There were, in addition, non-exercised control animals (of which thirty-six were trained and thirty-six untrained) which were studied on the same day.

2. Training occurred on a level treadmill at 0·2 m/s for 1 h/day, 5 days a week, for 6 weeks. The untrained animals ran on the treadmill every 3rd day for 5 min to maintain familiarity with treadmill running without training them.

3. At the end of the 6th week, the experimental animals ran for 1 h at 0·2 m/s on a level treadmill. Blood 3-hydroxybutyrate and tissue glycogen concentrations were measured at the beginning and immediately after exercise, and then every 30 min for 2 h.

4. Physically trained rats had higher pre- and immediate post-exercise liver glycogen concentrations than untrained rats: 413 ± 15 and 300 ± 8 μmol/g before exercise in trained and untrained rats respectively, and 225 ± 8 and 166 ± 3 μmol/g immediately after ($P < 0{\cdot}05$).

5. Muscle glycogen, which was also higher in trained than in untrained rats, was resynthesized at approximately the same rate in the two groups of animals (9 and 11 μmol/(g h), but the trained animals were able to achieve this without further depletion of liver glycogen beyond that which had occurred during exercise. In untrained animals liver glycogen concentrations continued to drop for 60 min beyond the end of exercise.

6. The difference between the immediate post-exercise blood concentrations of 3-hydroxybutyrate of trained and untrained animals was not significant. Thereafter the level rose, within 60–90 min, to $1{\cdot}31 \pm 0{\cdot}04$ mmol/l in the untrained animals, but to only $0{\cdot}62 \pm 0{\cdot}05$ mmol/l in the trained animals ($P < 0{\cdot}05$).

7. There was a striking inverse relationship between the blood 3-hydroxybutyrate concentrations of all the animals (trained and untrained) and their simultaneous liver glycogen concentrations during the experiment ($r = -0{\cdot}88$; $P < 0{\cdot}001$).

8. The results suggest that the phenomenon of post-exercise ketosis is associated with an inability to maintain the liver glycogen concentration after exercise.

INTRODUCTION

In the absence of food restriction, non-athletes are more prone than athletes to develop post-exercise ketosis (Johnson, Walton, Krebs & Williamson, 1969*a*, *b*; Johnson & Walton, 1971; Winder, Baldwin & Holloszy, 1973, 1975; Rennie, Jennett & Johnson, 1974; Rennie & Johnson, 1974; Winder, Beattie & Holman, 1982; Koeslag, 1982). The reasons are unclear. In this study we have determined the degree of post-exercise ketosis and simultaneous muscle and liver glycogen concentrations in *ad libitum*-fed trained and untrained rats, to establish what relationship, if any, there is between these three variables during the recovery from a standard bout of exercise. Rats were used as liver glycogen concentrations cannot be measured in healthy humans without what is considered by many to be an unacceptable risk.

We confined our enquiry to the measurement of the blood 3-hydroxybutyrate and tissue glycogen concentrations because we could only obtain very small quantities of blood from each rat with certainty, and because the plasma hormone, glucose, free fatty acid, and acetoacetate concentrations have already been studied exhaustively without leading to clear conclusions (Johnson *et al.* 1969*a*, *b*; Johnson, Rennie, Walton & Webster, 1971; Johnson & Walton, 1972; Johnson & Rennie, 1973; Winder *et al.* 1973; Rennie, Winder & Holloszy, 1976; Holloszy, Winder, Fitts & Rennie, 1978; Koeslag, Noakes & Sloan, 1980, 1982; Beattie & Winder, 1984, 1985; Koeslag, Levinrad, Lochner & Sive, 1985; Adams, Irving, Koeslag, Lochner, Sandell & Wilkinson, 1987). The effects of dietary manipulation on the tissue glycogen levels and post-exercise ketosis are described elsewhere (Adams & Koeslag, 1989).

METHODS

One hundred and forty-four male Long–Evans rats (bred in the Animal House of the Department of Physiology, University of Cape Town) were used for the study which was approved by the Ethics Committee of the Faculty of Medicine. The animals were housed five per cage in a temperature-controlled room (20 °C). All the animals ran for a few minutes per day on an electrically driven treadmill at 0·1 m/s for 1 week to familiarize them with treadmill running.

After 1 week, the animals were randomly divided into a trained and an untrained group. Rats assigned to the trained group ran at 0·2 m/s on a level treadmill for 1 h/day, 5 days a week for 6 weeks. Rats in the untrained group ran every third day for about 5 min at 0·1 m/s to maintain their familiarity with treadmill running, without physically training them. Both groups were fed *ad libitum* on normal laboratory chow (food-energy value = 12·75 kJ/g) consisting of 60% carbohydrate, 18% protein, 5% fat, 7% ash and 10% moisture. Both groups maintained their body weights on this regimen.

