POST-EXERCISE KETOSIS.

Thesis submitted to the University of Cape Town

for

MSc (Med)

by

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TABLE OF CONTENTS

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DECLARATION

I, Jacob Harold Adams, declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise), and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

I empower the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Part of this study was presented at the Physiological Congress held in Pretoria in October 1985.

J.H. Adams

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SUMMARY:

Koeslag, Noakes and Sloan (1980) showed that the plasma fuel-hormone concentrations during exercise, and the development of ketosis after exercise are critically dependent on the amount of carbohydrate consumed on the days preceding the exercise. This suggests that the development of post-exercise ketosis could be related to the liver glycogen content after exercise, especially since endurance training is known to increase hepatic glycogen concentrations and to provide immunity against post-exercise ketosis. As it is impractical and unethical to perform liver biopsies in healthy human subjects, it was decided to investigate the problem in an animal model.

Four hundred and thirty two Long-Evans rats were assigned to 3 groups according to the diet eaten during the week preceding the Experimental Day: normal, low and high carbohydrate diet. Each group was subdivided into a Trained (those that had run on a level treadmill at 0.2 m/s for 1 hour daily for 6 weeks) and an Untrained group. On the Experimental Day half of each subgroup ran on a treadmill at 0.2 m/s for an hour, while the other half (Controls) remained in their cages nearby. An animal from each subgroup was sacrificed by decapitation before the exercise, immediately afterwards and then at 30 minutes intervals for 2 hours into the recovery period.

The muscle malate dehydrogenase activity (an indication of oxidative enzyme induction) was significantly higher ($p<0.01$) in the Trained than in the Untrained animals, indicating that the training programme had been effective.

The liver glycogen levels of the Untrained animals were 450 ± s.e.m. 8 µmol glucosyl units/g wet mass in the high carbohydrate group, 300 ± 8 µmol/g in the normal diet group, and 150 ± 7 µmol/g in the low carbohydrate group. These levels fell during exercise to 270 ± 14; 166 ± 3 and 27 ± 1 µmol/g respectively. During the first 90 minutes of recovery the liver glycogen levels continued to fall (to 87 ± 3 µmol/g) in the normal group, but remained unchange in the other 2 groups. Thereafter liver glycogen levels rose in the normal and high carbohydrate groups but not in the low carbohydrate group.

Training caused higher pre-exercise liver glycogen levels, which dropped proportionally less with exercise, and recovered faster than in the Untrained group, for each dietary situation.

A highly significant negative correlation ($r = -0.9$; $p<0.01$) between the liver glycogen concentrations and simultaneous blood 3-hydroxybutyrate levels (of the combined Trained and Untrained results) was found in the normal diet group, as

well as in the low carbohydrate group ($r = -0.7$; $p<0.05$). This relationship was not clear in the high carbohydrate group, since the Untrained animals developed a marked post-exercise hyperketonaemia despite high liver glycogen concentrations. The Trained carbohydrate-fed animals had the highest post-exercise liver glycogen concentrations and the lowest blood 3-hydroxybutyrate concentrations found in the study.

These results show that: (1) The dominant cause for the differences in the degree of post-exercise ketosis between trained and untrained animals is probably the difference in liver glycogen concentration. (2) On any given diet, the blood ketone body levels of the trained rats were more resistant to changes in liver glycogen than were those of the untrained rats, especially after a high carbohydrate diet. This could be due to a greater availability of mobilizable carbohydrate (via the 3-carbon compounds) in the trained animals. (3) The relationship between the blood ketone body concentration and simultaneous liver glycogen content "shifted to the right" with carbohydrate-feeding and to the left with carbohydrate-deprivation. These "shifts" could be due to the induction (or repression) of the enzymes of ketone body oxidation by the week-long low (or high)

carbohydrate diet. Alternatively, the "shifts" could be due to an alteration of the relationship between the rate of ketogenesis and the hepatic glycogen concentration by, for instance, the supply of lactate and alanine (3-carbon compounds) to the liver.

CHAPTER 1.

LITERATURE REVIEW:

HISTORICAL BACKGROUND

Studies on the interaction between diet, training and post-exercise ketosis go back nearly a hundred years. Hirshfield (1895) found that the amount of acetone excreted in the urine of non-diabetic subjects was dependent on the amount of carbohydrate in the diet, but that exercise (such as turning a centrifuge handle for 2 hours) had no effect on the rate of ketone body excretion in the urine.

However, Forssner (1909) found that his urinary excretion of ketone bodies increased after a brisk walk of 4 km. At the time of the experiments he had been on a low carbohydrate diet (55-60 g carbohydrate per day) with a total energy value of about 14 MJ for more than 2 weeks. When 15 g of glucose was ingested on a day on which he did not exercise, a rapid decrease in acetonuria was observed, but no effect was seen when the glucose was taken immediately after exercise.

In agreement with Forssner's findings, Gemmill (1934) could not demonstrate a post-exercise increase in blood ketone body concentrations in three healthy subjects eating a

normal diet. But when these subjects ate a low carbohydrate diet for 4 days, their blood ketone body concentrations rose significantly after exercise.

Courtice, Douglas and Priestley (1939) found that 50 g glucose taken immediately after exercise did not abolish the development of post-exercise ketosis, corroborating the findings of Forssner, 30 years earlier. However, when the sugar was taken 1.5 to 4 hours later a significant reduction in the rate of excretion of ketone bodies was observed. The exercise was a 16 km walk at 7.2 km/h, which was performed alternately by Courtice and Douglas. Courtice was observed to develop post-exercise ketonuria only on days which had been preceded by dietary carbohydrate restriction. When his carbohydrate intake was increased on the day before the 16 km walk, ketonuria did not develop in the recovery phase.

It was found that the excretion of ketones tended to be biphasic in the recovery period. An early peak was observed about 3-4 hours after the walk followed by a dip and a second peak 5-6 hours later (Courtice and Douglas, 1936).

Mills (1938) used the same exercise as Courtice and Douglas (1936), a 16 km walk on almost level ground at a speed of 7.2 km/h. Mills performed the exercise while Douglas acted as the control. No post-exercise ketonuria could be demonstrated even after carbohydrate restriction. This was

ascribed to the large proportion of carbohydrate in Mill's regular diet.

The "Courtice-Douglas effect" as post-exercise ketosis came to be known, was investigated 20 years later by Passmore and R.E. Johnson (1958) under more rigorous experimental conditions. They studied post-exercise ketosis in 3 trained subjects (who were in training for rugby or squash at Cambridge University), and seven untrained but healthy university students. Their exercise test consisted of a walk of 16 km at 6.7 km/h on an indoor treadmill in carefully controlled environmental conditions. Before the exercise test all subjects had consumed the same balanced diet (12 MJ/day) for 48 hours; and after the test they all rested, lying down for 5 hours. The experiments were repeated at different environmental temperatures. On each occasion the excretion of ketone bodies in the urine was higher in the trained than in the untrained subjects, for up to 5 hours.

This was in agreement with an earlier study of Grollman and Phillips (1954) who showed that the immediate post-exercise blood ketone concentrations of physically exhausted rats are much higher in exercise-trained than in untrained animals, a finding which was later confirmed by Askew et al. (1975).

In contrast to these findings, the more recent investigations into post-exercise ketosis showed that

exercise training has an apparent anti-ketogenic effect. This was first demonstrated in 1969 by R.H. Johnson et al. who showed that the degree of post-exercise hyperketonaemia is more marked in non-athletes than in athletes. Winder et al. (1975 and 1979) confirmed these findings. Ignoring the contrary findings of Passmore and R.E. Johnson (1958) and Grollman and Phillips (1954), these researchers attempted to explain the anti-ketogenic effect of exercise training on the basis of the body's altered hormone response to exercise after training (Johnson et al., 1969a, 1970a, 1971, 1972a, 1972b, 1973a, 1973b, 1974a, 1974b, 1974c; Rennie et al., 1974a, 1974b, 1974c, 1976a, 1976b; Winder et al., 1979) and the training-induced changes in muscle enzyme activities (Winder et al., 1973a, 1973b 1974, 1975).

Their findings could not confirm the suggestion that the athletes' resistance to post-exercise ketosis is due to the higher plasma insulin, and lower blood glucagon, growth hormone and free fatty acid levels during exercise after training. Patients with hypopituitarism developed higher post-exercise blood ketone body concentrations than normal controls, despite the absence of growth hormone in these patients (R.H. Johnson et al., 1970 and 1971), whereas patients with acromegaly do not develop post-exercise ketosis (R.H. Johnson and Rennie, 1973). Induced hyperlipidaemia was similarly shown to counteract post-exercise ketosis in rats (Rennie et al., 1976).

There is, however, evidence that athletes metabolise ketone bodies at a faster rate than non-athletes do after exercise (Johnson and Walton, 1970 and 1972).

FACTORS THAT INFLUENCE POST-EXERCISE KETOSIS:

(1) ATHLETIC TRAINING:

Grollman and Phillips (1954) showed that the immediate post-exercise blood ketone body concentration is much higher in exercise-trained than in untrained rats (4.32 ± 1.84 mg/100 ml for the trained against 8.98 ± 2.74 mg/100 ml for the trained rats after exhausting exercise).

These findings were confirmed by Passmore and R.E. Johnson (1958), who studied 10 healthy University students of whom three were in training for rugby or squash. Each subject exercise on an indoor treadmill for 2.4 hours and then rested for 6 hours under carefully controlled conditions. The athletes excreted a total of 2.3 mmoles of ketone bodies in the urine during the post-exercise period compared with the 1.1 mmoles of the non-athletes under thermoneutral conditions (21^{o}C - 25^{o}C). Under cooler conditions (8^{o}C -

14°C), the athletes' urinary ketone body excretion was 3.1 mmoles, and that of the non-athletes 2.5 mmoles. The blood ketone body concentrations 5 hours after exercise were 0.98 and 1.04 mmol/l for the athletes compared to 0.68 and 1.28 mmol/l for non-athletes in the warm and cool conditions respectively. Askew et al. (1975) also found higher blood ketone body concentrations in trained than in untrained rats at exhaustion.

On the other hand, R.H. Johnson et al. (1969a, 1969b, 1970a, 1970b, 1971a, 1971b, 1972a, 1972b, 1973a, 1973b, 1974a, 1974b, 1974c) and Rennie et al. (1974a, 1974b, 1974c, 1976a, 1976b) found, in a series of extensive investigations, that post-exercise ketosis develops more readily in the untrained than in trained men.

When nine athletes and eighteen non-athletes ran for 1.5 hours on an outdoor track at their own speeds, the blood ketone body concentrations rose markedly in the non-athletes during the exercise and the recovery periods, reaching a mean value of more than 1.6 mmol/l, 90 minutes after exercise, compared to a value of 0.3 mmol/l in the athletes. The mean plasma free fatty acid concentration was also significantly higher in the untrained than in the trained group during the first 60 minutes of exercise. Blood glycerol levels were, however, similar in the two groups.

These results led to the conclusion that although both groups had mobilized triglycerides to the same extent during exercise (as evidenced by the equal rise in the blood of the glycerol levels in the two groups), the non-athletes did not utilize their free fatty acids as effectively as did the athletes. The higher free fatty acid levels in the blood of the non-athletes after exercise were presumed to have stimulated ketogenesis.

The obvious criticism of the above experiment was that the two groups had exercised at different absolute, as well as at different relative, intensities. The athletes ran further in the 1.5 hours, but at lower mean heart rates (130 beats/minute) than the non-athletes (165 beats/minute). However, when four trained cyclist and five untrained subjects exercised on a bicycle ergometer for 20 minutes, with work loads individually adjusted so that each subject worked at approximately the same heart rate (between 150 and 170 beats/minute), the blood lactate levels were similar in the two groups, but the blood ketone body concentrations rose to higher levels in the untrained subjects (0.19 mmol/l) than in the trained cyclist (0.07 mmol/l, $p<0.01$) (Rennie et al., 1974a).

In another experiment, Johnson and Walton (1971) confirmed that the blood ketone body concentrations two hours after exercise are negatively correlated with the subjects'

fitness indices, as determined by the Harvard Step Test ($r=-0.5$, $p<0.005$). However, in these experiments the plasma free fatty acid levels were higher in the athletes than in the non-athletes. This suggests that the relation between the post-exercise blood ketone body levels and the plasma free fatty acid levels found in their earlier studies (Johnson et al., 1969) may have been spurious and without physiological meaning. This has subsequently been confirmed by Rennie et al. (1976).

Rennie and Johnson (1974) studied eight young men who underwent a four week training programme. They found that the degree of post-exercise ketonaemia falls as training progresses. They concluded that the biochemical and endocrine differences observed between the trained and non-trained individuals are due to the regular exercise and not to a genetic characteristic of athletes. In a similar study, Winder et al. (1979) studied the degree of post-exercise hyperketonaemia in 6 healthy subjects during and after a 9 week training programme. Resting 3-hydroxybutyrate concentrations did not change significantly with training, but post-exercise 3-hydroxybutyrate concentrations fell from 0.42 mmol/l to 0.2 mmol/l during the first three weeks of training. Thereafter no further changes were observed.

Winder et al. (1975) also found that post-exercise ketonaemia is more pronounced in physically untrained rats

than in trained rats, even when the trained rats run at twice the speed of the untrained animals. They also found that with exercise training the rate of aceto-acetate oxidation by the gastrocnemius muscle homogenates obtained 24 - 27 hours after exercise increases two-fold and that of 3-hydroxybutyrate three-fold (Winder et al., 1973 and 1975). The activity of all the enzymes involved in ketone body oxidation were found to have increased. Thus 3-hydroxybutyrate dehydrogenase activity increased by 100%, and acetoacetyl CoA-thiolase by approximately 55%. The activity of 3-ketoacid CoA transferase also increased significantly in response to an endurance training programme (Winder and Beattie, 1984). This suggests that the rate of enzymic oxidation of ketone bodies might be an important factor determining differences in the degree of post-exercise ketosis between trained and untrained subjects. This has been confirmed by Johnson and Walton (1972) who showed that the rate of acetoacetate clearance from the blood is higher in athletes than in non-athletes after a bout of exercise. No difference in the rate of acetoacetate clearance exists between athletes and non-athletes at rest.

Askew et al. (1975) investigated the interaction of diet, training and exhausting exercise on ketone body metabolism in rats. Their training programme consisted of treadmill running at 0.49 m/s for 2 hours per day, 5 days a week for 12 weeks. This produced an increase in ketone body oxidizing

enzyme activities in the trained rats that were similar to those of Winder et al. (1973 and 1975). The final exercise consisted of 60 second periods of sprinting at 0.94 m/s on an 8 degree grade every 10 minutes until the animals were completely exhausted. Exhaustion was defined as the refusal to run after electrical stimulation, failure to right themselves upon being placed on their backs, or a body temperature higher than 40°C.

The mean 3-hydroxybutyrate concentration of the trained exhausted animals was significantly higher (2.1 mmol/l) than that of the untrained (0.8 mmol/l). This result could be due to the fact that Askew et al. (1975) kept their rats on the same total food intake throughout the training period, whereas Winder et al. (1975) allowed their animals to eat ad libitum.

A similar difference distinguishes Passmore and R.E. Johnson's (1958 and 1960) studies from those of R.H.Johnson et al. (1969). Passmore and Johnson's athletes and non-athletes ate matched diets (12.6 MJ/day) on the days preceding the exercise test, whereas the diets of R.H. Johnson's subjects were uncontrolled. The former dietary protocol resulted in the athletes developing higher levels of post-exercise ketone bodies than the non-athletes (as was the case in Askew et al's (1975) rats), whereas athletes on

a free diet show no signs of becoming ketotic after exercise (Johnson et al., 1969).

In summary, athletes eating a freely-chosen diet appear to be almost completely resistant to post-exercise ketosis, even under intensely competitive conditions (Pugh et al., 1967; Pugh, 1969). This appears to be at least partly the result of an increased rate of acetoacetate clearance from the blood, possibly by the exercise-trained skeletal muscles (Winder et al., 1973a, 1974, 1975). When athletes are paired-fed with non-athletic controls, however, they develop a higher degree of post-exercise ketosis than their non-athletic counterparts for the same absolute (Passmore and Johnson, 1969), or relative (Grollman and Phillips, 1954; Askew et al., 1975) intensity of exercise. This suggests that the training-induced immunity to post-exercise ketosis is strongly influenced by dietary factors.

(11) INTENSITY AND DURATION OF EXERCISE:

Courtice and Douglas (1936) believed that the intensity of the post-exercise ketonuria is proportional to the total oxygen consumption during the exercise. This was based on the finding that the post-exercise ketonuria was approximately the same after a 19 km walk at 5.6 km/h as it

was after a 16 km walk at 7.2 km/h, but the ketonuria was less after a 16 km walk at 5.6 km/h.

Inter-study comparisons appear to indicate that prolonged, moderate exercise (e.g. walking, or submaximal treadmill running) tends to give rise to post-exercise ketosis more readily than does intense exertion of short duration (Eriksson et al., 1971; Johnson et al., 1969; Johnson and Walton, 1970). However, the only systematic study of the influence of different forms of exercise on the post-exercise blood ketone body concentrations (Koeslag et al., 1980) failed to demonstrate significant deviations from control day blood 3-hydroxybutyrate levels in any tests which were performed on healthy, sedentary young men eating an ad libitum diet.

(111) HORMONAL REGULATION OF KETOSIS:

Regular physical training is known to influence the hormonal changes during exercise. For example, growth hormone tends to rise to higher concentrations in the blood with exercise in untrained than in trained subjects (Bloom et al., 1976; Buckler, 1972; Rennie et al., 1974a, 1974b). This led Johnson et al. (1970, 1972a, 1973, 1974b) and Rennie et al.

(1974a, 1974b, 1974c) to search for an endocrine cause of post-exercise ketosis.

Growth hormone was studied in particular because of its known lipolytic and anti-insulin properties. Six patients with hypopituitarism and eight non-athletic control subjects were therefore exercised on a bicycle ergometer for 30 minutes at a work load of 65 W (Johnson et al., 1970a and 1971a). The growth hormone concentrations increased from 5 ng/ml before the exercise to 35 ng/ml during the exercise in the controls, but remained lower than 1 ng/ml in the patients, while the blood ketone body concentration rose to higher levels in the patients than in the controls.

Similar experiments were carried out on patients with acromegaly (Johnson et al., 1973a). The blood ketone body concentration decreased during the first 10 minutes of exercise, but then rose to 0.18 mmol/l 15 minutes later. During the recovery phase a fall in ketone body concentration was observed in the patients with acromegaly indicating that growth hormone is not important in the development of post-exercise ketosis.

Insulin levels in the plasma fall during exercise (Adams et al., 1987; Ahlborg et al., 1974; Bottger et al., 1971, 1972; Cochran et al., 1966; Galbo et al., 1975, 1977; Hunter and Sukkar, 1968; Koeslag et al., 1980, 1982, 1985) but rise

again when the muscular activity stops and can reach higher than pre-exercise values for a short time during recovery (Bottger et al., 1972; Galbo et al., 1975; Koivisto et al., 1980; Wahren et al., 1973). Thereafter insulin levels return to, and remains at, the pre-exercise level, showing no correlation with the simultaneous changes in blood ketone concentrations (Koeslag et al., 1980, 1982, 1985).

The glucagon levels, in contrast with insulin, rise during muscular activity but fall at the end of exercise to reach pre-exercise levels within 1 to 1.5 hours (Adams et al., 1987; Koeslag et al., 1982; Gyntelberg et al., 1977)

The decrease in plasma insulin concentration and increase in blood glucagon concentration during exercise would be expected to increase the rate of hepatic ketogenesis (Foster, 1984; McGarry, 1978; McGarry et al., 1975), though the exercise period is in fact relatively antiketogenic (Balasse et al., 1978; Barnes et al., 1940; Bloom et al., 1976; Carlson et al., 1971; Drury et al., 1941; Gammeltoft, 1952; Hagenfeldt, 1979; Houghton et al., 1971; Johnson and Passmore, 1960; Neufeld and Ross, 1943; Sestoft et al., 1977; Winder et al., 1973b). During the first hour or 2 of recovery the blood ketone body concentrations rise steeply (Koeslag et al., 1980, 1982, 1985; Adams et al., 1987), while, contrary to expectation, the plasma insulin concentrations are rising and glucagon concentrations are

falling. Furthermore, if the insulin:glucagon ratio is increased still further by the ingestion of 50 g glucose immediately after exercise the development of post-exercise ketosis is not prevented (Forssner, 1909; Courtice et al., 1939; Koeslag et al., 1985).

On the other hand, when the plasma insulin:glucagon ratio is lowered by the ingestion of 50 g alanine immediately after exercise post-exercise ketosis fails to develop (Koeslag et al., 1985).

Thus, it is clear that the blood ketone body concentration after exercise is not a simple correlate of the human growth hormone, insulin, glucagon or insulin:glucagon ratio in the blood, but must be regulated by an independent mechanism.