After 6 weeks both the trained and untrained groups were randomly subdivided into an experimental and a control group. Thus, two experimental (trained and untrained) and two control (trained and untrained) groups existed after the second subdivision.

On the experimental day, the animals belonging to the two experimental groups ran on the treadmill at 0·2 m/s at a 0 deg gradient for 1 h. Immediately before the exercise, on completion of the exercise, and at 30 min intervals thereafter, six animals from each group (trained experimental, TE; trained control, TC; untrained experimental, UE; and untrained control, UC) were anaesthetized by a subcutaneous injection of sodium pentobarbitone, (dose = 4 mg/100 g body weight). As soon as each rat could be handled easily, it was killed by decapitation, in a room away from the other animals.

During the experiment the control rats were placed near the treadmill where their experimental counterparts were running, to keep the environmental noise, odour, light and temperature for the

different groups the same. None of the rats was given food to eat during the observation period on the experimental day.

After the animals had been killed they were bled and the blood collected for the determination of the 3-hydroxybutyrate concentrations (Williamson, Mellanby & Krebs, 1962). Portions of liver and gastrocnemius muscle were removed and frozen in liquid nitrogen within 2 min of the decapitation. The tissue was then stored at −80 °C until it could be analysed for its glycogen content (Good, Kramer & Somogyi, 1933) and the muscle malate dehydrogenase activity (Mehler, Kornberg, Grisolia & Ochoa, 1948).

Statistical analysis

The results are expressed as mean ± standard error of the mean (S.E.M.). Unless stated otherwise, the statistical significance of differences between groups was determined by analysis of variance, followed by Tukey's post-ANOVA test. $P < 0.05$ was considered to be an indication that the difference noted was not due to chance.

RESULTS

Body mass

The mean body weight of the untrained rats was 300 ± 15 g and that of the trained rats 325 ± 10 g after the 6 week training period. This difference was not statistically significant ($P > 0.1$; two-tailed Student's t test; d.f. = 142).

Muscle malate dehydrogenase

The muscle malate dehydrogenase (MDH) activity on the experimental day (before the day's exercise) was 11 ± 1·0 U/g wet mass for the untrained rats, and 24·0 ± 2·5 U/g wet mass for the trained rats ($P < 0.05$; two-tailed Student's t test; d.f. = 22).

This approximately 100% higher MDH activity in the trained animals suggests that the training programme had been effective, and is comparable with the results of Holloszy, Molé, Baldwin & Terjung (1973) and Holloszy, Booth, Winder & Fitts (1975), who showed that training produces increases of between 60 and 100% in the activities of the enzymes involved with the oxidative capacity of skeletal muscle.

3-Hydroxybutyrate (Fig. 1)

There was no significant difference between the blood 3-hydroxybutyrate concentrations of the trained and untrained groups before exercise.

The mean 3-hydroxybutyrate concentration immediately after exercise was significantly higher than before the exercise in both experimental groups ($P < 0.05$; Student's t test; d.f. = 10) and was also higher than those of the controls ($P < 0.01$; d.f. = 3, 20). No significant difference in mean blood 3-hydroxybutyrate concentration was observed between the trained and untrained experimental rats immediately on stopping of exercise (0·89 ± 0·05 mmol/l for the trained and 0·77 ± 0·02 mmol/l for the untrained; $P > 0.05$, d.f. = 3, 20).

In the trained experimental animals, the 3-hydroxybutyrate concentration did not change significantly for 90 min after exercise (0·77 ± 0·02 mmol/l at the end of exercise, and 0·62 ± 0·05 mmol/l at 90 min after exercise), but then fell to 0·58 ± 0·03 mmol/l ($P < 0.05$) at 120 min after exercise. The blood 3-hydroxybutyrate concentration increased significantly in the untrained experimental animals during the

recovery period, reaching 1·24±0·13 mmol/l at 30 min and 1·31±0·04 mmol/l at 60 min after exercise (Fig. 1). Thereafter it fell to 1·01±0·1 mmol/l at 90 min and 0·8±0·5 mmol/l at 2 h post-exercise.

The mean 3-hydroxybutyrate concentrations of the untrained experimental animals were significantly higher than those of the trained experimental animals, and both were significantly higher than those of their respective controls, during the entire 2 h recovery period.