(1V) EFFECT OF DIET ON POST-EXERCISE KETOSIS:

Post-exercise ketosis has been found to occur in subjects on high protein diets (McClellan and Toscani, 1928; Preti, 1911; Neufeld and Ross, 1943), and low carbohydrate diets (Askew et al., 1975; Courtice and Douglas, 1936; Koeslag et al., 1980) but never after a high carbohydrate diet.
Preti (1911) studied the interaction between diet, and post-exercise ketosis in dogs and found increased acetone

concentrations in the urine after exercise, when they were on a constant beef diet. Likewise, when human subjects were fed a high protein diet (100 g meat extracts, 3 eggs, 100 g chicken per day), a large increase in acetone excretion in the urine was noticed after running up and down the stairs.

Gemmill (1934) could not demonstrate post-exercise ketosis in three healthy subjects eating a normal diet, but when these subjects ate a low carbohydrate diet for 4 days, the blood ketone concentration rose significantly after exercise.

Courtice and Douglas (1936), showed that ketone bodies occurred in Courtice's urine after exercise only if the carbohydrate content of his diet on the day preceding the exercise test day has been restricted, but when his carbohydrate intake before the test day was increased ketone bodies would not appear.

Rennie and Johnson (1973 and 1974), studied 6 long distance runners, who ran 24 km in 90 minutes, first after eating their normal diet, and then after "glycogen loading". The glycogen loading process consisted of 3 days training on a high-protein, high-fat (low-carbohydrate) diet, followed by 3 days of rest, eating a high carbohydrate diet. Blood ketone body concentrations were similar on both occasions at the beginning of exercise, and also rose to the same extent

during the 90 minute run. However, the post-exercise blood ketone body concentration rose to 0.25 mmol/l at 105 minutes after exercise on a normal diet, whereas glycogen-loading caused the blood ketone level to remain unchanged at 0.07 mmol/l. The other metabolites (lactate and pyruvate) rose to higher levels after glycogen loading than after the normal diet. During the exercise period the insulin levels fell after both diets, but the fall was more marked after glycogen loading. Exercising blood glycerol, free fatty acid and growth hormone concentrations were also lower after glycogen loading.

Askew et al. (1975) studied the interaction of diet, training and exhausting exercise on ketone body metabolism in rats. The rats were fed three different diets before the exercise test (commercial laboratory chow; a high fat diet; and a high carbohydrate diet). Their results showed that rats fed on a high fat or high carbohydrate diet had lower muscle acetoacetyl CoA thiolase activities (the enzyme involved in ketone body oxidation) than those fed on the control diet. After exercise the blood 3-hydroxybutyrate concentrations in the trained, exhausted animals were higher than in the untrained exhausted animals (2.1 mmol/l vs 0.8 mmol/l). A high carbohydrate diet lowered the 3-hydroxybutyrate concentration in both trained and untrained rats, while a high fat diet raised them.

Forgac (1979) warns athletes that a low carbohydrate diet can increase the blood ketone body levels and advises them not to consume diets which are totally free of carbohydrate during the "stripping" phase of the "glycogen loading" process, in order to avoid the irritability and fatigue which she claims is caused by ketosis. Young (1978) similarly mentions that the "acetone-breath" that can occur after training, should be regarded as a signal that more carbohydrate needs to be consumed if performance is to be maintained.

In 1980 Koeslag, Noakes and Sloan studied the effect of exercise on blood ketone body concentrations in two trained athletes and a sedentary subject. The trained athletes were two marathon runners (whose 42 km times, at that stage, were less than three hours). The sedentary subject exercised at 100 W for 2 hours (from 7.00am to 9.00am) on 6 test days. Prior to the first test he had been on his normal diet which included about 250 g carbohydrates per day. For 2 days prior to the second and third tests he consumed an additional 80 g carbohydrates by adding sugar (sucrose) to his tea and coffee. The fourth and fifth days were preceded by 2 days of carbohydrate restriction, (carbohydrate content of about 80 g per day), while the test on the sixth experimental day was carried out after returning to his usual diet. The two marathon runners were studied on 2 test days each and a control day. Exercise on the test days

consisted of running at 12 - 13 km per hour for 2 hours (from 7.00am - 9.00am) on a level treadmill. The first test day was preceded by the athletes' normal diet (approximately 500 g carbohydrate per day) and the second test day by two days on a low carbohydrate diet consisting mainly of meat, fish, eggs and cheese, while continuing their training (the familiar "glycogen stripping" regimen described by Ahlborg et al. 1967). Blood samples were collected from all subjects at hourly intervals, for analysis of the acetoacetate, 3-hydroxybutrate, glucose, free fatty acids, insulin and growth hormone.

Their findings differed markedly from those of R.H. Johnson et al. (1969) and Rennie et al. (1974). The highly trained marathon runners showed very high blood ketone body concentrations after exercise on a carbohydrate-restricted diet (Koeslag et al., 1980). When they consumed their normal diet of about 500 g carbohydrate per day, the athletes did not develop post-exercise hyperketonaemia.

The untrained subject showed post-exercise hyperketonaemia after the low carbohydrate diet and also on his normal diet, while the addition of 60 - 90 g sucrose (approximately 1.0 - 1.5 MJ dietary energy) to the daily food intake abolished the post-exercise hyperketonaemia. The post-exercise hyperketonaemia which the untrained subject developed on a low carbohydrate diet was comparable to that reported by

R.H. Johnson et al. (1969) in exercising sedentary subjects who were consuming their normal diets.

What the study of Koeslag et al. (1980) seems to show, is that athletic training often involves the eating of more food per day (Davidson et al., 1973; Mayer and Bullen, 1960) and that it is the extra carbohydrate thus consumed which protects the subject against post-exercise ketosis. When unusually strenuous training is undertaken (Scheele et al., 1979) or when the carbohydrate content of the diet is decreased, (Bonen et al., 1979; Koeslag et al., 1980) athletes develop post-exercise ketosis as readily as non-athletes do.

Conversely, if a non-athlete increases his dietary carbohydrate intake he can also increase his liver and muscle glycogen stores (Bergstrom et al., 1971) and avoid the ketosis he would normally develop during the performance of unfamiliar exercise (Koeslag et al., 1980).

(V) CONCLUSION:

Post-exercise ketosis, together with ketoses of Type I-glycogenosis and glycogen synthase deficiency (Williamson, 1978) as well as the antiketogenicity of alanine, and the lack of antiketogenicity of glucose ingestion immediately after exercise (Koeslag et al., 1980; 1982 and 1985; Adams et al., 1987), occur independently of the so-called ketogenic hormones. Clearly, therefore, intrahepatic factors can over-ride the influence of insulin and glucagon on the rate of hepatic ketogenesis. Since the main determinants of post-exercise ketosis - i.e. athletic training (Corbett et al., 1969; Johnson et al., 1969a; 1969b; 1970; 1971 and 1972), the carbohydrate content of the diet during the preceding 48 hours (Rennie and Johnson 1974; Koeslag et al., 1980; Courtice and Douglas 1936; Askew et al., 1975), but not glucose ingestion immediately before, during, or immediately after exercise (Koeslag et al., 1980; 1982 and 1985; Adams et al., 1987) - also influence the glycogen content of the liver (Baldwin et al., 1973; Oscai et al., 1974; Rowell, 1974; Arnall et al., 1986), it would appear that the post-exercise ketosis might be critically dependent on the liver glycogen content.

There is, however, no direct evidence from liver or muscle biopsy data that post-exercise ketosis is directly

correlated with glycogen depletion in the body. As it is impractical to test the hypothesis in humans - liver biopsies in healthy subjects being ethically questionable - an animal model of post-exercise ketosis was developed.

The aim of the present investigation was thus to investigate a possible relationship between post-exercise ketosis and the liver carbohydrate content during the consumption of three different diets, viz. Normal, Low and High carbohydrate diets.

CHAPTER 2:

NORMAL DIET

INTRODUCTION:

This study was undertaken to investigate the hypothesis that endurance-trained animals develop a lesser degree of post-exercise ketosis than untrained controls on a free, balanced diet, and the relationship between the ketosis and liver and muscle glycogen levels before and after exercise.

METHOD:

One hundred and forty male Long Evans rats, bred in the Animal House of the Department of Physiology, University of Cape Town were used. The animals were housed five per cage in a temperature controlled room (20°C). All the animals ran for a few minutes 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 hour per day, 5 days a week for 6 weeks. Rats in the Untrained

group ran every third day for about 5 minutes at 0.1 m/s to maintain their familiarity with treadmill running, without physically training them. Both groups were fed ad libitum on a normal Epol Laboratory Chow (Energy value = 12.75 kJ/g) consisting of the following:

60% carbohydrate (56% soluble and 4% crude fibre),
10% moisture,
18% protein,
7% ash,
5% fat.

The two groups maintained their initial body weights on this regimen. After 6 weeks both the Trained and Untrained groups were randomly assigned either into an Experimental or 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 2 Experimental groups ran on the treadmill at 0.2 m/s at a 0° gradient for 1 hour. Immediately before the exercise, on completion of the exercise, and at 30 minute intervals thereafter, one animal from each group (Trained Experimental, Trained Control, Untrained Experimental, and Untrained Control) was anaesthetized by a subcutaneous

injection of pentobarbitol (dose = 4 mg/100 g body weight). As soon as the rats could be handled easily, they were sacrificed by decapitation, in a room away from the other animals.

During the experiment the Control rats were placed near the treadmill where their Experimental partners were running, to keep the environmental conditions for the different groups as similar as possible. None of the rats were given food to eat during the observation period on the Experimental Day.

After sacrifice, the animals were bled and the blood collected for the determination of the 3-hydroxybutyrate concentrations. Portions of liver and gastrocnemius muscle were removed and frozen in liquid nitrogen within 2 minutes of the decapitation. The tissue was then stored at -80°C until it could be analysed for its glycogen content and the muscle malate dehydrogenase activity.

PREPARATION OF MATERIAL FOR BIOCHEMICAL ANALYSIS:

(a) For the liver and muscle glycogen assays the frozen tissue was ground to powder at the temperature of liquid nitrogen. The powder was added to 1 ml of 40% KOH solution for digestion of the tissue and boiled for 30 minutes, after

which 4 - 6 ml 90% (vol:vol) alcohol was added to precipitate the glycogen.

The mixture was centrifuged and washed using alcohol. 1 ml 2M HCl was then added to the tubes and boiled for 3 hours, to hydrolyse the glycogen to glucose. The glucose concentration was then determined using an enzyme (hexokinase:glucose 6-phosphate dehydrogenase mixture) method (See Biochemical Analysis for a more detailed description).

(b) The blood sample were deproteinized by adding 10 ml of 70% (w:w) perchloric acid to each aliquot of whole blood. This extract was centrifuged and the supernatant was stored in plastic tubes at -20°C until determination of 3-hydroxybutyrate concentration. Perchloric acid extracts were neutralized with KOH and the 3-hydroxybutyrate concentration determined with an enzyme method (see Biochemical Analysis).

The muscle tissue was also analysed for malate dehydrogenase (MDH) activity to determine the effectivity of the training programme (Holloszy et al., 1973).

The results are expressed as mean ± standard error of the mean (S.E.M). Statistical significance of differences between groups was determined by the Student's t-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 six week training period. This difference was not statistical significant ($p>0.1$; 2-tailed Student's t-test).

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 ($p<0.05$).

This approximately 100% increase in MDH activity in the trained animals suggests that the training programme had been effective, and is comparable with the results of Holloszy et al. (1973 and 1975), and Mole et al. (1973), who

showed that training produces an increase of between 60 - 100% in the activities of the enzymes involved with the oxidative capacity of skeletal muscle.

Blood Ketone Bodies:

There was no significant difference between the blood 3-hydroxybutyrate concentrations of the Trained and Untrained groups before exercise (Fig.1a; Table 1).

The mean 3-hydroxybutyrate concentration immediately after exercise was significantly higher than before the exercise in both animal groups ($p<0.05$) and was also higher than those of the controls ($p<0.01$). 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; $0.1>p>0.05$).

In the Trained Experimental animals, the 3-hydroxybutyrate concentration did not change significantly for 90 minutes after exercise (0.77 ± 0.02 mmol/l at the end of exercise, and 0.62 ± 0.05 mmol/l at 90 minutes after exercise), but then fell to 0.58 ± 0.03 mmol/l ($p<0.05$) at 120 minutes 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 minutes and 1.31 ± 0.04 mmol/l at 60 minutes after exercise (Fig. 1a; Table 1). Thereafter it fell to 1.01 ± 0.1 mmol/l at 90 minutes and 0.8 ± 0.5 mmol/l at 2 hours post-exercise.

The mean 3-hydroxybutyrate concentrations of the Untrained Experimental animals were significantly higher than those of the Trained Experimental animals during the entire 2 hour recovery period.

Liver Glycogen:

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; $p<0.05$. Table 2) at rest.

The 1 hour of exercise caused 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 ($p<0.05$), the rate of glycogen utilization

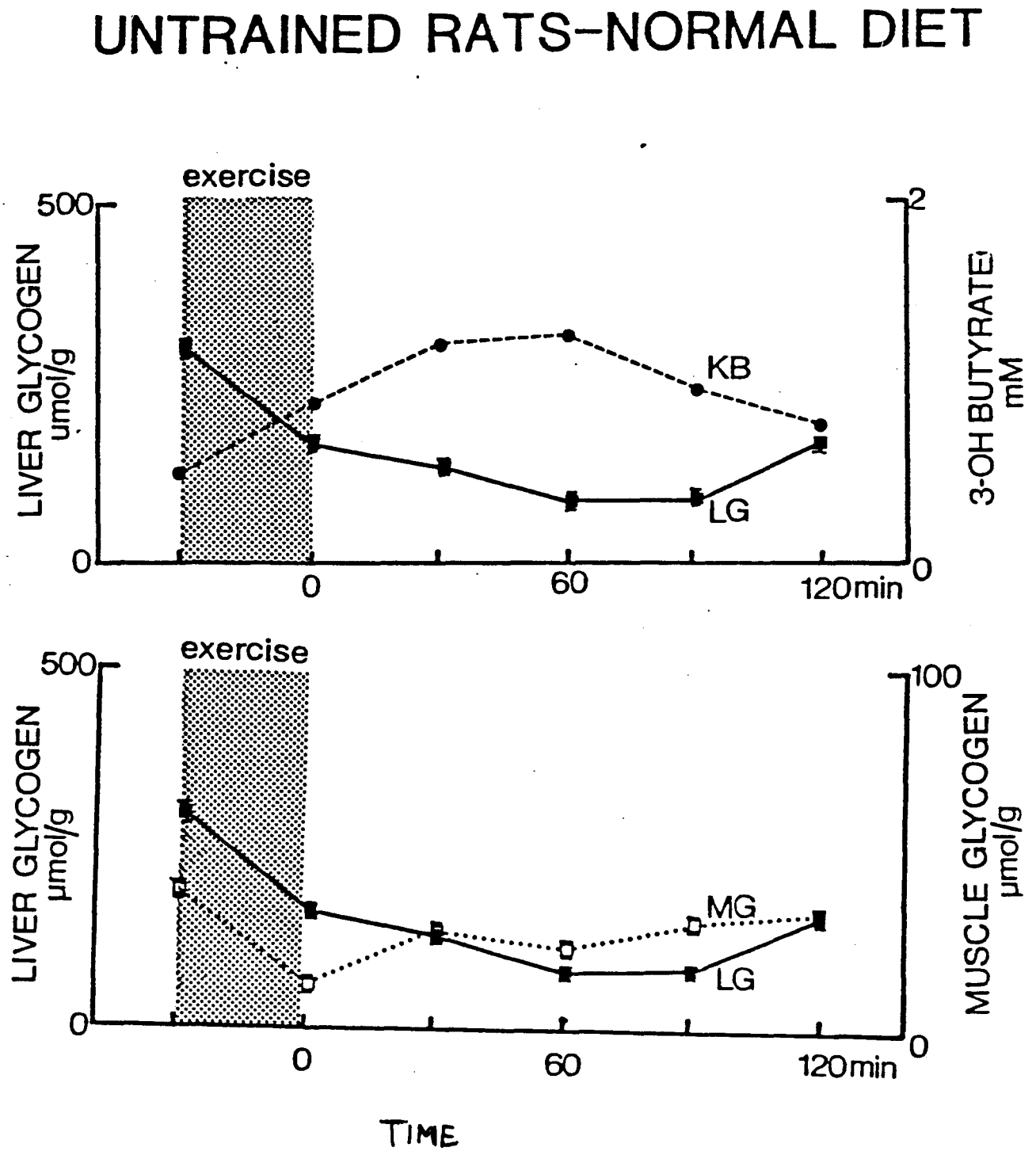


Fig 1 (a) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrates (MG) before and after 60 minutes of exercise in untrained rats fed a normal diet.

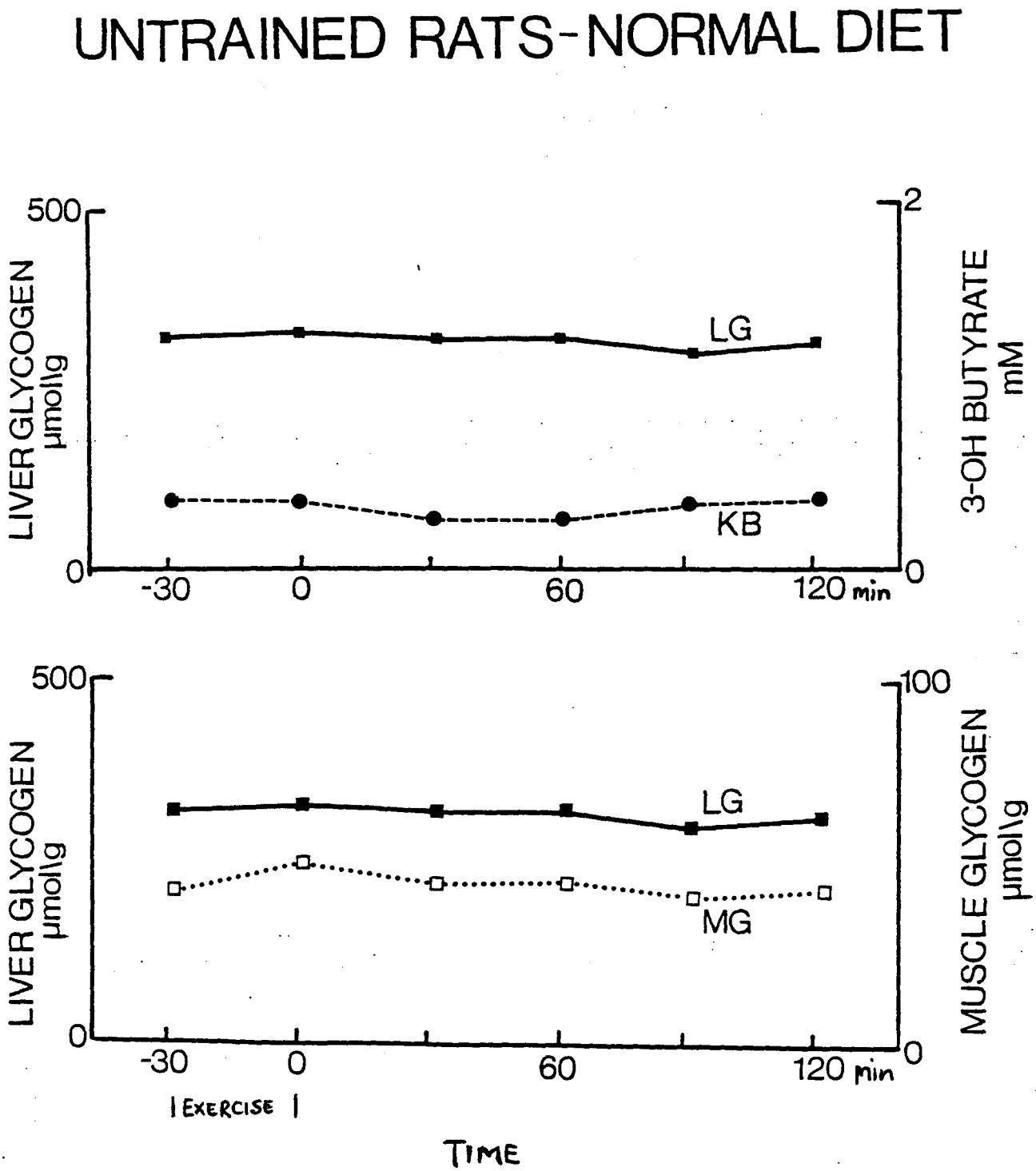


Fig 1 (b) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) in untrained non-exercised rats fed a normal diet.

TRAINED RATS – NORMAL DIET

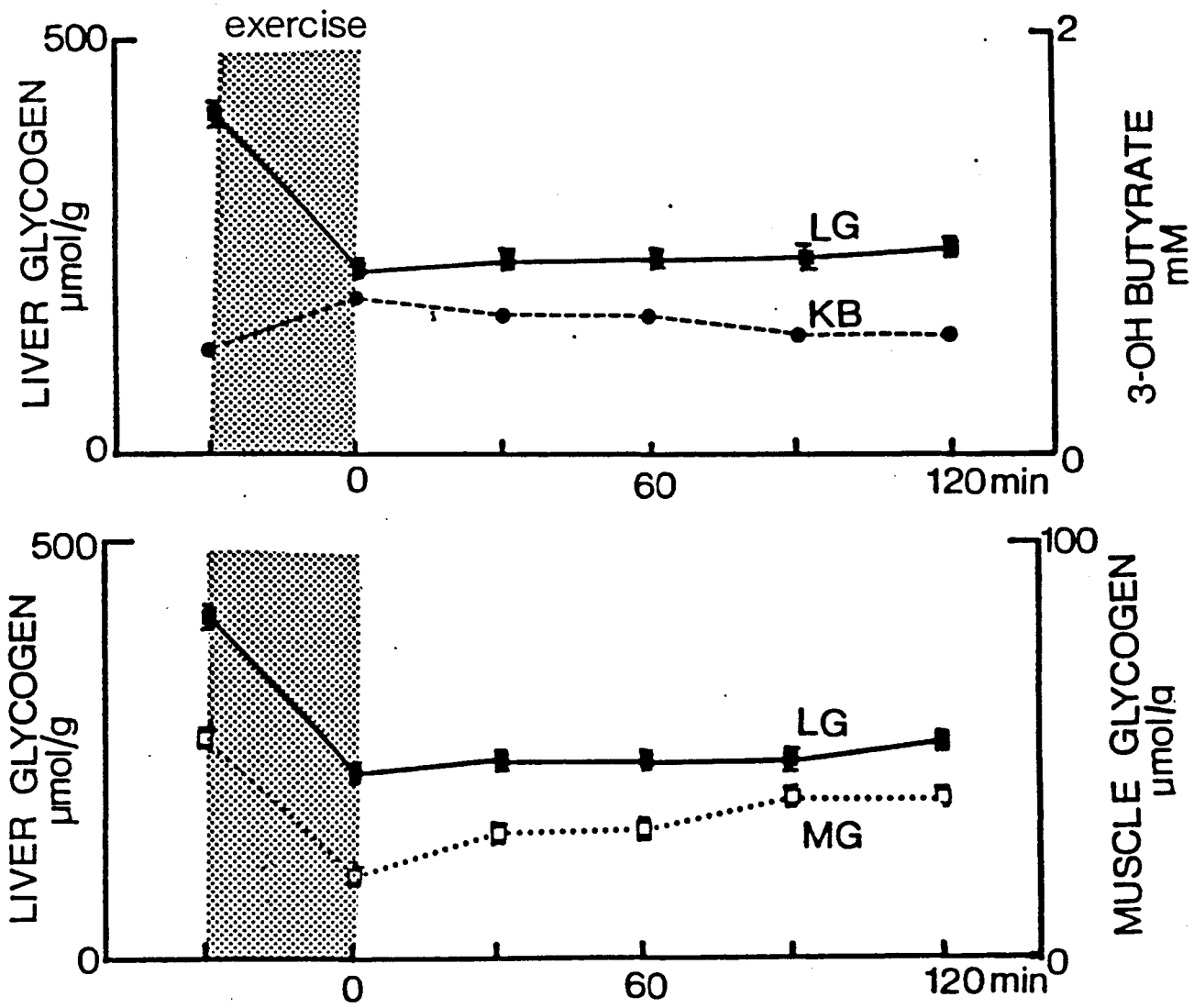
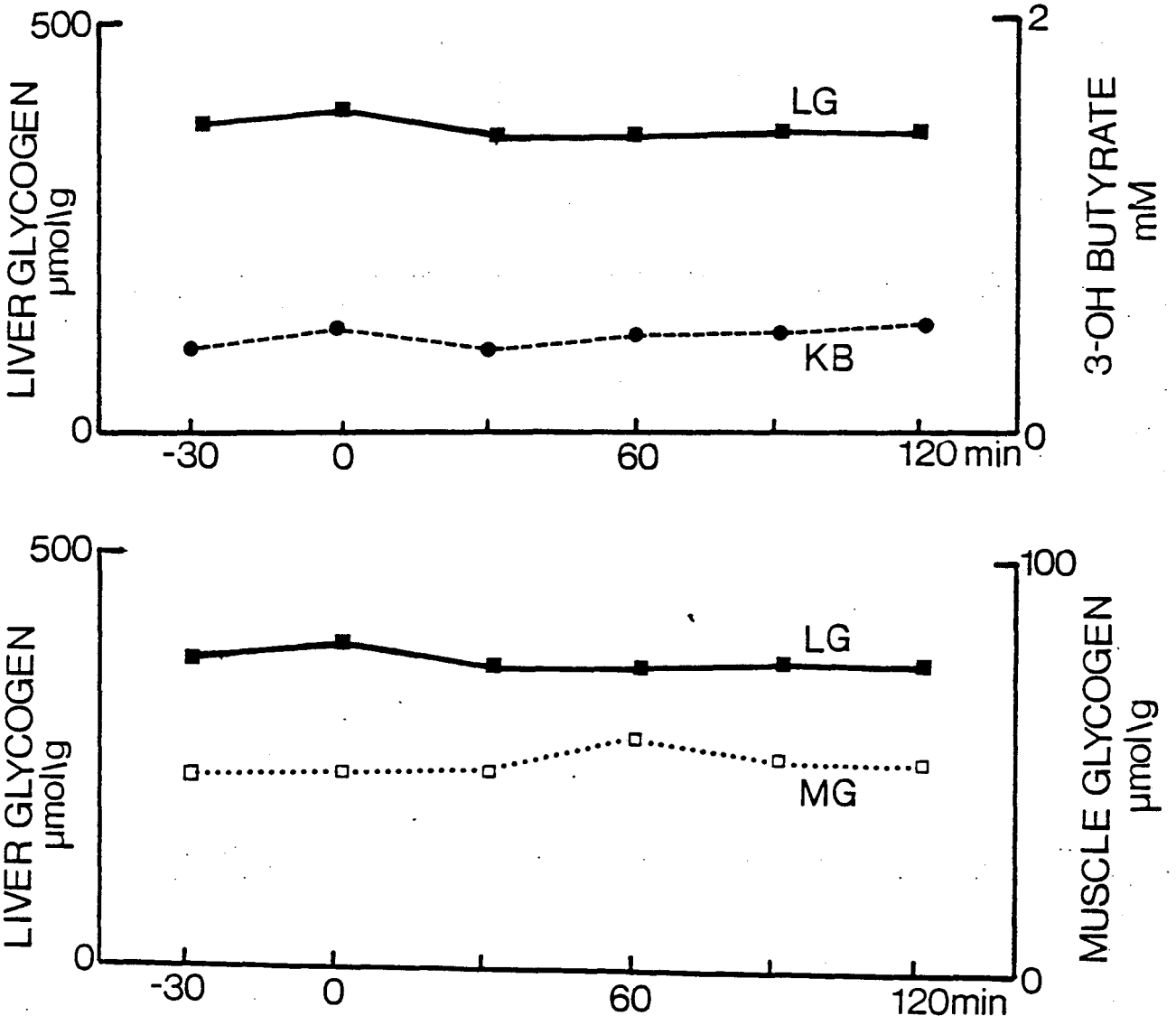


Fig 2 (a) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) before and after 60 minutes of exercise in trained rats fed a normal diet.

TRAINED RATS-NORMAL DIET



2 (b) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) in trained non-exercised rats fed a normal diet.

during exercise was slightly higher in the Trained than in the Untrained rats (188 µmol/g/h 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 minutes post-exercise, which was significantly lower than the 166 ± 3 µmol/g at the end of exercise ($p<0.01$). After 2 hours of recovery, the liver 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 minutes after exercise to reach 261 ± 4 µmol/g at 2 hours of recovery. This means that the Trained animals replenished 19% of the liver glycogen used during exercise in the 120 minutes of recovery (without food) (Fig.2a). The Untrained animals regained none of the liver glycogen used during exercise (Fig. 1a). The mean liver glycogen concentrations of the Trained animals were significantly higher than those of the Untrained rats during the entire recovery period (Table 2).

Muscle Glycogen:

The mean resting muscle glycogen concentration was significantly higher in the Trained rats (54 ± 1 μmol/g) than in the Untrained rats (39 ± 1 μmol glucosyl units/g wet mass; $p<0.05$, Table 3).

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 μmol/g/h 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 wet mass) than did the Untrained rats (13 ± 1 μmol/g wet mass; $p<0.05$; Table 3, Figs. 1a and 2a) .

Muscle glycogen resynthesis started almost immediately after exercise in both groups. The mean rate of glycogen resynthesis was approximately the same in both groups during the first hour of recovery (10 μmol/g/h in the Untrained and 11 μmol/g/h in the Trained), but slightly slower in the Untrained animals than in the Trained animals during the second hour of recovery (10 μmol/g/h in the Trained and 7.0 μmol/g/h in the Untrained).

DISCUSSION:

The major findings of the present study are that physically trained rats store more liver and muscle glycogen than untrained rats. Though the trained rats used slightly more glycogen (from their liver and muscle stores) than the untrained rats did during exercise, the liver and muscle glycogen concentrations immediately after exercise were significantly higher in the trained than in the untrained animals. The rate of muscle glycogen resynthesis was approximately the same in the 2 groups of animals, but the untrained rats apparently achieved this at the expense of their liver glycogen concentrations, whereas the trained animals were able to replenish their liver and muscle glycogen stores simultaneously. The mean blood 3-hydroxybutyrate concentration was the same in both the trained and untrained animals before the exercise as well as immediately after exercise (Figs. 1a and 2a; Table 1). Thereafter, the mean blood 3-hydroxybutyrate concentrations were significantly higher in the untrained than in the trained animals Figs 1a and 2a; Table 1).

Endurance training has been shown to modify hepatic and muscle glycogen metabolism. Trained rats accumulate increased amounts of liver and muscle glycogen following each training session, so that on non-exercising days their liver and muscle glycogen concentrations are higher than

those of sedentary rats (Baldwin et al., 1975; Fitts et al., 1975; Galbo et al., 1977; Winder et al., 1981).

The exact mechanism for this enhanced carbohydrate storage with training is uncertain, but could be due to the induction of the enzymes involved in glycogen synthesis. On the other hand, Oscai et al. (1974), and Mayer and Bullen (1960) have found that rats subjected to regular exercise eat more food (by up to 80%) than sedentary controls. Since, in a subsequent chapter (Chapter 4), we show that (sedentary) rats fed a high carbohydrate diet accumulate approximately 50% more liver and muscle glycogen than on a normal diet, it is possible that the elevated glycogen levels of exercised-trained animals are largely the result of eating more food and, therefore, more carbohydrate. Teleologically, nevertheless, the higher liver glycogen concentrations of trained animals would be expected to protect them against total liver glycogen depletion and the development of hypoglycaemia during prolonged exercise (Terjung et al., 1974; Baldwin et al., 1975).

Previous studies have shown that trained rats utilize liver and muscle glycogen more sparingly during exercise than untrained animals do (Baldwin et al., 1975; Fitts et al., 1975; Winder et al., 1981; Beattie and Winder, 1985). These findings are in contrast with the results of the present study in which trained rats utilized slightly more liver and

muscle glycogen than the untrained rats for the same absolute amount of exercise. This difference between our results and those of others might be related to the intensity of the test exercise. Baldwin et al. (1975), for instance, ran his rats at 0.53 m/s up a 15° incline (our rats ran at 0.2 m/s, on a level treadmill). The rats in Winder et al's (1981) study ran at 0.35 m/s up a 9° incline, and Beattie and Winder's (1985) at 0.27 m/s, also up a 9° incline. That this might influence the relative rates at which trained and untrained animals use glycogen is shown by Baldwin et al's (1975) results of 3 different work loads. At the lowest work load the untrained rats used muscle glycogen at a 57% faster rate than the trained animals did. At the highest work load the untrained rats utilized muscle glycogen 200% faster than the trained animals. Despite their faster rate of glycogen utilization during exercise, our trained rats nevertheless had higher immediate post-exercise liver and muscle glycogen concentrations, which is in keeping with the findings of others (Terjung et al., 1974; Baldwin et al., 1975; Winder et al., 1981).

Fell et al. (1980) showed that despite carbohydrate starvation, considerable glycogen accumulation, ranging from 20 to 30 μmol glucosyl units/g occurred in the hindlimb muscles of rats following exhausting exercise that caused severe muscle and liver glycogen depletion and hypoglycaemia. In contrast, the liver remained depleted of

glycogen in the rats fasted after exercise. This indicates that the glucose made available via liver gluconeogenesis is preferentially used for muscle glycogen resynthesis.

In our study, the rate of muscle glycogen resynthesis started immediately exercise ceased, and occurred at approximately the same rate in the trained as in the untrained animals (11 and 9 μmol glucosyl units/g/h respectively) during the 2 hours of recovery (in the absence of food). The rate of liver glycogen repletion was, however, different. The liver glycogen levels of the untrained rats fell by almost 50% during the first 90 minutes of recovery (from 166 ± 3 μmol/g immediately after exercise to 87 ± 3 μmol/g at 90 minutes post-exercise; $p<0.01$). This suggests that muscle glycogen repletion can occur at the expense of the liver glycogen stores in the untrained animal in the absence of exogenous carbohydrate.

Even when exogenous carbohydrate is available during recovery, most of it escapes hepatic retention, allowing repletion of muscle glycogen to take precedence over hepatic glycogen repletion (Maehlum et al., 1978).

Exercise has a powerful insulin-like effect on muscle that results in enhanced glucose uptake for a considerable period after the cessation of exercise (Holloszy and Narahara, 1965; Terjung et al., 1974). A low glycogen concentration

has been shown to enhance glycogen synthesis (Danforth, 1965), an effect which is probably mediated by the conversion of glycogen synthase from the b to the a form (Villar-Palasi and Larner, 1966; Roach, 1981; Cohen, 1981).

Glycogen synthase exist in 2 forms: synthase a and b. Synthase a , the unphosphorylated form, is physiologically active whereas the phosphorylated b form is is relatively inactive. The rate of glycogen synthesis therefore depends largely upon the relative concentration of synthase a. Various protein kinases catalyse the conversion of synthase a to synthase b, whereas phosphoprotein phosphatase catalyses the reverse reaction.

Insulin decreases the activity of protein kinase (Villar-Palasi and Larner, 1966), which increases the concentration of synthetase a and thus promotes muscle glycogen synthesis. Since low muscle glycogen levels stimulate the formation synthase a by activation of a protein phosphatase, there will be a higher rate of glycogen synthesis, for any given plasma concentration of insulin, when muscle glycogen levels are low than when they are high (Bergstrom et al., 1972; Conlee et al., 1978). It is thus possible for muscles to synthesize glycogen in a hormonal milieu which is catabolic for hepatic glycogen.

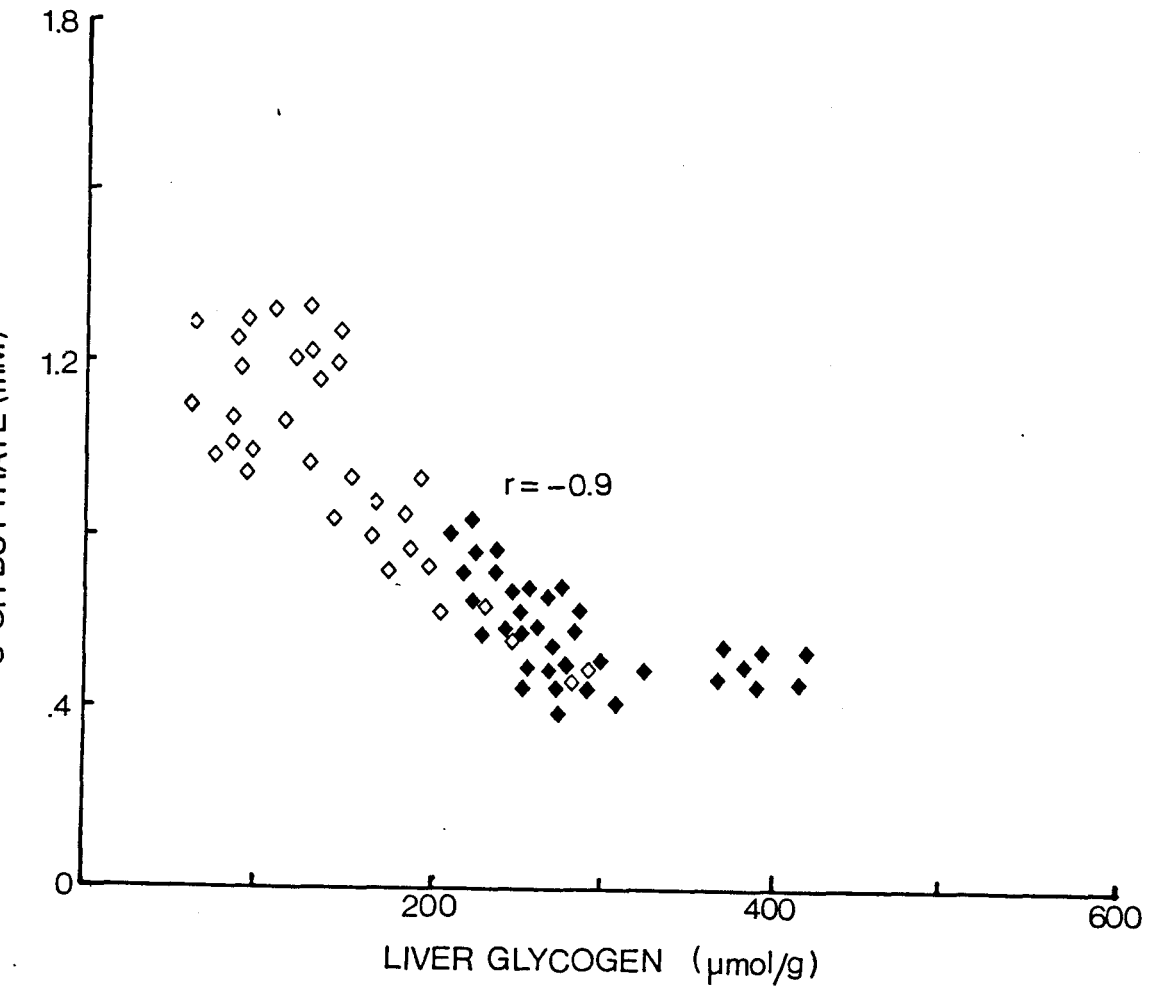


Fig. 3

The relationship between the liver glycogen concentrations and the simultaneous blood 3-hydroxybutyrate concentrations of chow-fed trained and untrained rats which were sacrificed at various times before and after 60 minutes of exercise (6 x 5 = 30 trained, and 6 x 5 = 30 untrained animals were sacrificed). The coefficient of correlation (r) for the results as a whole is -0.9.

◆ Trained ; ◇ Untrained

The 1 hour treadmill run increased the 3-hydroxybutyrate concentrations in both the trained and untrained animals to the same extent. However, in the recovery period, the 3-hydroxybutyrate concentration increased further in the untrained animals while remaining the same in the trained rats (Table 1; Fig.1a).

These findings are in agreement with earlier studies which have reported 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; Holloszy et al., 1978; Winder et al., 1974, 1975, 1979 and 1982).

Figure 3 depicts the relationship between liver glycogen concentration and blood 3-hydroxybutyrate concentration in the animals used in this study. This clearly shows that the low post-exercise blood ketone concentrations of normal-fed trained animals is related to their higher liver glycogen concentrations ($r = -0.9$, $p<0.01$); and that for the same hepatic carbohydrate levels trained and untrained rats have similar blood ketone body concentrations.

CONCLUSION:

Rapid muscle glycogen resynthesis occurs during the first 2 hours of recovery (The present study; Maehlum et al., 1978;

Fell et al., 1982; Richter et al., 1982) even in fasting subjects (The present study; Maehlum and Hermansen, 1978; Maehlum et al., 1977; Fell et al., 1980) and much of the body's mobilizable carbohydrate is probably diverted to this purpose (Wahren et al., 1973; Fell et al., 1980; Maehlum et al., 1978). In our untrained animals, muscle glycogen repletion occurred, at least partly, at the expense of liver glycogen, and this caused the animals to become ketotic. The trained animals repleted their muscle glycogen stores probably using other substrates, possibly via the "3-carbon pool" (viz: plasma alanine,and lactate) (Newgard et al., 1983; Katz et al., 1986; Brooks, 1986), thereby keeping their liver glycogen stores elevated, and not developing post-exercise ketosis.

CHAPTER 3:

LOW CARBOHYDRATE DIET:

INTRODUCTION:

To test the hypothesis that a sedentary life-style and a low carbohydrate diet predispose subjects to post-exercise ketosis via a common mechanism - namely a low liver (and/or muscle) glycogen content, we studied the effect of a low carbohydrate diet on trained and untrained rats, measuring blood ketone body concentrations, and liver and muscle glycogen levels before and after exercise.

METHOD:

One hundred and forty four male Long Evans rats, bred in the Animal House of the Physiology Department at the University of Cape Town were used. The atmospheric conditions in the Animal House were the same as those described in Chapter 3 (Normal diet group).

After the familiarization period, (See Methods Chapter 3), the animals were randomly divided into a "Trained" and an "Untrained" group. The Trained group ran on a treadmill five days a week for six weeks at 0.2 m/s for one hour. The

Untrained group ran every third day for about 5 minutes at 0.1 m/s to keep them accustomed to treadmill running without physically training them.