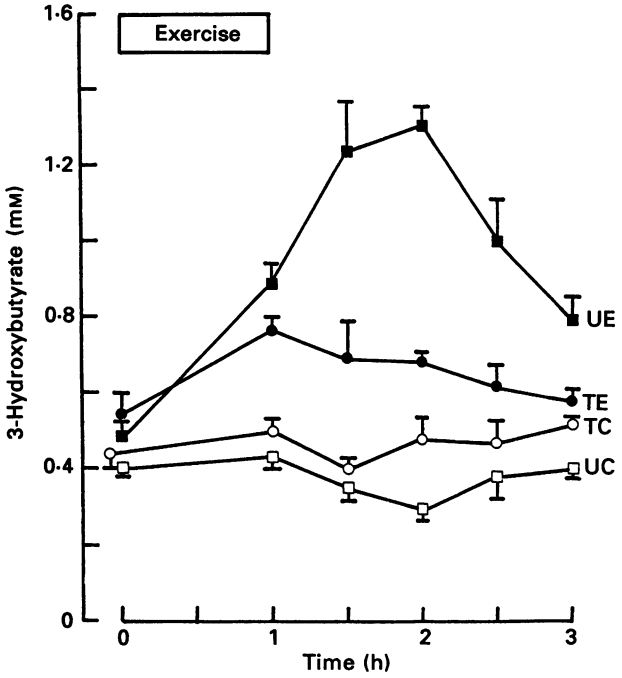

Fig. 1. The mean±S.E.M. blood 3-hydroxybutyrate concentrations of four groups of rats who had all eaten a normal diet up to the time of the experiment. Two groups, TE (●) and TC (○), underwent a 6 week physical training programme before the experiment; the remaining two groups, UE (■) and UC (□), did not train. The closed symbols (groups TE and UE) depict the results of the animals that ran on a level treadmill at 2 m/s for 1 h at the beginning of the observation period, as indicated. The open symbols (groups TC and UC) did not exercise during the observation period, but were housed in cages next to the treadmill.

Liver glycogen (Fig. 2)

The liver glycogen concentrations of the trained animals were significantly higher (413±15 μmol glucosyl units/g wet mass) than those of the untrained animals (300±8 μmol glucosyl units/g wet mass) ($P < 0·05$; two-tailed Student's t test; d.f. = 22).

The 1 h of exercise cause a significant decrease in liver glycogen concentration in both the trained and untrained rats. Although the amount of glycogen left in the trained livers (225±8 μmol/g) was significantly higher than that in the untrained livers (166±3 μmol/g) at the end of exercise ($P < 0·05$; d.f. = 3, 20), the rate of

glycogen utilization during exercise was slightly higher in the trained than in the untrained rats (188 and 134 μmol/(g h) respectively).

The liver glycogen concentration continued to fall during the post-exercise recovery phase in the untrained experimental animals to reach 87 ± 3 μmol/g at 90 min post-exercise, which was significantly lower than the 166 ± 3 μmol/g at the end of exercise ($P < 0{\cdot}01$; Student's t test; d.f. = 10). After 2 h of recovery, the liver

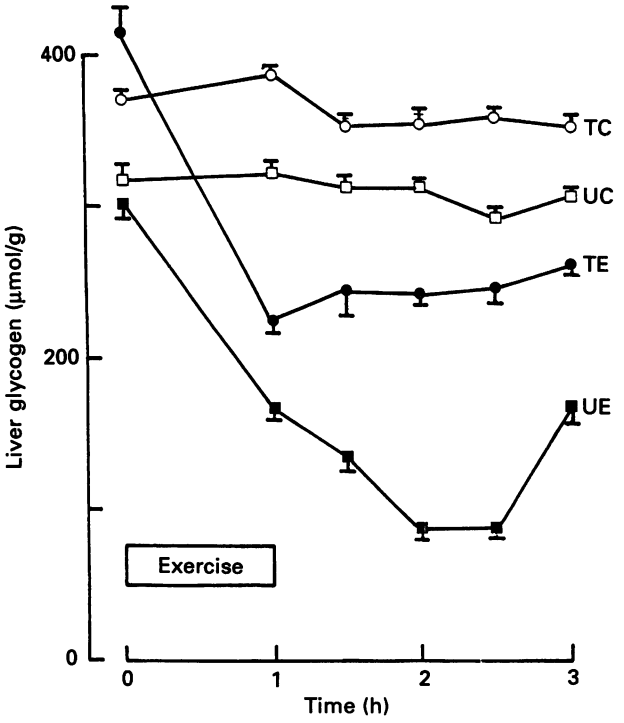

Fig. 2. The effect of exercise on the mean $\pm$ S.E.M. liver glycogen concentrations of the trained (TE), and untrained (UE) rats whose blood 3-hydroxybutyrate concentrations are depicted in Fig. 1 (using the same symbols). The exercise consisted of running at 2 m/s for 1 h on a level treadmill, at the time indicated on the graph. The changes in liver glycogen concentration of unexercised control animals are depicted by the curves labelled TC (trained controls) and UC (untrained controls).