Both groups were fed ad libitum on normal Epol Laboratory Chow for the first 5 weeks of the programme, as described in Chapter 2. The energy value of the Chow as well as the constitution of the diet was the same as described in Chapter 2.

On this regimen the two groups maintained equal body weights. During the 6th week, a low carbohydrate diet was substituted for the normal chow. The low carbohydrate diet consisted of minced meat (Table Top Beefburger) plus vegetables.

The burgers and vegetables were presented raw because the rats appeared to prefer them that way. The purpose was to decrease the daily carbohydrate intake without causing a significant loss of weight.

At the end of the 6th week, the Trained and the Untrained groups were both randomly subdivided into an Experimental and a Control group, as described in Chapter 2.

On the Experimental Day, the animals belonging to the 2 Experimental groups ran on a level treadmill at 0.2 m/s for 1 hour.

The collection, storage and analyses of the blood and tissue samples for the determination of the blood 3-hydroxybutyrate level, the liver and muscle glycogen concentrations, and the muscle malate dehydrogenase activity were the same as described in Chapter 2. The results are expressed as mean ± standard error of the mean (S.E.M).

Statistical significance was determined by Student's 2-tailed t-test, the chosen level of significance being $p<0.05$.

RESULTS:

Body mass:

The final body weights were 320 ± 20 g for the Trained rats and 312 ± 18 g for the Untrained rats ($p>0.1$).

Muscle Malate Dehydrogenase:

The training programme resulted in a significantly higher muscle malate dehydrogenase activity in the Trained than in the Untrained rats (30 ± 3.0 U/g wet massfor the Trained and 14 ± 3.0 U/g wet mass for the Untrained, $p<0.05$, after the 6 weeks training period).

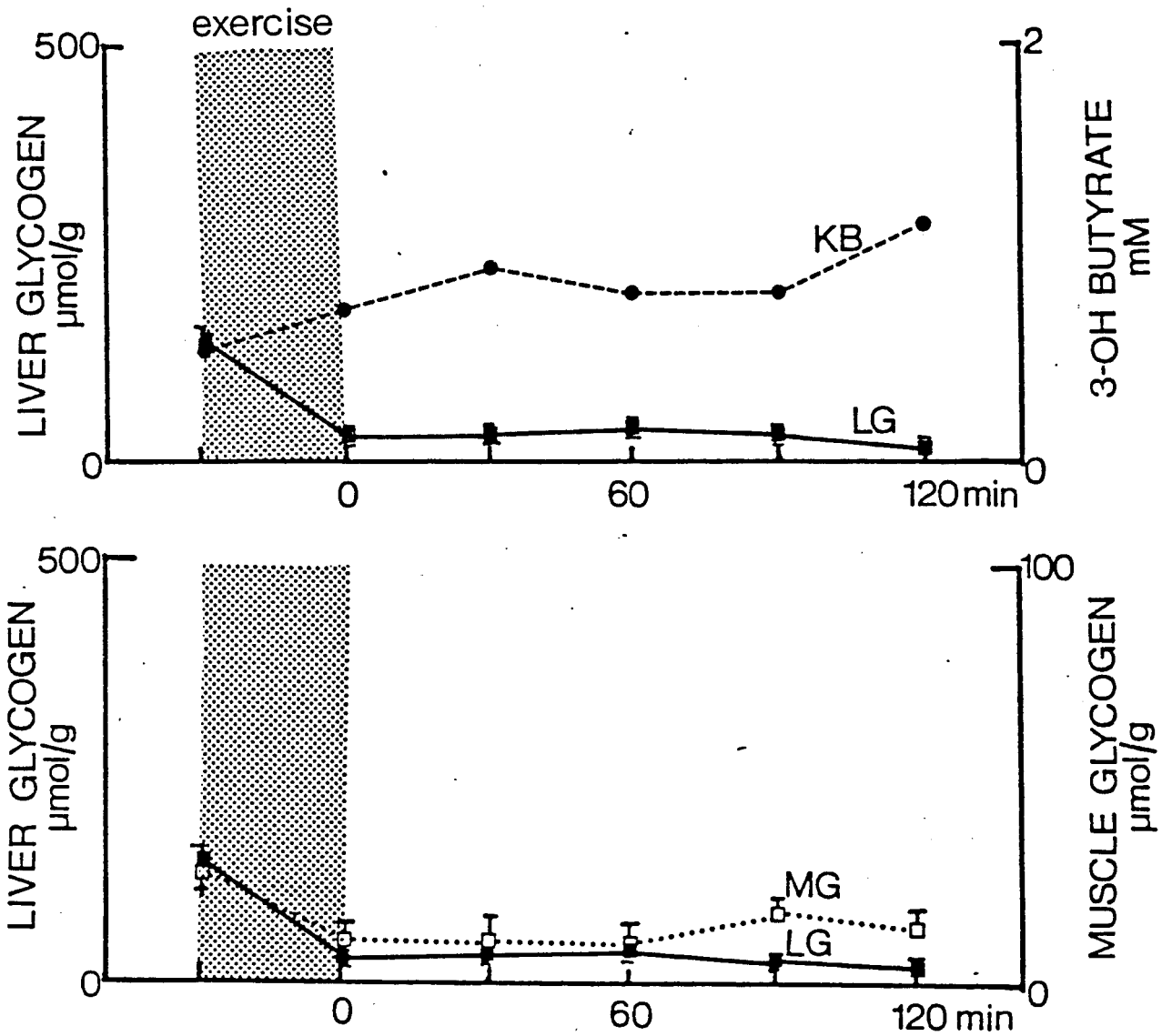
Blood ketone bodies:

The pre-exercise blood 3-hydroxybutyrate concentration was slightly higher in the Trained Experimental (0.62 ± 0.11 mmol/l) than in the Untrained Experimental group (0.49 ± 0.06 mmol/l).

After exercise the blood 3-hydroxybutyrate concentration was lower (0.54 ± 0.09 mmol/l) than before the exercise (0.62 ± 0.11 mmol/l) in the Trained animals (Fig. 5a; Table 4), but significantly higher (0.72 ± 0.14 mmol/l) than the pre-exercise concentration (0.49 ± 0.06 mmol/l, $p<0.05$) in the Untrained animals (Fig. 4a; Table 4).

A marked rise in the mean blood 3-hydroxybutyrate concentration occurred in both groups during the first 60 minutes after exercise. In the Untrained group the blood 3-

UNTRAINED RATS -LOW CHO DIET



g 4 (a) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) before and after 60 minutes of exercise in untrained rats fed a low carbohydrate diet during the preceding week.

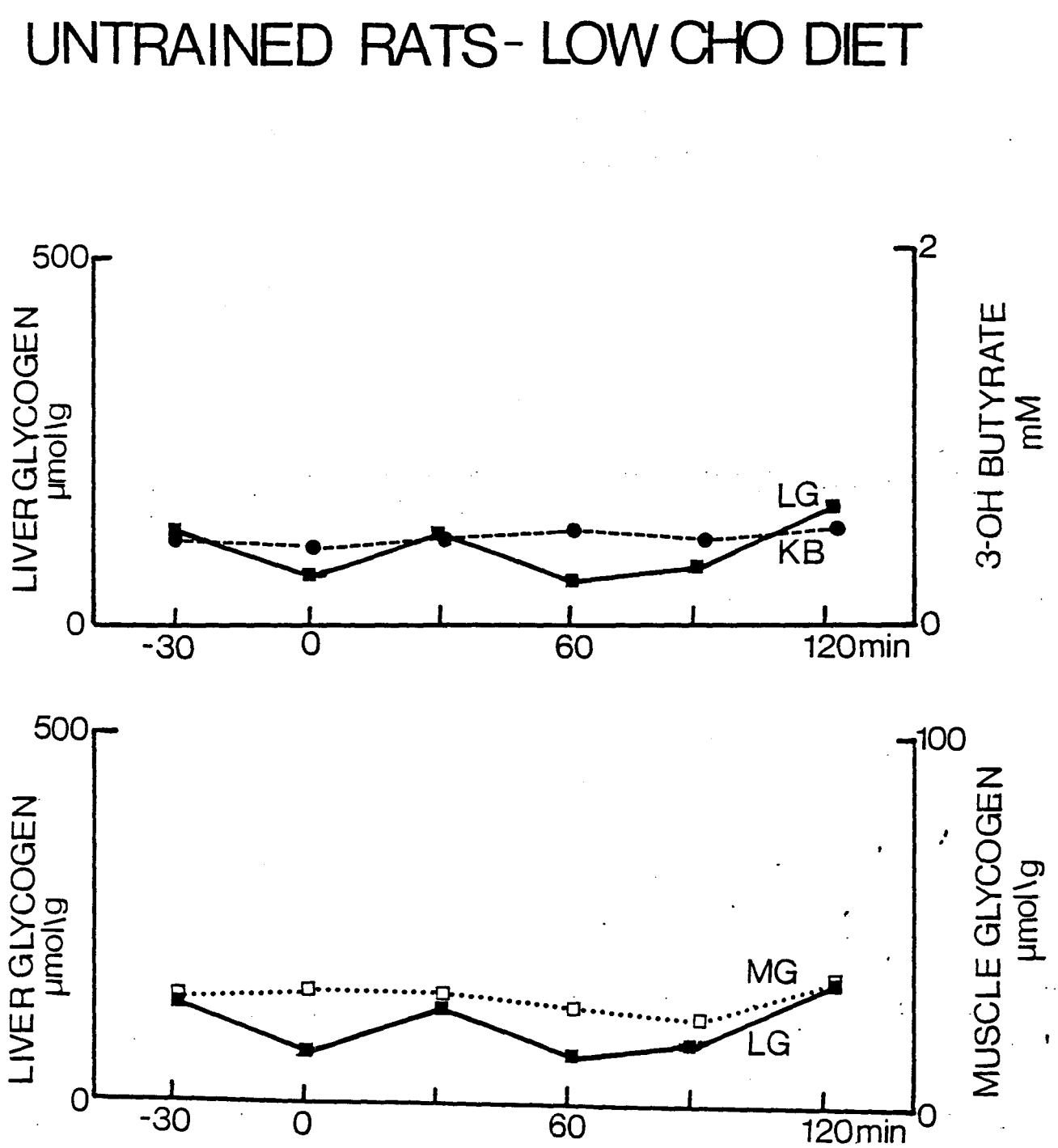


Fig 4 (b) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) in untrained non-exercised rats fed a low carbohydrate diet during the preceding week.

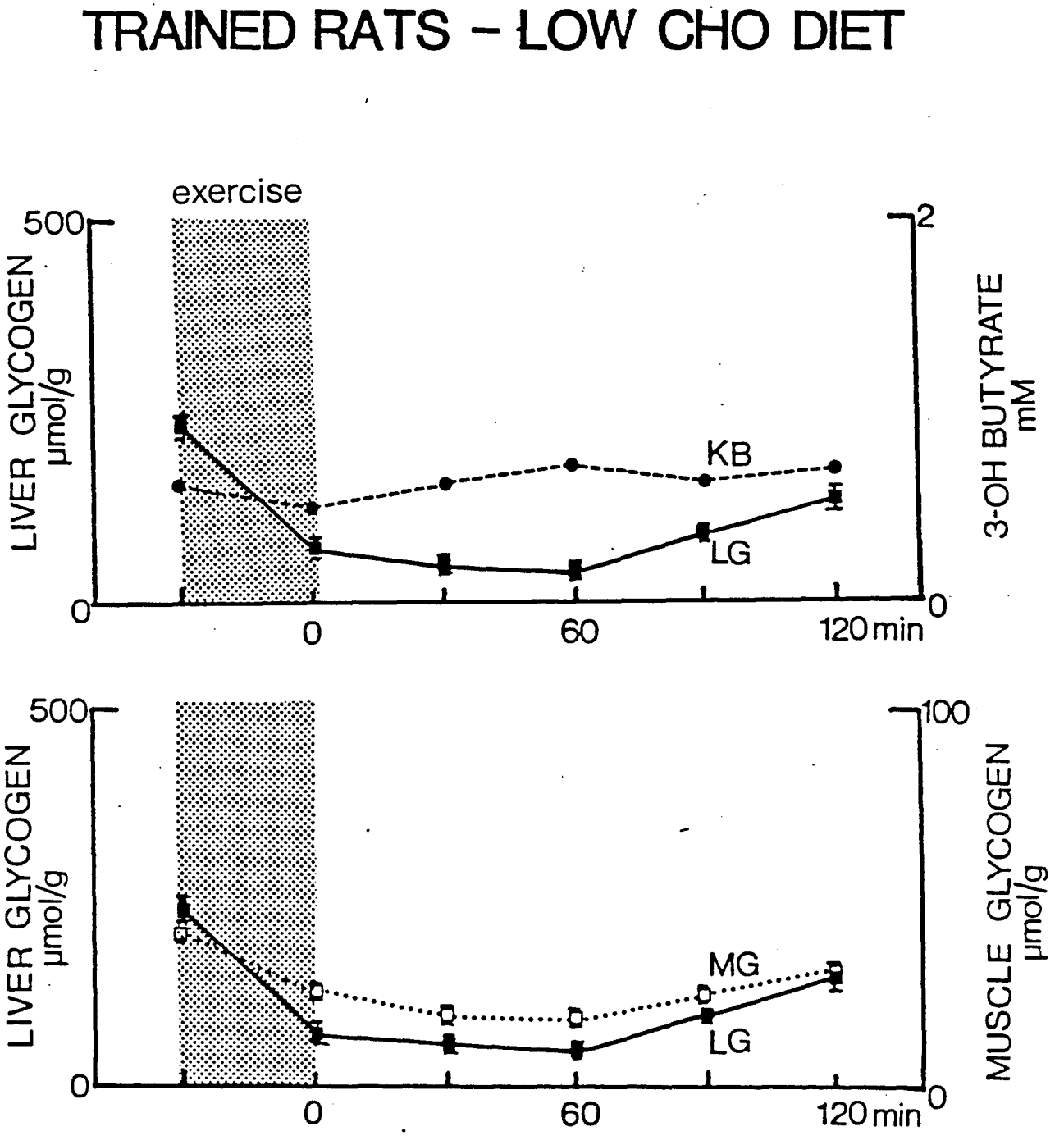
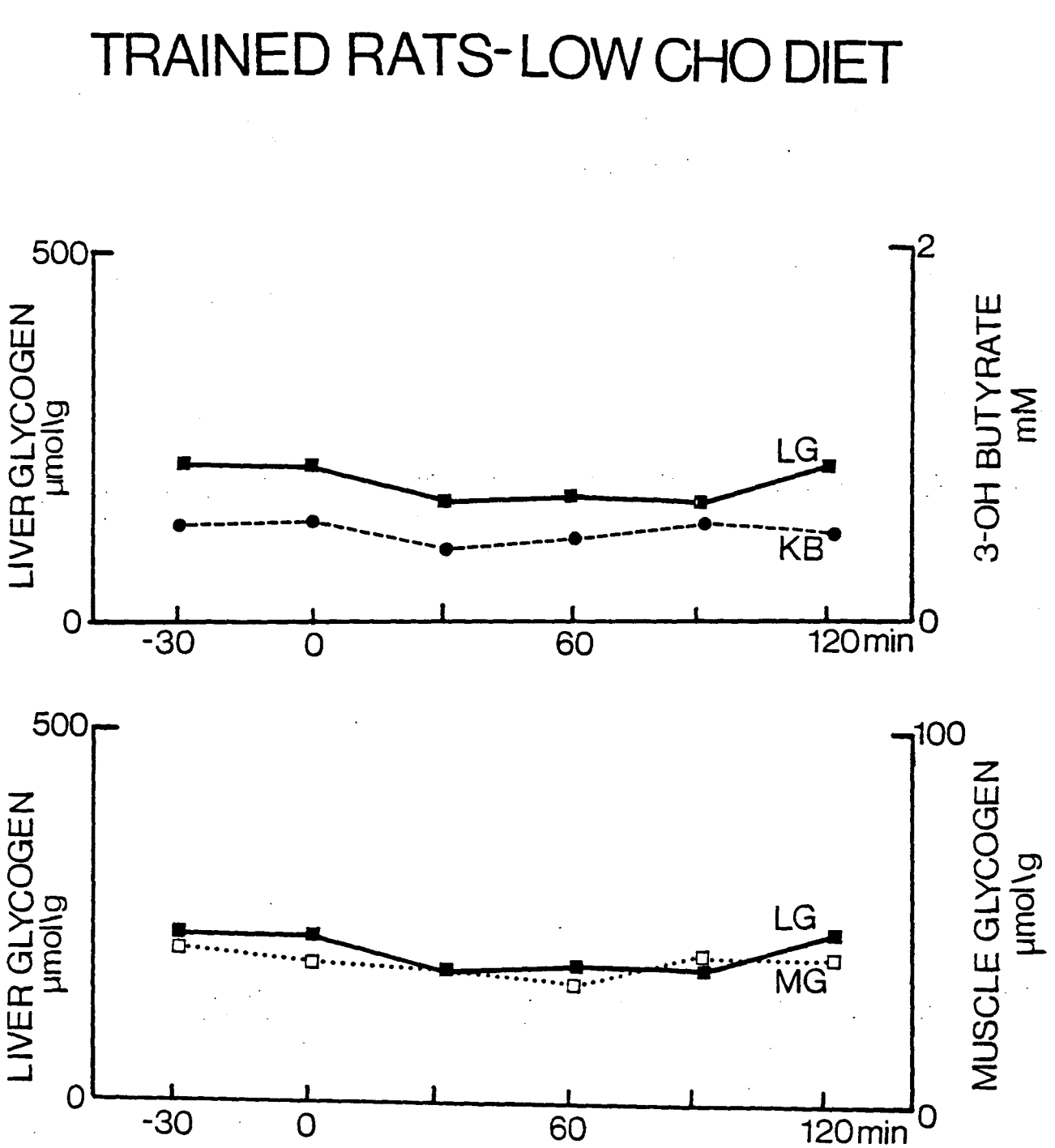


Fig 5 (a) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) before and after 60 minutes of exercise in trained rats fed a low carbohydrate diet during the preceding week.



ig 5 (b) Blood 3- hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) in trained non-exercised rats fed a low carbohydrate diet during the preceding week.

hydroxybutyrate levels increased from 0.72 ± 0.14 mmol/l immediately after exercise, to 0.95 ± 0.2 mmol/l at 60 minutes after exercise. In the Trained animal, the rise was from 0.54 ± 0.09 mmol/l to 0.80 ± 0.15 mmol/l at 60 minutes after the 1 hour exercise period (Table 4, Figs. 4a and 5a). The 3-hydroxybutyrate concentration did not change significantly after the first 60 minutes in the Trained rats, reaching 0.78 ± 0.17 mmol/l at 2 hours post-exercise (Fig. 5a).

In the Untrained group, however, the 3-hydroxybutyrate concentration continued to rise during the 2nd hour of recovery to reach a concentration of 1.14 ± 0.25 mmol/l 2 hours after exercise (Fig. 4a).

The 3-hydroxybutyrate concentration at the end of exercise and at all intervals during the post-exercise period were significantly higher in the Untrained than in the Trained animals (0.92 ± 0.2 mmol/l for the Untrained vs. 0.70 ± 0.15 mmol/l for the Trained group at 30 minutes post-exercise; 1.14 ± 0.25 mmol/l for the Untrained vs. 0.78 ± 0.17 mmol/l for the Trained at 2 hours after exercise; $p<0.05$, (Table 4)).

The 3-hydroxybutyrate concentrations of the Experimental animals were significantly higher than those of the Controls at all intervals during recovery (Table 4, Figs. 4a and 4b).

Muscle glycogen:

The pre-exercise muscle glycogen concentrations were significantly higher in the Trained (43 ± 2 μmol glucosyl units/g wet mass) than in the Untrained animals (28 ± 1 μmol glucosyl units/g wet mass; $p<0.05$; Table 5).

One hour of exercise significantly decreased the muscle glycogen concentration of both groups by 15 - 18 μmol/g, so that the immediate post-exercise glycogen levels were 10 ± 1 and 28 ± 1 μmol/g in the Untrained and Trained rats respectively (Figs. 4a and 5a).

The muscle glycogen concentration remained at these low levels throughout the 2 hour recovery period (10 ± 1 μmol/g at the end of exercise, and 15 ± 2 μmol/g at 2 hours after exercise) in the Untrained group (Table 5, Fig. 4a). The Trained animals lost some glycogen during the first hour of recovery but regained it during the second hour to reach the same level at 2 hours after exercise as immediately after exercise (28 ± 1 μmol/g at the end of exercise; 19 ± 2 μmol/g at 60 minutes post-exercise, and 29 ± 2 μmol/g at 2 hours after exercise). Thus, there was no nett glycogen resynthesis in the exercised muscles of carbohydrate-starved Trained and Untrained animals during recovery.

Muscle glycogen concentrations were significantly higher in the Trained animals than in the Untrained animals during the entire 2 hour post-exercise recovery period (Table. 5).