glycogen content of the untrained animals had risen again to 167 ± 5 μmol/g. In the trained rats, the liver glycogen concentration started to rise within the first 30 min of recovery to reach 261 ± 4 μmol/g at 2 h after exercise. This means that the trained animals replenished 19% of the liver glycogen used during exercise in the 120 min of recovery (without food). The untrained animals regained none of the liver glycogen used during exercise.

Muscle glycogen (Fig. 3)

The mean resting muscle glycogen concentration was significantly higher in the trained rats (54 ± 1 μmol glucosyl units/g wet mass) than in the untrained rats (39 ± 1 μmol/g) ($P < 0{\cdot}05$, two-tailed Student's t test; d.f. = 22).

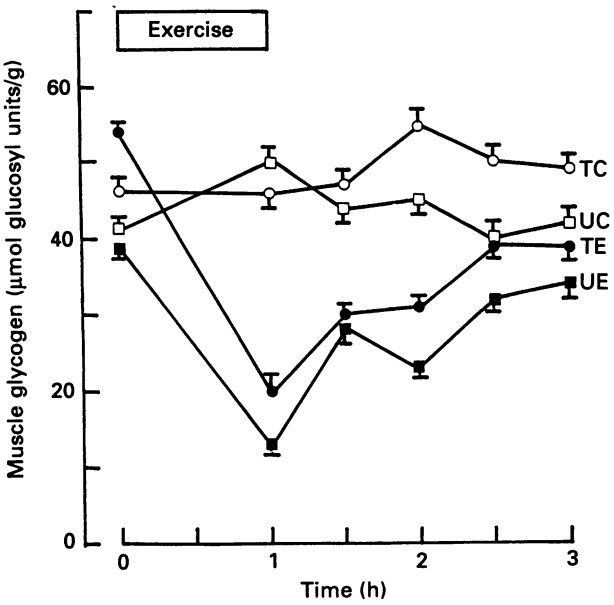

Fig. 3. The mean ± S.E.M. gastrocnemius muscle glycogen concentrations of the rats whose liver glycogen and 3-hydroxybutyrate concentrations are illustrated in Figs 1 and 2. The experimental conditions and symbols are the same as in Fig. 1. (TE = trained animals which exercised at the time indicated; UE = untrained rats which exercised at the beginning of the observation period; TC = trained rats which did not run during the observation period; UC = untrained rats which did not exercise during the experiment.)

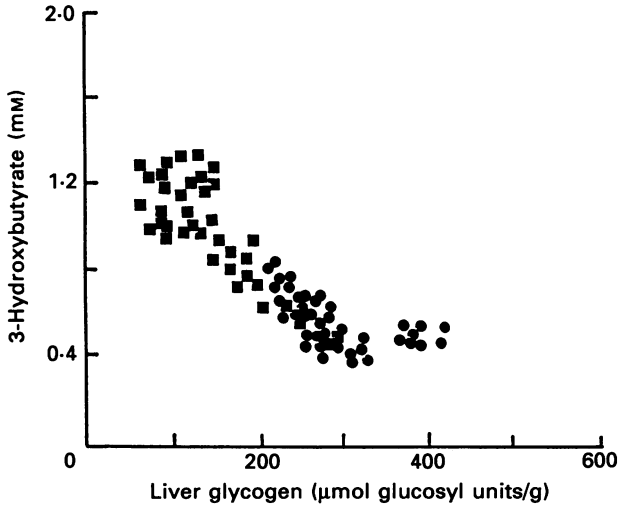

Fig. 4. The simultaneous blood 3-hydroxybutyrate and liver glycogen concentrations of each of the thirty-six trained (●) and thirty-six untrained (■) rats who constituted the two exercise groups (TE and UE) described in Fig. 1. The coefficient of correlation (r) is $-0{\cdot}88$; $P < 0{\cdot}001$.

Exercise significantly lowered the muscle glycogen content in both experimental groups. As was the case with liver glycogen, the rate of muscle glycogen utilization was slightly higher in the trained than in the untrained animals (33 and 26 μmol/(g h) respectively). Nevertheless, because of their higher resting levels, the trained rats retained more glycogen in their muscles after exercise (20 ± 2 μmol/g) than did the untrained rats (13 ± 1 μmol/g) ($P < 0{\cdot}05$; d.f. = 3, 20).