Liver glycogen:

The pre-exercise liver glycogen concentrations of the Trained animals were significantly higher than those of the Untrained animals (150 ± 7 μmol glucosyl units /g tissue for the Untrained, compared with 236 ± 10 μmol/g for the Trained; $p<0.05$; Table 6). Exercise lowered the liver glycogen concentration in both groups, but the immediate post-exercise liver glycogen concentration was significantly higher in the Trained (72 ± 12 μmol/g) than in the Untrained (26 ± 1 μmol/g) rats (Figs. 4a and 5a).

The mean liver glycogen concentration of the Untrained animals remained low throughout the post-exercise period, but in the Trained animals the mean liver glycogen levels decreased significantly during the first hour of recovery, (72 ± 12 μmol/g at the end of exercise compared with 57 ± 7 μmol/g at 60 minutes after exercise), but rose during the second hour of recovery to reach 142 ± 15 μmol/g at 2 hours after exercise (Figs. 4a and 5a). This means that the Trained Experimental animals regained 43% of the liver

glycogen utilized during exercise in the 2-hour observation period. This liver glycogen resynthesis occurred in the total absence of food intake.

The liver glycogen concentrations were significantly higher in the Trained than in the Untrained animals at all times (Table 6).

DISCUSSION:

The principal findings of the present study are that endurance-trained rats store more liver and muscle glycogen than untrained rats even when both have consumed a low carbohydrate diet for 1 week. The amount stored is, however, less than when a normal diet is consumed (Chapter 2). During the one hour exercise period, approximately the same absolute amount of muscle glycogen was used by both animal groups regardless of training (18 μmol/g/h in the Trained and 15 μmol/g/h in the Untrained), but the Trained animals lost more liver glycogen (164 μmol/g/h and 123 μmol/g/h in the Trained and Untrained animals respectively). In contrast to what was seen after a normal diet (Chapter 2) there was no significant muscle glycogen resynthesis during the 2 hours of recovery in any of the Experimental animals. Post-exercise hyperketonaemia was more pronounced in the Untrained than in the Trained rats.

A low carbohydrate diet for 1 week lowered the liver glycogen content of our Trained and Untrained rats by between 43 and 50% of that found in laboratory rats eating normal chow (Chapter 2), and muscle glycogen by 32 - 35%. Similar findings have been reported in humans. Hultman and Bergstrom (1967), Costill (1974) and Pruet (1970) have shown that the muscle glycogen content of human leg muscles decreases by about 30 - 40% after 1 week of total carbohydrate deprivation.

The Trained animals utilized approximately the same amount of muscle glycogen (15 vs 18 μmol glucosyl units/g wet mass), but more liver glycogen (164 vs 123 μmol glucosyl units/g) than the Untrained animals during the 1 hour of running on a level treadmill. However, as the Trained rats had higher pre-exercise liver and muscle glycogen concentrations, the immediate post-exercise levels were nearly twice those of the Untrained rats.

In contrast, both the Trained and Untrained animals utilized 29% less liver and 24% less muscle glycogen during the 1 hour of running than the normal-fed rats described in Chapter 2. This suggests that the rate of liver and muscle glycogen utilization during exercise is concentration dependent. This is confirmed by Sherman et al. (1981), who studied the effect of three different diets (low

carbohydrate; high carbohydrate and mixed diet) on the carbohydrate stores and performances of 6 well trained runners during a 20.9 km run, and found that despite dissimilar pre-race muscle glycogen levels, the post-race muscle glycogen levels were similar in all 3 dietary groups.

Jansson and Kaijser (1982) similarly studied the effect of diet on muscle glycogen utilization in humans. They found that the rate of muscle glycogen depletion during submaximal exercise of short duration (65% VO_2max) is lower after a high fat diet than after a high carbohydrate diet. The rate of blood glucose utilization was, however, higher after the fat than after the carbohydrate diet.

It should be noted that the duration of the exercise differed markedly in the three studies. In our study, rats ran at 0.2 m/s for 1 hour. In Sherman's study (1981), humans completed 20.9 km in 90 minutes, while the duration of exercise in Jansson's study (1982) was only 6 minutes. This suggests that the apparently lower rate of glycogen utilization by carbohydrate-starved individuals is not an experimental artifact due to the rapid reduction of the liver or muscle glycogen levels to irreducible minimum concentrations in the early part of exercise, and thereafter remaining constant.

The lower rate of muscle glycogen utilization when the muscle glycogen levels are low indicates that blood glucose (derived from gluconeogenesis) and/or fats can to a large extent substitute for glycogen (Terjung et al., 1974; Rennie et al., 1976). These substitute fuels are probably mobilized by the high plasma catecholamine and glucagon concentrations found in carbohydrate-depleted exercising individuals (Winder et al., 1979 and 1982; Koeslag, 1980; Galbo, 1983).

Minimal muscle glycogen resynthesis occurred in both Trained and Untrained animals during recovery, which is in sharp contrast to what was found after a normal diet (Chapter 2). Gaesser and Brooks (1980) studied the rate of muscle glycogen resynthesis in rats that had run to exhaustion after a 12 hour fast. Pre-exercise muscle glycogen concentrations were similar to those of our low-carbohydrate fed rats. The rate of glycogen resynthesis during recovery remained less than 1.3 μmol/g/h. (Muscle glycogen was resynthesized at a rate of about 10 μmol/g/h in our normal-fed rats after exercise.) Similar results were obtained by Maehlum and Hermansen (1978) who measured the rate of muscle glycogen resynthesis in 5 human subjects who had exercised to exhaustion after fasting for 12 hours. Glycogen resynthesis occurred at a rate of 1.75 μmol/g/h. These results are not significantly different from ours (Table 5). This indicates that the rapid muscle glycogen repletion which occurs after exercise following a normal diet is

primarily due to the redistribution of the body's carbohydrate reserves (probably via the plasma "3-carbon pool" of Newgard et al. (1983); Brooks (1986) and Katz et al.,(1986)), rather than the result of de novo carbohydrate synthesis from protein reserves.

Similarly, when food is eaten during recovery, the rate of muscle glycogen resynthesis is increased only if the diet contains a substantial quantity of carbohydrate (Suzuki et al., 1984; Maehlum et al., 1978). However, this appears to hold only if the pre-exercise muscle glycogen levels (and therefore, presumably, total body carbohydrate reserves) are low, since MacDougall et al. (1977) found that carbohydrate feeding during recovery had no influence on the rate of muscle glycogen repletion when resting muscle glycogen concentrations were between 80 and 100 μmol/g. (The pre-exercise muscle glycogen concentrations of Suzuki et al's rats (1984) were about 50 μmol/g; and those of Maehlum et al's men (1978) 65 μmol/g.)

The liver demonstrated no appreciable glycogen repletion after exercise during the food-restricted recovery period in our untrained rats. Since these rats did not show any symptoms suggestive of hypoglycaemia (i.e collapse or unconsciousness), despite their persistently low liver glycogen levels, the blood glucose concentration must have been maintained entirely by gluconeogenesis, either from the

"plasma 3-carbon pool" (derived from unexercised muscle carbohydrate stores) or from the body's protein reserves. The fact that these reserves were plundered only to maintain the blood glucose level, and not to replenish depleted glycogen stores, suggests that they were probably not primary fuel reservoirs.

That the trained animals did partially replenish their liver glycogen stores under similar circumstances suggests that they had retained dispensible extra-hepatic carbohydrate reserves (probably in the form of glycogen in unexercised muscle).

As was the case on a normal diet (Chapter 2), when carbohydrate-starved rats exercised the blood ketone body concentrations rose to higher levels in the untrained than in the trained animals during recovery (Figs. 4a and 5a). This is in contrast with the results of the only other study in which carbohydrate-starved trained and untrained rats performed exercise (Askew et al., 1975). Here the immediate post-exercise blood ketone body concentrations of the trained animals were more than twice as high as those of the untrained rats. (Ketone body concentrations were not measured during recovery.) There are, however, some striking differences between this study and ours. Askew et al's rats all ran to exhaustion, which meant that their trained rats performed significantly more work than their

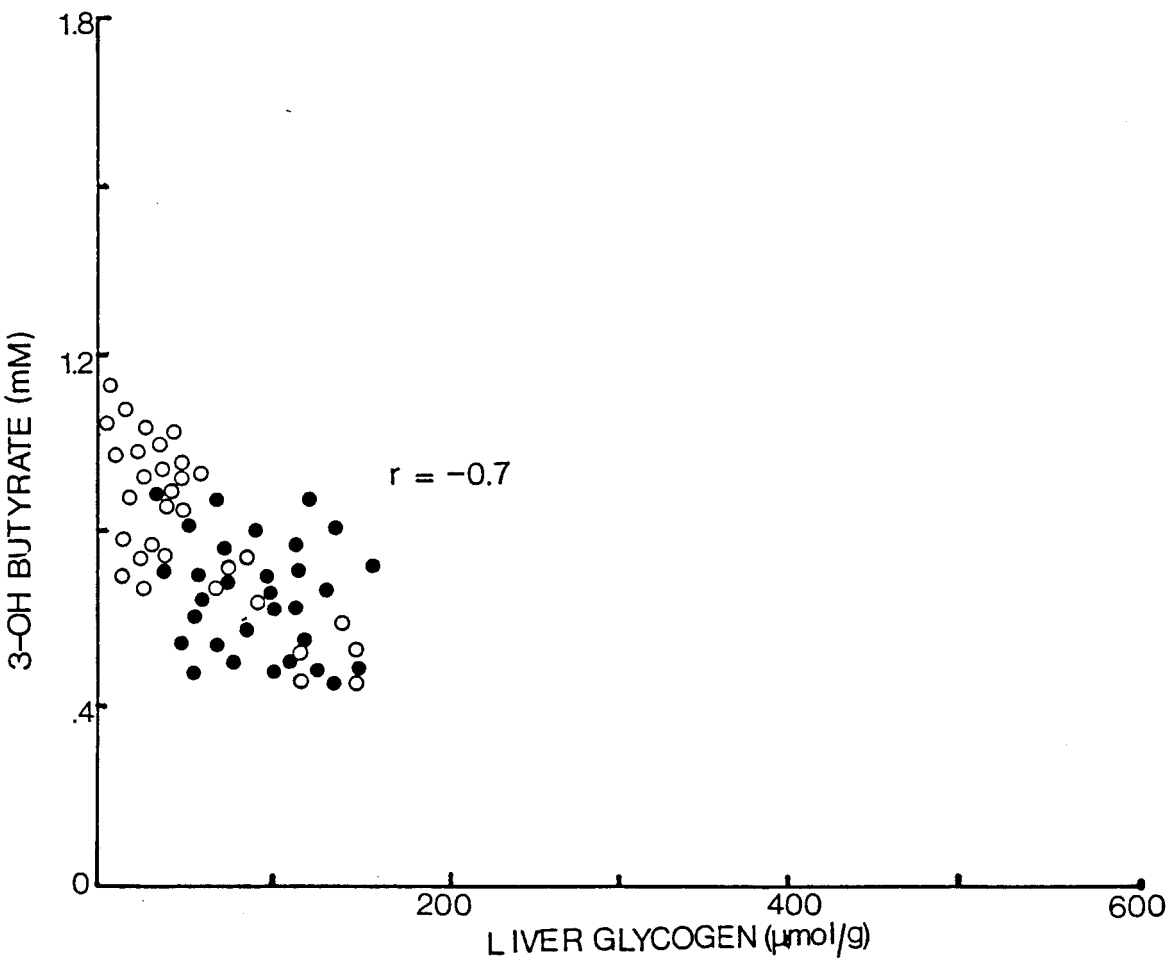


Fig 6 (a)

The Relationship between liver glycogen concentration and simultaneous blood 3-hydroxybutyrate concentration before and after 60 minutes of exercise in rats fed a Low Carbohydrate Diet.

● Trained ; O Untrained

untrained rats. Our rats all performed exactly the same amount of exercise. The training regimen used by Askew et al. was also considerably more intense than ours: 12 weeks of running at 0.5 m/s up a 5° gradient for 2 hours per day versus our 6 weeks of running at 0.2 m/s on a level treadmill for 1 hour per day. Askew et al. noted that this amount of exercise caused the trained rats to eat 20% less food than their sedentary controls. We did not measure food consumption in our rats, but Mayer and Bullen (1960) have shown that food intake is positively correlated with daily exercise intensity except at very high work loads, when animals lose their appetite and are unable to maintain their body weights. Since there was no significant difference between the weights of our trained and untrained rats (see: Bullen and Mayer, 1960), but there was a 23% difference between those of Askew et al. (his trained rats weighed less than his untrained animals), we are inclined to believe that our trained animals ate more than their sedentary counterparts, and were therefore more resistant to post-exercise ketosis than either our untrained rats, or Askew et al's trained animals.

Figure 6a shows that for the same liver glycogen concentration the blood ketone body concentrations of carbohydrate-starved trained and untrained animals rise to approximately the same levels. The higher post-exercise blood ketone body concentrations of the untrained animals

are associated with their lower liver glycogen contents. However, though we succeeded with our low carbohydrate diet to reduced the trained animals' liver glycogen concentrations to those of untrained animals on a normal diet, the trained rats still did not display the same degree of post-exercise ketosis as sedentary rats (compare Fig. 3 with Fig. 6a). This will be discussed in Chapter 5 (GENERAL DISCUSSION).

Beattie and Winder, (1985) found significantly higher blood 3-hydroxybutyrate concentrations in untrained than in trained rats despite similar liver glycogen levels in the 2 groups after exercise. Nor were there significant differences between the hepatic malonyl CoA concentrations of the 2 groups of animals. However, the resting liver glycogen concentrations of these rats were very low (46 and 40 μmol/g in their trained and untrained rats respectively) compared with those of even our low carbohydrate fed rats (236 and 150 μmol/g in the trained and untrained animals respectively). This implies that their animals were probably more food-restricted than ours. Their training programme was comparable to that of Askew et al. (1975), which leads us to suspect that their animals also lost weight during training. This suspicion is supported by the fact that Beattie and Winder had to food-restrict their sedentary controls to bring their weights down to those of the trained animals.

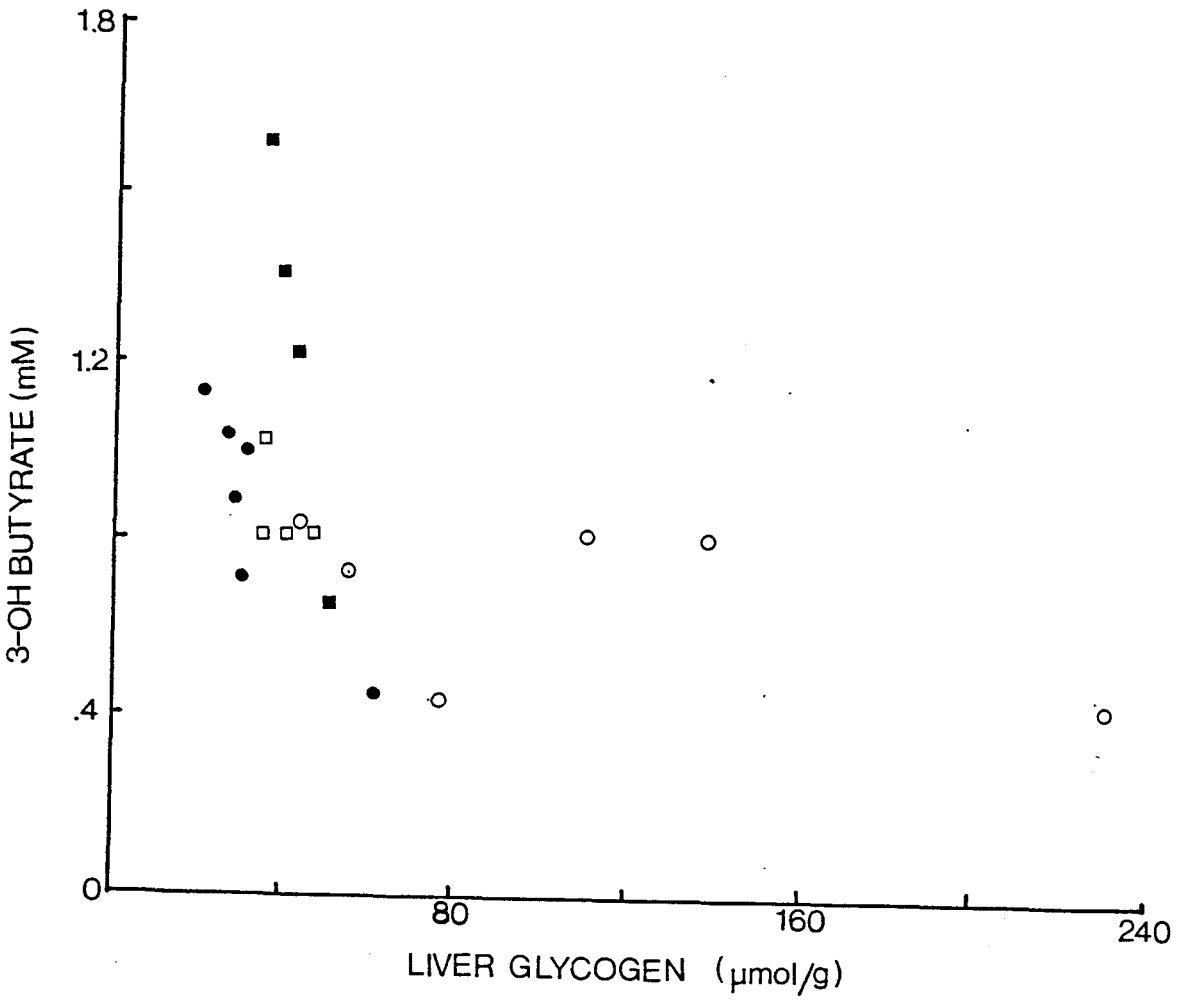


Fig. 6 (b)

The relationship between the mean liver glycogen concentrations at each sampling time during the experiment (before, during and after 60 minutes of exercise) and the simultaneous mean blood 3-hydroxybutyrate concentrations of trained and untrained rats which had been fed a low carbohydrate diet during the week preceding the exercise, compared with the results of Beattie and Winder (1985).

○ Trained (n = 6) ; ● Untrained (n = 6)

□ Beatties' Trained (n = 5) ; ■ Beatties' Untrained (n = 5)

When Beattie and Winder's liver glycogen-blood ketone body concentrations are compared with ours (Fig. 6b), the negative correlation noted in Figure 6a, between the liver glycogen concentration and the simultaneous ketone body level in the blood of food-restricted exercised rats, is accentuated by the inclusion of Beattie and Winder's results. This would lead us to believe that their inability to demonstrate a relationship between liver glycogen content and blood ketone body level is due to the bugbear which haunts every researcher's protocol at some time or other: the examination of too narrow a range of the independent variable.

CHAPTER 4:

HIGH CARBOHYDRATE DIET:

INTRODUCTION:

The factors which enhance or suppress the ketogenicity of the post-exercise period are only partially understood. The composition of the pre-exercise diet appears to be important: a low carbohydrate diet enhances post-exercise ketosis (Askew et al., 1975; Courtice and Douglas, 1936; Gemmil, 1934; Neufeld and Ross, 1943; Koeslag et al., 1980) and a high carbohydrate diet reduces it (Askew et al., 1975; Courtice and Douglas, 1936; Rennie and Johnson, 1974; Koeslag et al., 1980).

To investigate the possibility that the resistance of endurance-trained subjects to post-exercise ketosis is diet induced, we studied the post-exercise changes in blood 3-hydroxybutyrate concentration as well as liver and muscle glycogen levels of trained and untrained rats on a high carbohydrate diet.

METHOD:

One hundred and forty male Long Evans rats, bred in the Animal House of the Physiology Department, University of Cape Town were used. The environmental conditions of the Animal House as well as the subdivision of the rats into different groups were the same as described in Chapter 2. Both groups were fed ad libitum on normal Epol Laboratory Chow for 5 weeks of the training period. The energy value was the same as in Chapter 2. On this regimen the two groups maintained their body weights.

During the 6th week, special home-made fudge, consisting mainly of glucose, was added to their diet, to increase the proportion of carbohydrate in the diet. After the 6th week the Trained and Untrained groups were each randomly divided into an Experimental and a Control group (see Chapter 2). On the Experimental Day the animals belonging to the 2 Experimental groups ran on a level treadmill at 0.2 m/s for one hour. Six animals from each group were sacrificed immediately before the exercise, and again immediately after exercise, and at half-hourly intervals thereafter. The collection of blood for 3-hydroxybutyrate concentration

determination, liver and muscle tissue for the measurement of the glycogen and muscle malate dehydrogenase activity were the same as described in Chapter 2.

The preparation for the material for biochemical analysis is described in the Appendix. The statistical significance of differences between groups was determined by the Student's 2-tailed t-test. The chosen level of significance was $p<0.05$.

RESULTS:

Body Mass:

No significant difference in body mass was found between the Trained (330 ± 20 g) and Untrained (335 ± 22 g) rats on the Experimental Day ($p>0.05$). These weights were also not significantly different from those of the rats which were fed a normal diet up to the Experimental Day (see Chapter 2).

Malate Dehydrogenase:

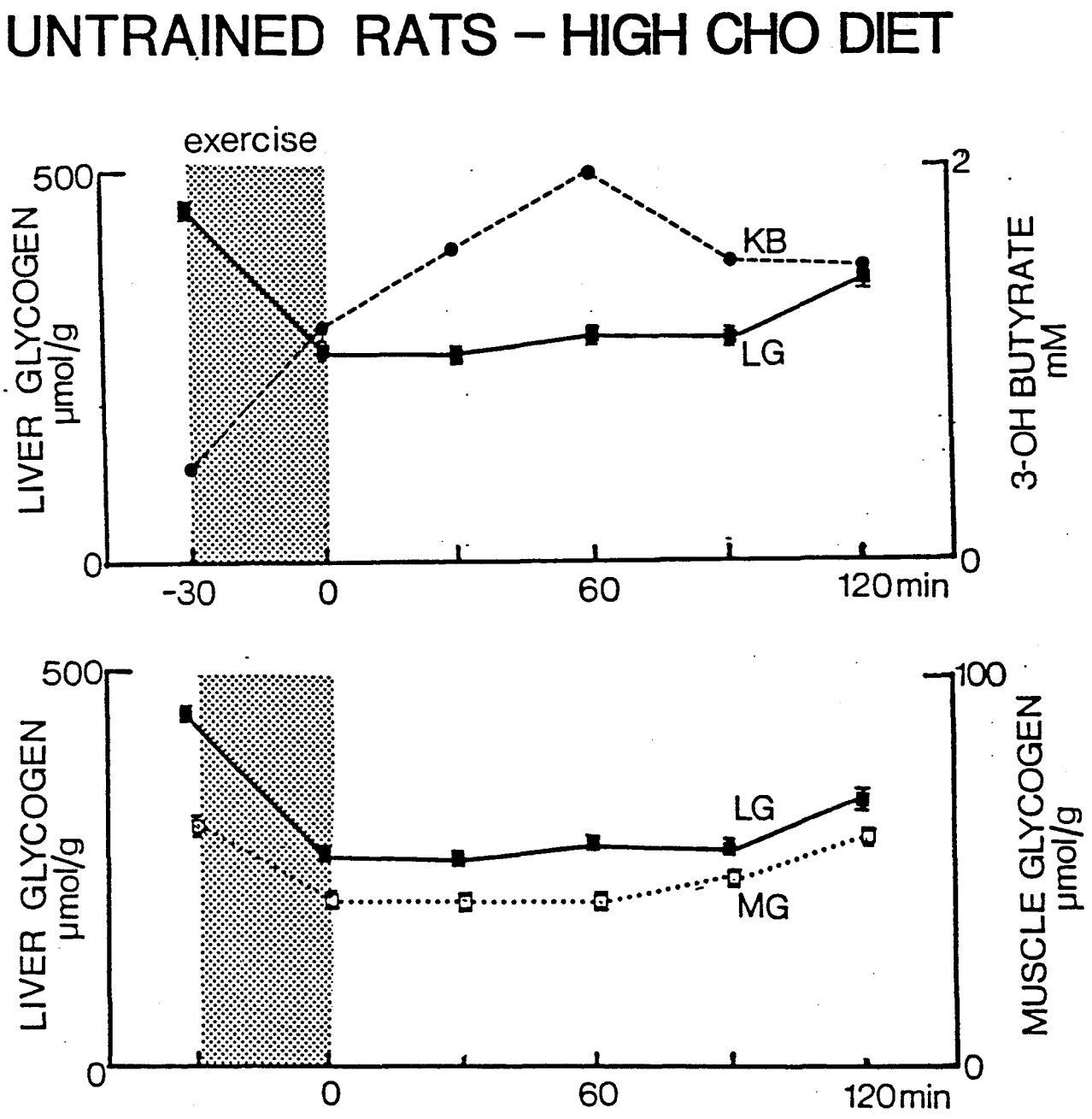
The muscle malate dehydrogenase activity was 19 ± 2.0 U/g wet mass in the Trained, and 8.7 ± 2.0 U/g wet mass in the

Untrained animals on the Experimental Day ($p<0.01$). This 100% higher activity in the Trained group suggests that the training programme had been effective, and is comparable with the training programmes of others (Holloszy et al., 1973; Mole et al., 1973).

Blood Ketone Bodies:

There was no statistically significant difference between the mean blood 3-hydroxybutyrate concentrations of the Trained (0.04 ± 0.01 mmol/l) and Untrained rats (0.08 ± 0.02 mmol/l) before the exercise (Table 7; Figs 7a and 8a). The mean 3-hydroxybutyrate concentration immediately after exercise was significantly higher than it was before the exercise in the Untrained rats (0.08 ± 0.02 vs 1.2 ± 0.18 mmol/l; $p<0.05$; Table 7), but there was no significant change in blood ketone concentration with exercise in the Trained group (0.04 ± 0.01 vs 0.05 ± 0.01 mol/l at the end of exercise; $p>0.01$; Table 7).

For the first 90 minutes of the post-exercise period the 3-hydroxybutyrate concentration in the blood of the Untrained rats did not change significantly (1.2 ± 0.18 mmol/l at the end of exercise; and 1.3 ± 0.3 mmol/l at 90 minutes after exercise), and then fell to 0.8 ± 0.2 mmol/l at 120 minutes after exercise, which was, however, still significantly



; 7 (a) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) before and after 60 minutes of exercise in untrained rats fed a high carbohydrate diet during the preceding week.

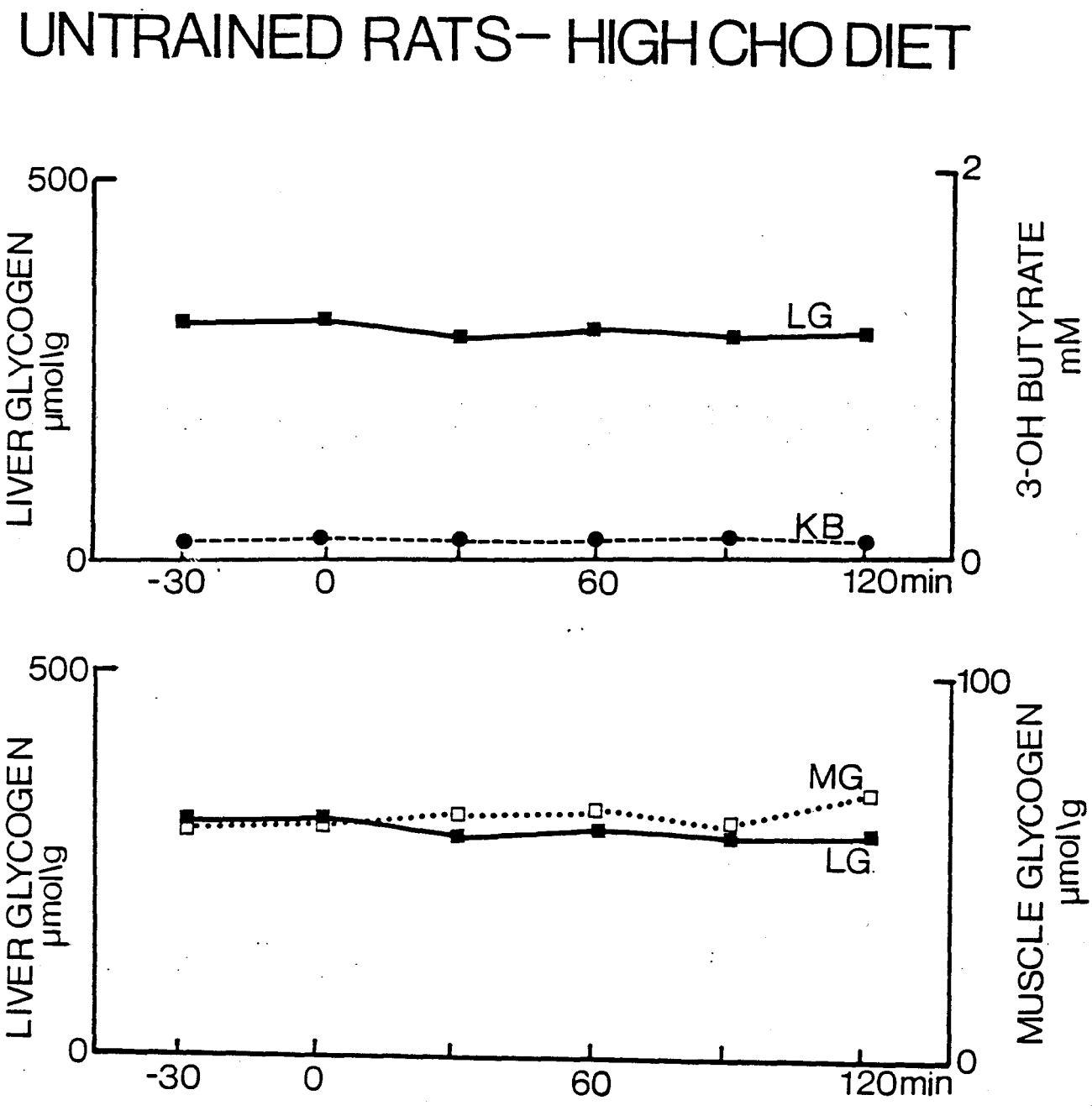


Fig 7 (b) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) in untrained non-exercised rats fed a high carbohydrate diet during the preceding week.

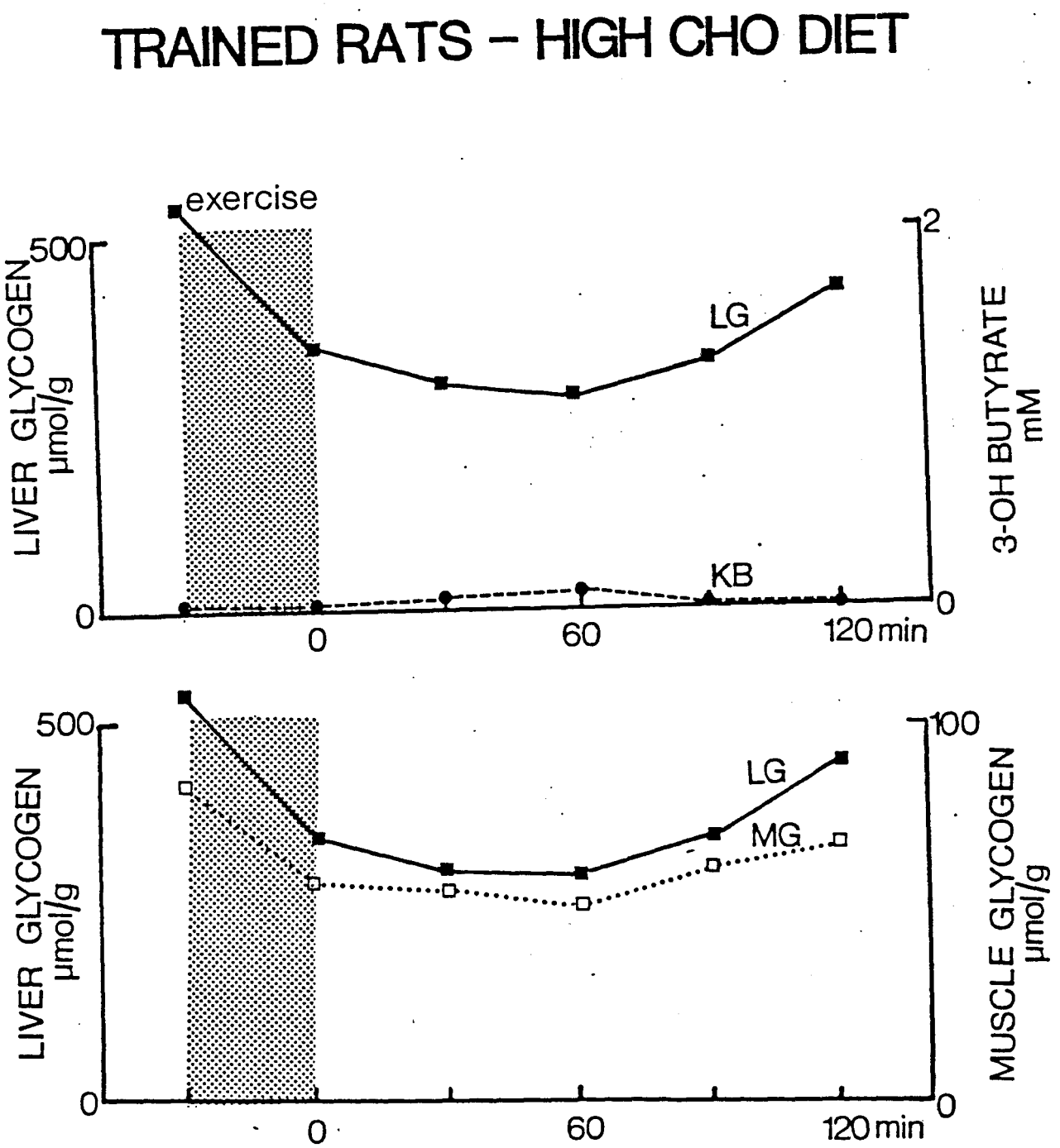
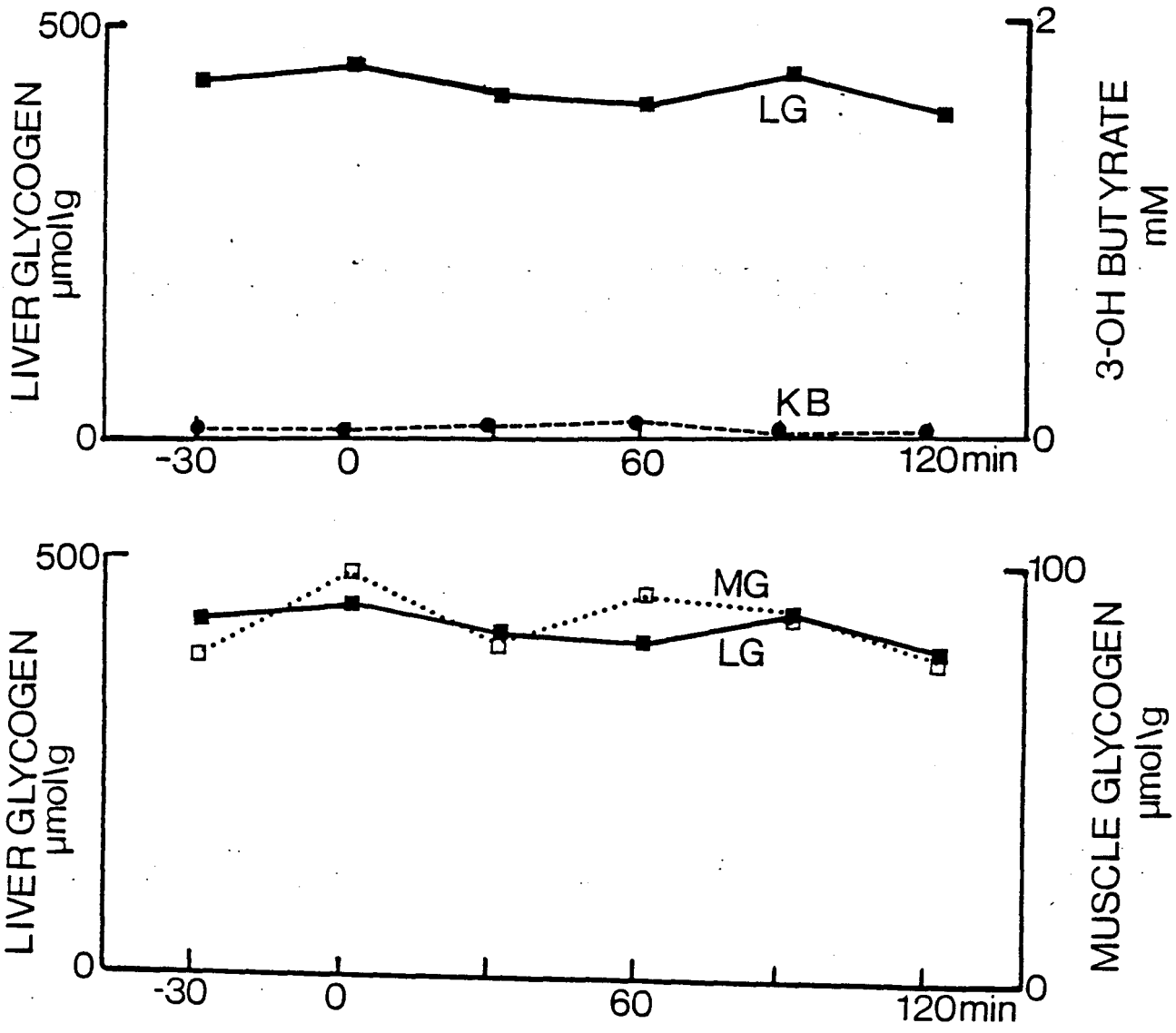


Fig 8 (a) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) before and after 60 minutes of exercise in trained rats fed a high carbohydrate diet during the preceding week.

TRAINED RATS - HIGH CHO DIET



g 8 (b) Blood 3-hydroxybutyrate (KB), liver glycogen (LG) and muscle glycogen concentrations (MG) in trained non-exercised control rats fed a high carbohydrate diet during the preceding week.

higher than the pre-exercise level ($p<0.05$), or the 3-hydroxybutyrate levels of the non-exercising Controls ($p<0.05$).

The blood 3-hydroxybutyrate concentration of the Trained Experimental group remained less than 0.1 mmol/l for the entire observation period, which was not significantly different from those of the Trained Controls (Figs. 8a and 8b). These values were all significantly lower than those of the Untrained Experimental group.

Liver Glycogen:

The Trained animals had significantly higher glycogen concentrations (546 ± 9 µmol glucosyl units/g wet mass) than the Untrained animals at rest (450 ± 8 µmol glucosyl units/g wet mass; $p<0.05$, Table 8; Figs. 7a and 8a).

Exercise significantly decreased the liver glycogen concentrations of both Experimental groups. In the Untrained group, the liver glycogen concentration fell from 450 ± 8 µmol/g to 270 ± 14 µmol/g and in the Trained group from 546 ± 10 µmol/g to 348 ± 19 µmol/g ($p<0.05$) after the 1 hour of treadmill running. Although the Trained animals utilized slightly more liver glycogen (198 µmol/g) than the Untrained (180 µmol/g) rats, their liver glycogen content

was significantly higher than that of the Untrained animals after the 1 hour of exercise on a level treadmill (348 ± 19 μmol/g for the Trained compared to 270 ± 14 μmol/g for the Untrained; $p<0.05$, Table 8).

In the Untrained Experimental group, no significant change in liver glycogen was observed for about 90 minutes after the exercise period, but thereafter it rose significantly to reach 367 ± 10 μmol/g at 2 hours of the recovery period. For the entire recovery period, the liver glycogen content of the Untrained Controls was significantly higher than that of the Untrained Experimental group (Table 8).

The liver glycogen concentration of the Trained rats fell slightly during the first 60 minutes of recovery, but started to increase thereafter so that there was no significant difference between the 90 minute value and that of the immediate post-exercise concentration (348 ± 19 μmol/g at the end of exercise compared to 350 ± 10 μmol/g at 90 minutes recovery; $p>0.1$). It increased further to reach 446 ± 28 μmol/g after 2 hours of recovery.

The liver glycogen concentrations of the Trained Controls were significantly higher than those of their Experimental counterparts, throughout the entire recovery period (Table 8, Figs. 7b and 8b).

Muscle Glycogen:

The Trained rats had significantly higher muscle glycogen concentrations (83 ± 5 μmol glucosyl units/g wet mass) than the Untrained animals at rest (62 ± 3 μmol/g glucosyl units/g wet mass; $p<0.05$) (Table 9; Figs. 7a and 8a).

Exercise lowered the muscle glycogen concentration significantly in both Experimental groups. In the Untrained group it decreased from 62 ± 3 to 44 ± 4 μmol/g ($p<0.05$), and in the Trained from 83 ± 5 to 48 ± 7 μmol/g ($p<0.05$). Thus, the Trained rats utilized approximately 25 and the Untrained about 18 μmol glucosyl units/g in the 1 hour exercise period.

No significant change in muscle glycogen concentration was observed for the first 90 minutes of the recovery period in the Untrained group (44 ± 4 at the end of exercise compared to 49 ± 4 μmol/g at 90 minutes after exercise; $p>0.1$), but during the final half hour of observation the muscle glycogen content increased significantly to 59 ± 4 μmol/g ($p<0.05$).

The muscle glycogen content of the Trained animals also showed no significant change for the first 90 minutes of the

recovery period, but then the level rose to reach 68 ± 3 μmol/g at 120 minutes after exercise. The muscle glycogen concentrations of the Trained and Untrained non-exercised Controls was significantly higher than those of their respective Experimental counterparts throughout the 2 hour recovery (Table 8, Figs. 7b and 8b).

The rate of muscle glycogen resynthesis was the same in the Trained animals as in the Untrained rats (approximately 16 μmol/g/h) during the final hour of the recovery period.