Muscle glycogen resynthesis started almost immediately after exercise in both groups, being slightly slower in the untrained than in the trained animals.

Blood ketone body and liver glycogen concentrations (Fig. 4)

The changes in the blood concentrations of 3-hydroxybutyrate during the recovery in both the trained and untrained animals were the approximate inverse of their liver glycogen concentrations (compare Fig. 1 with Fig. 2). This is emphasized when the blood ketone body concentrations of the individual trained and untrained rats are plotted against their simultaneous liver glycogen concentrations (Fig. 4). The correlation between these two variables is highly significant ($r = -0{\cdot}88$; $P < 0{\cdot}001$; d.f. = 71).

DISCUSSION

The present results confirm previous reports than overnight-rested trained animals store more liver and muscle glycogen than untrained animals do (Baldwin, Fitts, Booth, Winder & Holloszy, 1975; Fitts, Booth, Winder & Holloszy, 1975; Galbo, Richter, Christensen & Holst, 1975; Winder, Holman & Garhart, 1981; Adams & Koeslag, 1989). Since both groups of animals used approximately the same amount of liver and muscle glycogen during exercise (which was of exactly the same intensity and duration for both) the immediate post-exercise tissue glycogen concentrations were between 35 and 55% higher in the trained than in the untrained rats (Figs 2 and 3).

Net resynthesis of muscle glycogen started during the first 30 min of recovery, and occurred at approximately the same rate in the trained as in the untrained animals (11 and 9 μmol glucosyl units/(g h) respectively), in the total absence of food. The rate of liver glycogen repletion was, however, very different. The liver glycogen concentrations of the trained animals rose slowly during recovery, whereas those of the untrained rats fell by almost 50% during the first 90 min of recovery (from 166 ± 3 μmol/g immediately after exercise to 87 ± 3 μmol/g at 90 min post-exercise; $P < 0{\cdot}01$) before starting to rise during the final 30 min of the observation period. At 120 min after exercise the untrained rats' liver glycogen concentrations were no higher than they had been immediately after exercise (Fig. 2). Muscle glycogen replenishment is therefore clearly given a far higher priority than the restoration of the liver levels of glycogen. When carbohydrate is scarce (e.g. after exercise in untrained animals) the latter can in fact continue to be plundered while, in the same hormonal milieu, glycogen is actively being laid down in the muscles (Figs 2 and 3). This even occurs, though to a lesser extent, in carbohydrate-starved untrained rats challenged with exactly the same exercise as the present animals (Adams & Koeslag, 1989). Teleologically, this makes good sense, since a glycogen-depleted liver produces

only minor handicaps under normal circumstances, whereas a glycogen-depleted muscle is almost functionless.

The 1 h of treadmill running increased the blood 3-hydroxybutyrate concentrations of both the trained and untrained animals to about the same extent (Fig. 1). However, the 3-hydroxybutyrate concentration increased further during recovery in the untrained animals while remaining the same in the trained rats. These findings are in agreement with earlier studies which have shown that endurance-trained animal and human subjects on a free diet exhibit a lesser degree of post-exercise ketosis than untrained controls (Johnson *et al.* 1969*a*; Winder *et al.* 1973, 1975). The present study shows, however, that this phenomenon is closely related to the differences in liver glycogen metabolism of trained and untrained animals. Thus, not only are the changes in the post-exercise blood ketone body concentrations of the different groups the approximate inverse of the changes in their mean liver glycogen levels (Figs 1 and 2), but for the same hepatic carbohydrate levels trained and untrained rats have similar blood ketone body concentrations (Fig. 4). The high degree of correlation between the blood ketone body concentration and simultaneous liver glycogen content ($r = -0{\cdot}88$) suggests that the link between the two is very close indeed after exercise. Such a close link has never previously been shown to exist between the post-exercise blood ketone body concentration and any other biochemical variable measured to date (Johnson *et al.* 1969*a*, *b*; Johnson & Walton, 1971; Johnson & Rennie, 1973; Winder *et al.* 1973; Rennie *et al.* 1974, 1976; Holloszy *et al.* 1978; Koeslag *et al.* 1980, 1982, 1985; Beattie *et al.* 1985; Adams *et al.* 1987; Adams & Koeslag, 1989). The nature of the link, and the mechanisms which drive the system, remain matters for speculation beyond the scope of this article.

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