DISCUSSION:

The present study shows that trained rats store more glycogen than untrained rats when both eat a high carbohydrate diet for one week. As on the other diets (Chapters 2 and 3), the trained animals utilized slightly more muscle (25 vs 18 μmol/g/h) and liver (198 vs 180 μmol/g/h) glycogen than the untrained animals did during identical submaximal exercise. However, the trained rats had significantly more muscle and liver glycogen left at the end of the exercise. The rate of liver and muscle glycogen repletion was the same in the trained and the untrained animals (approximately 49 and 16 μmol/g/h in liver and muscle respectively). The post-exercise 3-hydroxybutyrate

concentrations were significantly higher in the untrained than in the trained rats.

The finding that carbohydrate-loaded trained rats have higher pre-exercise muscle and liver glycogen concentrations than carbohydrate-fed untrained rats, suggests that liver and muscle subjected to a combination of regular exercise and a high carbohydrate diet, store more glycogen than livers and muscles exposed to a high carbohydrate diet alone. This is in keeping with the findings of several investigations conducted in the late 1960's and 1970's which showed that muscle glycogen stores could be elevated to above-normal values, by 2 bouts of exercise on a low carbohydrate diet, followed by 3 days of eating a high carbohydrate diet and rest (Astrand, 1971; Hultman, 1967; Saltin and Hermansen, 1967; Bergstrom and Hultman, 1966; Saltin, 1977).

Sherman et al., (1981) studied the effect of 3 exercise-diet regimens on muscle glycogen of athletes. The diets consisted of either 15% carbohydrate (low), 50% carbohydrate (mixed) or 70% carbohydrate (high). This corresponded to 104, 353 and 542 g carbohydrate per day respectively. The effect of 6 days of training, while on 3 different combinations (A, B and C) of these diets, on a 20.9 km run on day 7 was investigated. During trial A the athletes ate the low carbohydrate diet (15%) for 3 days followed by the high

carbohydrate diet (70%) for the next 3 days. During trial B the athletes ate the mixed diet for 3 days followed by the high carbohydrate (70%) for the next 3 days. The athletes on trial C ate the mixed diet for the entire 6 days.

The results showed that similar muscle glycogen concentrations were achieved when a mixed or low carbohydrate diet (trials A and B) was followed by 3 days of high carbohydrate consumption. Eating a mixed diet during the final 3 days of training (trial C) produced muscle glycogen concentrations which were 25% lower than those of the athletes in trials A and B. The muscle glycogen concentrations of the subjects in trials A and B were comparable to those of described by Astrand, (1971), Saltin and Hermansen, (1967), and Saltin, (1977), in subjects who had followed the more rigorous "carbohydrate-stripping/ carbohydrate-loading" regimens. This suggests that carbohydrate-restriction (followed by a high carbohydrate diet) is not necessary to attain supra-normal muscle glycogen concentrations. Regular training on its own appears to stimulate glycogen synthesis during carbohydrate loading as effectively as does a preceding "carbohydrate-stripping" routine.

In our study, the muscle glycogen concentrations achieved by training on a high carbohydrate diet (without prior "carbohydrate-stripping") were 60% higher than those

achieved by training on a normal diet (Chapter 2). A high carbohydrate diet alone (i.e. without training) also raised the mean muscle glycogen concentration: by 50% compared with a normal mixed diet.

Our trained animals used slightly more liver- (198 vs 180 µmol/g/h) and muscle- (25 vs 18 µmol/g/h) glycogen than the untrained rats during the one hour of exercise. In comparison, the trained and untrained carbohydrate-restricted rats (Chapter 3) lost approximately 164 and 123 µmol/g/h of liver-, and 15 and 18 µmol/g/h of muscle-glycogen during the 1 hour run respectively. The corresponding losses of glycogen when a normal diet was consumed (Chapter 2) were 188 and 134 µmol/g/h for liver-, and 17 and 21 µmol/g/h for muscle-glycogen respectively. The rate of glycogen utilization during standardized exercise is therefore positively correlated with the pre-exercise tissue glycogen concentration: the higher the glycogen concentration, the faster it is utilized during submaximal exercise. However, despite their fast rate of glycogen utilization, our trained animals, and those fed a high carbohydrate diet (i.e. the animals with the highest glycogen stores), had the highest immediate post-exercise liver and muscle glycogen concentrations, which is in keeping with the findings of earlier investigations (Terjung et al., 1974; Baldwin et al., 1975; Winder et al., 1981). This therefore makes the attainment of high liver and muscle

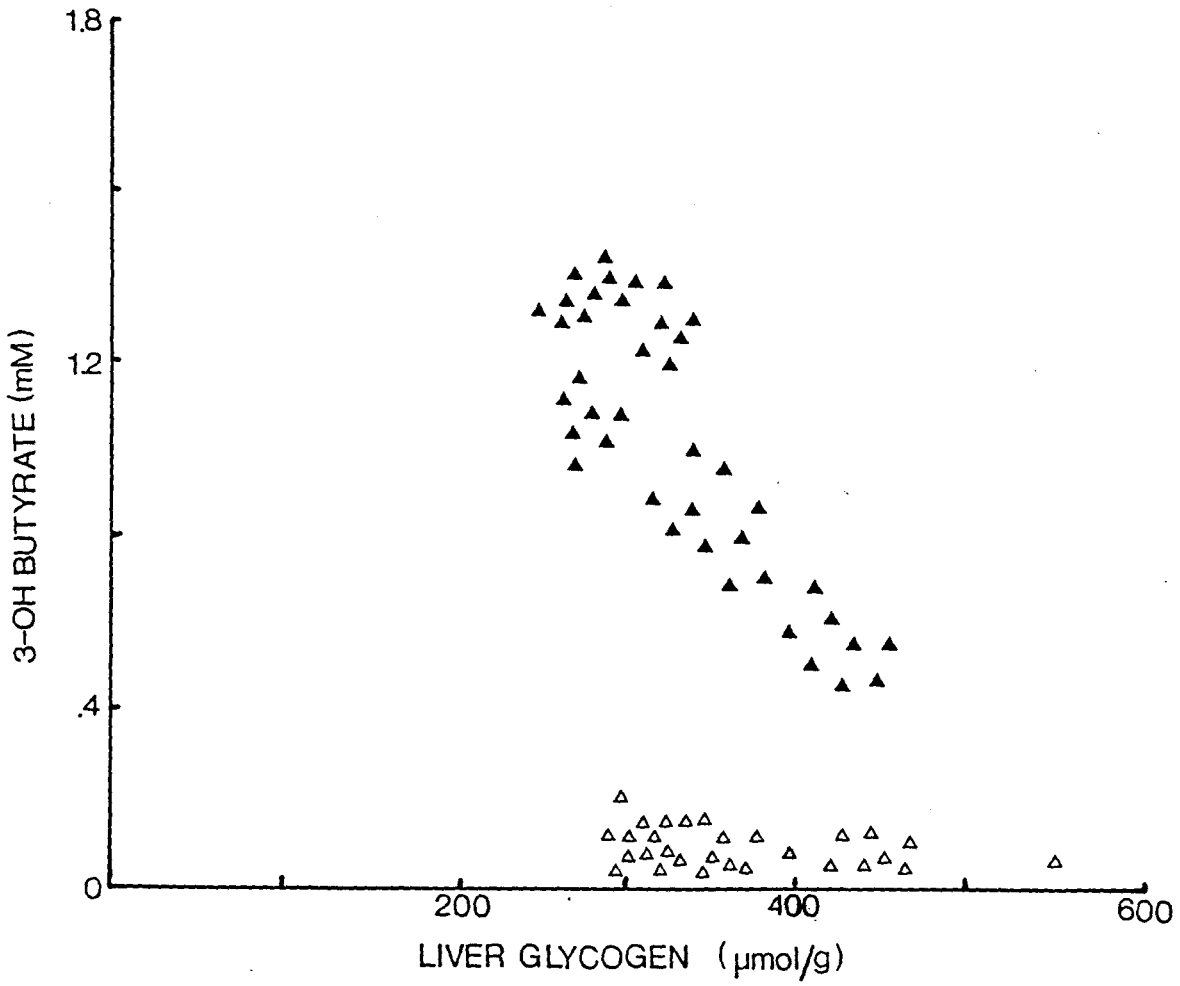


Fig. 9

The relationship between the liver glycogen concentrations and the simultaneous blood 3-hydroxybutyrate concentrations of carbohydrate-loaded trained and untrained rats which were sacrificed at various times before and after 60 minutes of exercise. The coefficient of correlation for the results as a whole is -0.3; the corresponding value for the untrained results on their own is -0.9.

△ Trained ; ▲ Untrained

glycogen concentrations worthwhile in athletes, since they do postpone exhaustion (Baldwin et al., 1975; Fitts et al., 1975; Winder et al., 1981).

Rapid muscle glycogen resynthesis occurred in both the untrained and trained rats (7.5 and 10 µmol/g/h during the entire 2 hour of recovery, respectively), despite the absence of food. This is slightly higher than the rate of resynthesis after a normal diet (Chapter 2: 5.5 and 9.5 µmol/g/h for untrained and trained rats respectively), and higher still than after a low carbohydrate diet (Chapter 3), when rates of muscle glycogen resynthesis did not exceed 3 µmol/g/h. This confirms our earlier postulate (Chapter 3: Discussion) that muscle glycogen is probably replenished primarily by the redistribution of the body's carbohydrate stores, rather than by de novo glycosynthesis from non-carbohydrate depots.

Untrained rats which had been on a low-carbohydrate diet before exercise lost 10 µmol glycogen/g of liver tissue during the 2 hour recovery period (Chapter 3). On a normal diet these rats gained 1 µmol (Chapter 2), and on a high carbohydrate diet they regained 97 µmol/g of liver tissue, indicating that the body's carbohydrate status is of even greater import to the rate at which liver glycogen is resynthesized, than it is to the rate of muscle glycogen repletion. The trained animals showed nett liver glycogen

resynthesis after all 3 diets: the highest rate of resynthesis occurring after a high carbohydrate diet, though the lowest rate occurred after a normal mixed diet. The reason for this anomaly is not clear.

Figure 9 shows a very marked disparity between the blood ketone body concentrations of carbohydrate-loaded trained and untrained animals at the same liver glycogen concentrations. Despite a nearly 100% difference between the individual highest and individual lowest liver glycogen concentration (during the entire observation period), the blood ketone body concentrations of the trained rats appeared to vary randomly between 0.04 and 0.2 mmol/l. Over a similar range of liver glycogen concentrations the blood ketone body concentrations of the untrained rats showed a highly significant negative correlation ($r = -0.9$; $p<0.01$) with the simultaneous hepatic glycogen levels (Fig. 9). This discrepancy will be discussed in Chapter 5 - The GENERAL DISCUSSION.

CHAPTER 5:

GENERAL DISCUSSION:

Previous investigations have shown that the blood 3-hydroxybutyrate concentration rises gradually during prolonged exercise, and then increases to higher levels in the post-exercise period: the phenomenon post-exercise ketosis (Courtice and Douglas, 1936; Johnson et al., 1969; Winder et al., 1975 and 1979; Koeslag et al., 1980). Endurance training has been found to decrease the degree of post-exercise ketosis (Johnson et al., 1969, 1970 and 1971). The exact mechanism by which physical training decreases the blood 3-hydroxybutyrate concentration after exercise is not known but a number of suggestions have been made:

1. Increased rate of removal of ketone bodies from the blood

One possible reason for the reduction in ketone body concentration with training can be the effect which training has been shown to have on the skeletal muscle enzymes involved in ketone body oxidation.

2. Decreased rate of hepatic ketogenesis

It has been suggested that the liver glycogen levels might be higher in trained than in untrained subjects and animals at the end of exercise, thus preventing high rates of ketogenesis.

1. Ketone Body Utilization:

Baldwin et al. (1972) have shown that skeletal muscle adapts to exercise training by increasing its capacity to oxidize pyruvate, fatty acids and ketone bodies. The activities of the enzymes of the citric acid cycle and the mitochondrial respiratory chain are increased (Holloszy, 1967; Holloszy et al., 1973; Mole et al., 1971; Winder et al., 1974). Thus, training (treadmill running for 2 hours/day for 3 months) was found to increase the concentration of cytochrome c and the activities of cytochrome oxidase, citrate synthase, 3-hydroxybutyrate dehydrogenase, 3-ketoacid transferase and aceto CoA-thiolase almost 2-fold in all three types of muscle in rats (Baldwin et al., 1972; Holloszy et al., 1975; Oscai et al., 1971; Winder et al., 1974). These changes are associated with increases in both the size and number of

mitochondria (Gollnick and King, 1969; Hoppeler et al., 1973; Morgan et al., 1971).

That athletes do indeed metabolize ketone bodies faster than non-athletes do has been shown by Johnson and Walton (1972), who studied the rate of clearance of a 700 mmol oral dose of acetoacetate from the blood in trained long distance runners and sedentary subjects before, during and after a 90 minute run. Both groups had similar rates of utilization of acetoacetate at rest. The clearance rate increased during exercise and decreased again after exercise in both groups, but the decrease was more marked in the untrained subjects.

In the present study, malate dehydrogenase activity (MDH) was used as a marker for the oxidative capacity of the skeletal muscles when animals become trained. The approximately 100% increase in malate dehydrogenase concentration with endurance training showed that our training programme was comparable to those of previous investigators (Holloszy et al., 1973 and 1975), and it is therefore very likely that the activities of the other oxidative enzymes, particularly those involved with ketone body oxidation, were also increased to a similar extent.

2. Ketone Body Production:

Ketones are synthesized primarily in the liver, by the process of ketogenesis. They are formed from free fatty acids that have undergone β-oxidation in the hepatic mitochondria. The prevailing view (McGarry, 1978, Robinson and Williamson, 1980; McGarry et al., 1979, Foster, 1984) is that the rate of ketone body synthesis is regulated at the site of entry of long chain fatty acids into the liver mitochondria. This step is catalyzed by the enzyme carnitine acyl transferase 1 (CAT 1). This enzyme is inhibited by high malonyl CoA concentrations in the hepatic cytosol. Malonyl CoA is the first committed intermediate in the conversion of glucose into fat, and its concentration in the cytosol varies, by a variety of mechanisms, with the liver cell's carbohydrate content. When carbohydrate is plentiful the hepatic malonyl CoA concentration is high; when carbohydrate is scarce the malonyl CoA level is reduced (Robinson and Williamson, 1980). This causes low rates of β-oxidation and ketogenesis in animals with high liver glycogen concentrations, and high rates of β-oxidation and ketogenesis in animals with low liver glycogen concentrations (McGarry, 1978, Foster and McGarry, 1983). (The major assumption made by McGarry and others is that malonyl CoA concentration is proportional to flux through malonyl CoA.)

Glycogen is converted to pyruvate in the hepatic cytosol. This is decarboxylated to acetyl CoA in the mitochondrion, from which citrate is then formed by condensation with oxaloacetate. Part of the citrate is consumed in the citric acid cycle within the mitochondrion, but some of it is exported (by an active transport mechanism) to the cytoplasm, where it is reconverted into acetyl CoA (and oxaloacetate) as the preliminary step in the formation of malonyl CoA (Foster and McGarry, 1983; Foster, 1984).

The enzyme which catalyses the conversion of cytosolic acetyl CoA into malonyl CoA is acetyl CoA carboxylase, which exists in 2 interchangeable forms: a phosphorylated and an unphosphorylated form. An insulin-dependent phosphatase dephosphorylates, and activates, the enzyme. Two endogenous protein kinases are responsible for its phosphorylation: a cAMP-dependent protein kinase (which can be activated by glucagon and the catecholamines), and a cAMP-independent kinase. Both convert acetyl CoA carboxylase into a form which is more dependent on allosteric activation by citrate than the unphosphorylated form is (Cohen, 1979). This means that citrate plays a central role in the regulation of hepatic ketogenesis, not only as the penultimate intermediate for malonyl CoA synthesis, but also as a regulator of the enzyme which forms malonyl CoA. In the presence of abundant citrate both the phosphorylated and unphosphorylated forms of acetyl CoA carboxylase are

activated, their substrate concentrations will be high, and the cytosolic malonyl CoA levels will be elevated. When citrate is scarce, the production of malonyl CoA will be inhibited whether acetyl CoA carboxylase is phosphorylated or not, through lack of substrate.

Citrate levels in the hepatocyte are dependent on the carbohydrate status of the liver through the conversion of glycogen and glucose to mitochondrial acetyl CoA, and also on the availability of oxaloacetate, which is an important intermediate in the gluconeogenic pathway from lactate and alanine to glucose (Nosadini et al., 1980, 1981; Sugden and Watts, 1982). Both glucose and alanine are thus powerful inhibitors of hepatic ketone body production (Genuth, 1973; Genuth and Castro, 1974; Koeslag et al., 1982) despite producing opposing insulin/glucagon ratios in the plasma (Koeslag et al., 1982, 1985).

The blood ketone body concentrations of our rats were negatively correlated ($p<0.01$) with their simultaneous liver glycogen concentrations in each experimental situation (Figs. 3, 6a, and 9), except after a high carbohydrate diet, when the trained animals' blood ketone body concentrations were randomly associated with their liver glycogen contents (Fig. 9). However, there was no correlation between the animals' liver glycogen levels and their simultaneous blood 3-hydroxybutyrate concentrations when all 3 dietary regimens

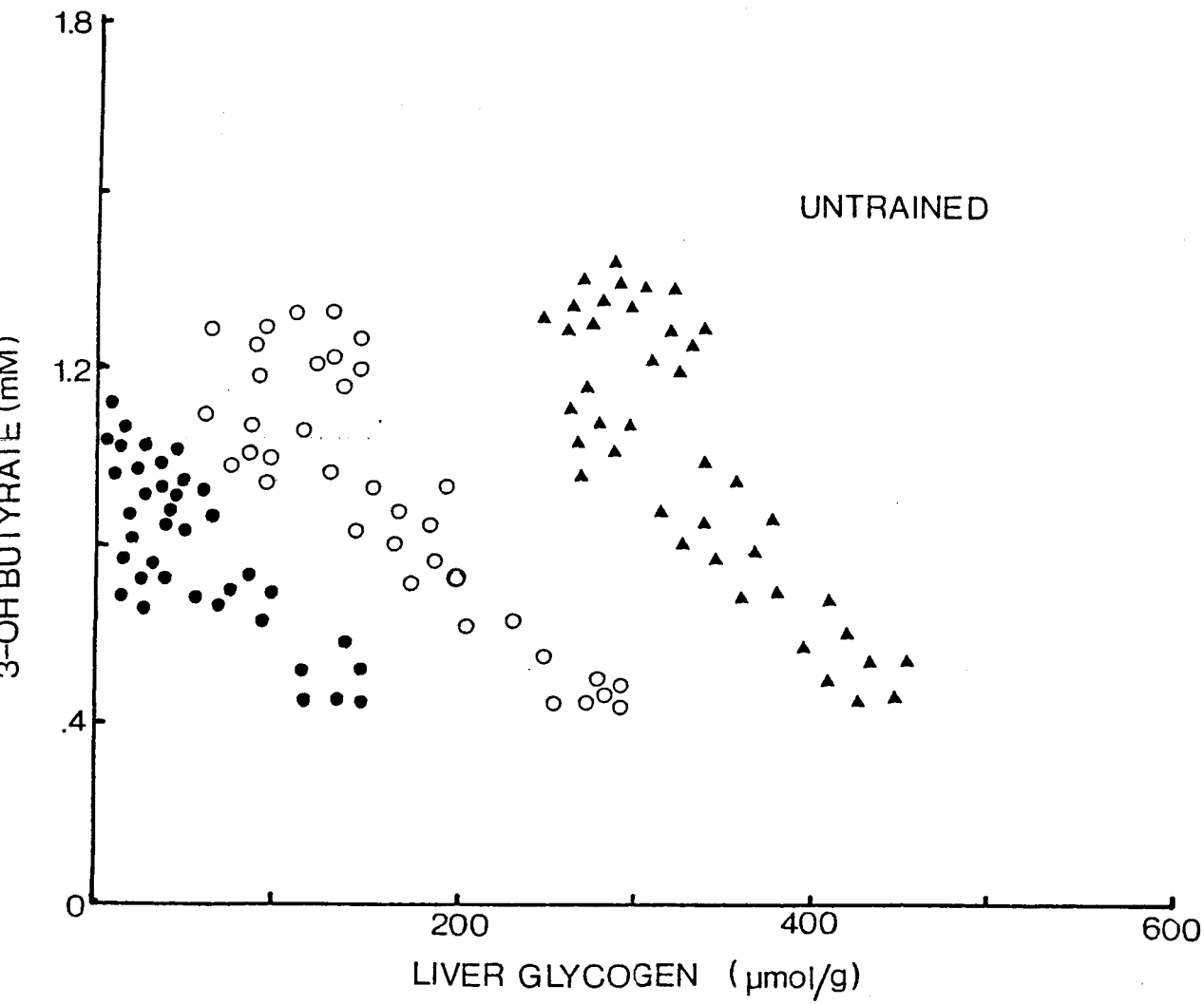


Fig 10

The Relationship between liver glycogen concentration and simultaneous blood 3-hydroxybutyrate concentration before and after 60 minutes of exercise in untrained rats fed 3 different diets during the preceding week.

○ Normal Diet; ● Low Carbohydrate Diet; ▲ High Carbohydrate Diet

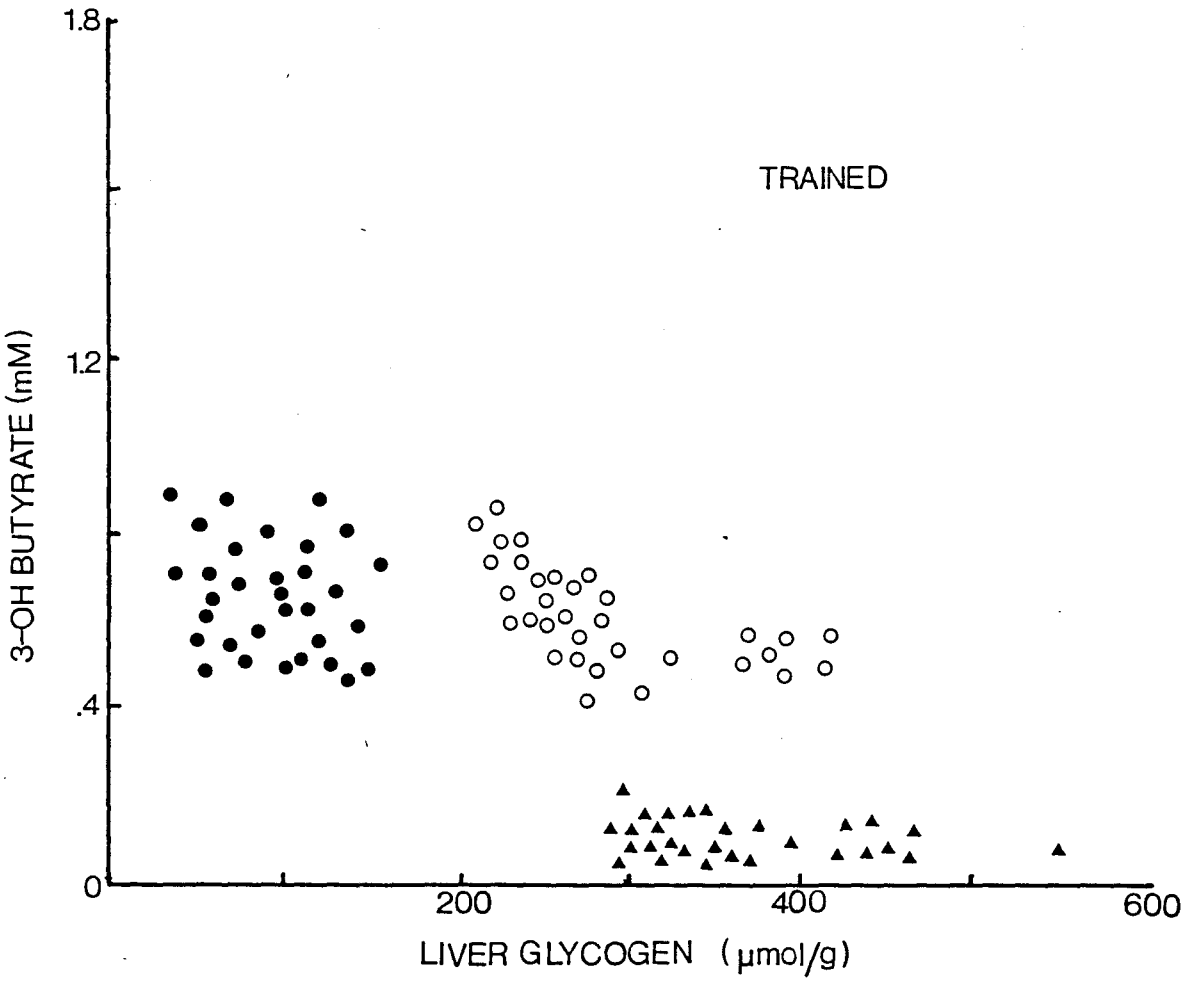


Fig 11

The Relationship between liver glycogen concentration and simultaneous blood 3-hydroxybutyrate concentration before and after 60 minutes of exercise in trained rats fed 3 different diets during the preceding week.

○ Normal Diet; ● Low Carbohydrate Diet; ▲ High Carbohydrate Diet

were considered together (Figs. 10 and 11). Resting blood ketone body concentrations tended not to be affected by the diet of the preceding week or by training, despite an approximately 300% difference in pre-exercise liver glycogen concentrations between carbohydrate-restricted and carbohydrate-fed rats. Similarly, post-exercise blood ketone body concentrations, particularly in the untrained animals (Fig. 10), rose to similar levels after each diet, despite large differences in liver glycogen concentration during recovery. The trained animals (Fig. 11) showed some overall (all 3 experiments seen together) correlation between the simultaneous blood ketone body and liver glycogen concentrations, but not in individual experiments.

It should be noted that we measured the blood concentration of 3-hydroxybutyrate, and not its rate of synthesis by the liver. We are thus, as it were, observers of the level of a point on a river (i.e. the blood ketone body concentration) whose source (the rate of ketogenesis) and end (ketone body catabolism) are invisible to us. We can, however, observe the sky (the liver glycogen concentration), though not the rainfall, above what we believe is the catchment area of the river, and are also aware that there are sluice-gates down stream (the activity of the enzymes of ketone body catabolism). If the sluice-gates remain unaltered the level of the river will be directly proportional to the rainfall

in the catchment area. This proportionality will remain at every settings of the sluice-gates, though the overall level of the river will change with each new "setting" of the sluices.

Continuing with this analogy, we have observed 2 such rivers (endurance trained and untrained rats), on 3 different occasions (low, normal and high carbohydrate diets). On each occasion we observed a substantial change in the amount of cloud cover over the presumptive catchment areas of the 2 rivers, as well as changes in the levels of the rivers themselves. The cloud cover over the source of the Rio Athleta was always less than over the source of the Rio Sedentarius at the beginning and end of each of the 3 observation periods, but for the same amount of cloud cover, on any particular day, the rivers were approximately equally full. The changes in the levels of the rivers, during any given observation period, were directly proportional to the amount of cloud cover over the putative sources of the 2 rivers. The most likely interpretation of these observations is that on any given observation day the settings of the sluices on the 2 rivers were approximately the same, and that the rainfall in the 2 catchment areas was directly proportional to the clouds seen from our vantage point.

It is conceivable, though highly unlikely, that no changes occurred to the rates of inflow to the rivers, and that the

increases in the levels of the streams were due to alterations in the settings of the sluices in response to the cloud cover. As we can think of no mechanism whereby this would occur, we will ignore this possibility as the prime cause of our observations.

Over the 3 observation days, however, there was no correlation between the amount of cloud cover and the levels of the rivers: the rivers started at approximately the same depth at the beginnings of each of the observation periods, despite large differences in initial cloud cover. The 2 most likely explanations are that the sluices were set differently on the 3 occasions, or that the clouds that we could see were not the only source of water for the rivers (i.e. liver glycogen is not the only determinant of the rate of ketogenesis).

More detailed inspection of the data reveals that the proportionality between cloud cover and the level of the river, on any given day, was clearer in the Rio Sedentarius than in the Rio Athleta. This difference between the rivers was particularly marked on the day with the lowest cloud cover (High-Carbohydrate Diet). This could mean that there was more adjustment of the sluices in the Rio Athleta than in the Rio Sedentarius during a given observation period, or that the other sources of inflow to the rivers were more variable in the Rio Athleta than in the Rio Sedentarius.

In both cases the changes to the inflow or outflow from the the Rio Athleta tended to counteract the changes in rainfall over its catchment area.

Translated back into the Biochemistry of Ketone Body Metabolism, the analogy of the rivers suggests:

1. On a given diet the blood ketone body concentration is probably governed primarily by the rate of hepatic ketogenesis, which is in turn inversely proportional to the liver glycogen content (Foster, 1984). Differences in the rate of removal of ketone bodies from the blood (Johnson and Walton, 1972) are probably small in comparison with the differences in the rates of ketogenesis between trained and untrained animals, because the proportionality between the liver glycogen concentration and simultaneous blood ketone body levels of the 2 groups of animals is very high.

Our results therefore show that, though differences in the ability to oxidize ketone bodies exist between trained and untrained animals (Johnson and Walton, 1972; Baldwin et al., 1972; Holloszy et al., 1975; Oscai et al., 1971; Winder et al., 1974) the dominant cause of the differences in post-exercise ketosis between trained and untrained animals is the difference in liver glycogen concentration. For the same level of liver glycogen (on a normal, or on a carbohydrate-

restricted diet) trained and untrained animals have approximately the same blood ketone body levels (Figs. 3 and 6a)

2. The "shift to the left" of the relationship between the blood ketone body concentration and simultaneous liver glycogen content with carbohydrate-deprivation (and "shift to the right" with carbohydrate-feeding) (Fig. 10), could be due to the induction of the enzymes of ketone body oxidation by the week of carbohydrate-restriction, and their repression by the week of carbohydrate-loading. Alternatively, the shifts could be due to an alteration of the relationship between the rate of ketogenesis and the hepatic glycogen concentration, by, for instance, the supply of lactate and alanine (3-carbon compounds) to the liver.

That the rate of ketone body oxidation might be influenced by the carbohydrate content of the diet has been shown by Askew et al. (1975). They examined the activities of 3-oxoacid CoA transferase and acetoacetyl CoA thiolase in heart and skeletal muscle after rats had been fed 3 different diets for 4 weeks. The skeletal muscle 3-oxoacid CoA transferase activity and heart muscle acetoacetyl CoA thiolase activity were both inversely related to the carbohydrate content of the diet. Muscle acetoacetyl CoA thiolase and heart 3-oxoacid CoA transferase activity showed no recognizable relation to the composition of the diet. We

would therefore conclude that this could shift the graph to the left or to the right, but probably only to a minor degree.

The blood lactate concentration at rest and during exercise is positively related to the proportion of carbohydrate in the diet. A low carbohydrate diet is associated with low resting blood lactate levels, which rise only modestly with submaximal exercise, whereas a high carbohydrate diet gives rise to high resting blood lactate concentrations, which rapidly rise with exercise (Wasserman, 1967; Rennie and Johnson 1974; Kell et al., 1975; Yoshiba, 1984 and 1986). Since lactate is not only a major gluconeogenic substrate, but, together with alanine, is also a potent inhibitor of hepatic ketogenesis (McGarry and Foster, 1979; Boyd et al., 1981; Foster, 1984), we would expect the rate of ketone body production, for any given liver glycogen concentration, to be higher after a low carbohydrate diet than after a high carbohydrate diet, as indeed is suggested by our results.

3. Though there is no escaping the fact that the relative immunity of trained animals to post-exercise ketosis is largely due to their higher liver glycogen concentrations, there are qualitative metabolic differences between the trained and untrained rats (see Figs. 10 and 11). On any given diet the blood ketone body levels of the trained rats

were more resistant to changes in liver glycogen, than were those of the untrained rats, especially after a high carbohydrate diet.

Accepting that liver glycogen is a proven determinant of the rate of hepatic ketogenesis (Foster, 1984), the relative insensitivity of the trained animals' blood ketone body concentrations to changes in liver glycogen must either be due to alterations in the rate of ketone body catabolism during a given experiment, or due to the confounding of the relationship between liver glycogen and ketogenesis by, for instance, a changing supply of 3-carbon compounds (lactate and alanine) to the liver.

Since the trained animals replenished their muscle and/or liver glycogen stores more adequately than the untrained animals did during recovery from exercise, they probably commanded larger carbohydrate reserves than sedentary animals did after exercise (see "Discussions" of Chapters 2, 3 and 4). Mobilization of these reserves, mainly in unexercised muscles, presumably raised the plasma levels of alanine and lactate (Brooks et al., 1986; Katz et al., 1986; Newgardt et al., 1983), which, on being taken up by the liver, inhibited ketogenesis (by increasing the hepatic citrate concentration). The untrained animals, possessing smaller extrahepatic carbohydrate reserves, presumably mobilized fewer 3-carbon compounds during recovery, with the

result that the rate of ketogenesis was determined largely, if not entirely, by the liver glycogen content.

Changes in the activities of the enzymes involved in ketone body oxidation occur during and after exercise, and these changes have been shown to be more marked in trained subjects than in untrained subjects (Johnson and Walton, 1972; Baldwin et al., 1972; Holloszy et al., 1975; Oscai et al., 1971; Winder et al., 1974). This could also contribute to the relative homeostasis of the blood ketone body concentrations of athletes during recovery, though, as discussed under item 1, we are inclined to believe that it plays a relatively minor role. The mechanism and teleology of the changes in these enzyme activities are unclear.

CONCLUSION:

In conclusion, we have observed that: (1) the blood 3-hydroxybutyrate concentration is significantly higher in the untrained rats compared to the trained rats in all 3 dietary (normal, low carbohydrate and high carbohydrate) situations. In addition, the trained animals had significantly higher liver glycogen levels than the untrained animals, under any experimental condition. This suggests that the dominant cause for the differences in degree of ketosis in trained and untrained animals, at the end of exercise and during recovery could be the differences in liver glycogen concentration. (2) In the trained animals, the blood 3-hydroxybutyrate levels were relatively more resistant to changes in liver glycogen than in the untrained animals, on any given diet. This could be due to a greater availability of mobilizable carbohydrate (probably via the 3-carbon compounds) in the trained animals, or to a greater variability in the activities of the enzymes of ketone body catabolism in the trained animals than in the untrained rats. (3) When a high carbohydrate diet was consumed the relationship between the blood 3-hydroxybutyrate concentration and simultaneous liver glycogen content "shifted to the right", while carbohydrate-restriction "shifted" the relationship "to the left". These "shifts" could be due to the induction (or repression) of the enzymes

of ketone body oxidation by the week-long carbohydrate feeding or carbohydrate-deprivation. On the other hand, these "shifts" could be due to an alteration of the relationship between the rate of ketogenesis and the hepatic glycogen concentration by, for instance, the supply of lactate and alanine (3-carbon compounds) to the liver.

APPENDIX

BIOCHEMICAL ANALYSIS:

I. DETERMINATION OF D-3-HYDROXYBUTYRATE CONCENTRATION IN THE BLOOD:

The determination of the D-3-hydroxybutyrate concentration in the blood was carried out according to the method of Williamson, Mellanby and Krebs (1962), using 3-hydroxybutyrate dehydrogenase prepared from Rhodopsuedomonas spheroides (Boehringer Mannheim Gmb, West Germany. Cat. No. 127841).

The enzymatic determination of 3-hydroxybutyrate is specific for this substance. It is also very sensitive, and allows this substance to be determined in the blood of normal postabsorptive subjects (Wildenhoff, 1970).

Principle

This method is based on the fact that 3-hydroxybutyrate dehydrogenase catalyses the reaction

$$\text{3-hydroxybutyrate} + NAD^+ \rightleftharpoons \text{acetoacetate} + NADH + H^+$$

The equilibrium constant at 25° is 1.42×10^{-9} (Wildenhoff, 1970). To determine the 3-hydroxybutyrate concentration the reaction is made to proceed from left to right, using a ketone trap (hydrazine), and a pH of 8.5 in the reaction mixture. In this way all the 3-hydroxybutyrate is converted to acetoacetate. The rise in absorbance at 340nm, due to the conversion of NAD^+ to NADH is then an expression of the 3-hydroxybutyrate concentration.

Reagents and Apparatus

Perchloric acid, 0.7 moles/l.

10ml 70% perchloric acid, $HClO_4$ (E. Darmstadt, Art. 519) made up to 100 ml with distilled water.

Potassium hydroxide, 4.3 moles/l.

25g KOH dissolved in 100 ml distilled water.

Phenol red indicator. (The British Drug Houses Ltd. London).

DL-β-hydroxybutyric acid sodium salt, A grade (Calbiochem, San Diego, U.S.A. Cat. No. 3905).

Tris buffer, 0.1 M, pH 8.5.:

Dissolve 1.21 g Tris-(hydroxymethyl)-methylamine (AnalR, BDH Chemicals Ltd, Poole, England. Cat. No. 10315) in 50 ml distilled water. Adjust the pH to 8.5 with 0.2 M HCl, and make up to 100 ml with distilled water. Check the pH.

Hydrazine buffer, pH 8.5:

To 1 ml hydrazine hydrate 99 - 100% (AnalR, BDH Chemicals Ltd, Poole, England. Cat. No. 10327) add 1.0 M HCl till pH is 8.5. Prepare fresh daily.

NAD:

Dissolve 50 mg Nicotinamide Adenine Dinucleotide (Miles Laboratories (Pty) Ltd, South Africa, Cat. No. 36/299) in 10 ml distilled water. Prepare fresh daily.

3-hydroxybutyrate dehydrogenase Grade II (Boehringer Mannheim GmbH, West Germany. Cat. No. 127841).

Spectronics 20 spectrophotometer. (Bausch & Lomb, obtained through Laboratory & Scientific Equipment Co. (Pty) Ltd, Cape Town).

Procedure

Ten ml perchloric acid is pipetted in a plastic centrifuge tube standing in an ice tray. To this is added 4 ml of blood. The mixture is stirred with a glass rod, and kept in ice till it is centrifuged. The specimen is then centrifuged at 3000 g_n for 10 minutes, to remove the precipitated protein.

Exactly 10 ml of the supernatant solution is then pipetted into a clean plastic centrifuge tube. A drop of phenol is added, and enough 4.3 M KOH solution to neutralize the perchloric acid. The volume of KOH solution is noted.

After mixing well the specimen is stored overnight at -20°C to ensure maximal precipitation of $KC10_4$.

The next morning the specimen is thawed and centrifuged at 3000 g_n for 10 minutes.

1.0 ml of the supernatant is transferred to a glass cuvette, to which is added 3.7 ml Tris buffer, 0.1 ml hydrazine buffer and 0,2 ml NAD solution. The mixture is thoroughly stirred on a vortex mixer, and the absorbance is measured at 340 nm, using distilled water as reference. When the reading is stabilized, the absorbance is noted - A_0.

0.04 ml 3-hydroxybutyrate dehydrogenase is then added to the mixture which is again thoroughly stirred on a vortex mixer. It is then left to stand for 60 minute at room temperature. Readings of the absorbance are then taken every 10 minutes till the rise in absorbance becomes undetectable. This reading is noted - A_1. A blank, prepared from the perchloric acid solution without blood added to it,, and a standard prepared from a solution of DL-β-hydroxybutyrate in perchloric acid, is treated in the same way.

The concentration of 3-hydroxybutyrate in the cuvette is then calculated according to the formula:

$$\text{3-hydroxybutyrate } (\mu\text{mol/5ml}) = \frac{A \times 5.0}{6.22 \times 1.17}$$

where A is the difference in absorbance ($A_1 - A_0$) of the specimen minus the increase in the absorbance in the blank; 6.22 is the coefficient of extinction for NAD ($cm^2/\mu mole$); 1.17 is the light path in cm; and 5,0 in the assay volume (ml).

Dilution of specimens

The dilution is such that the amount of 3-hydroxybutyrate in the cuvette has to be multiplied by

$$\frac{10 + \text{volume of KOH used (ml)}}{\text{volume of sample in cuvette (ml)}} \times 350$$

to obtain the concentration in 1 litre blood.

If the concentration of ketone bodies in the blood is expected to be high, or if the change in absorbance in the cuvette exceeds 0.3 units, the neutralized supernatant solution is diluted with Tris buffer.

Reproducibility of the analyses

On determination of the 3-hydroxybutyrate concentration in the blood, a coefficient of variation of 7.3% was found on multiple analyses (up to 10 x) of 10 different blood samples, when the concentration in the blood exceed 0.2 mmol/1. At concentrations less than 0,1 mmol/1 the coefficient of variation was 17.1%.

Recovery Studies

A fresh standard solution of sodium DL-3-hydroxybutyrate in 0.7 M perchloric acid was prepared, for each set of analyses. The mean recovery of D-3-hydroxybutyrate was 78.7% (± SD 5.5). The assay showed a fall off in recovery with high concentrations of this substance in the cuvette.

The reason for the low recoveries is not known, but they were consistent, and the readings of the blood ketone body concentrations in the text are the unadjusted values

calculated directly from the changes in absorbance. There is therefore a possibility that all our readings are about 20 - 25% lower than the actual blood ketone body concentrations.

Stability of the ketone bodies

When the standard solutions were kept at -20°C for 3 weeks, there was no difference in the recovery of 3-hydroxybutyrate.

The blood samples were always analysed within 24 hours.

11. MEASUREMENT OF GLYCOGEN CONCENTRATIONS IN THE LIVER AND MUSCLE TISSUES:

The weighed liver or muscle sample was macerated with 1 ml KOH (40%) and added to a plastic tube. The tube was placed in a water bath of 100°C for 30 minutes, after which 4 ml of 95% ethanol was added to the solution and the tube was again placed into the water bath for 30 minutes to precipitate the glycogen. The solution was left overnight in the fridge (4°C) and then the supernatant removed after centrifuging (10 minutes at 5000 r.p.m.). One ml 2N HCl was added to the precipitate to hydrolyse the glycogen to glucose and the solution was placed in the water bath at 100°C for 3 hours. 0.2M Tris buffer was added to the solution to bring the pH to 7.5, 200µl of the resultant solution was mixed with 2.8 ml of the reagent mix (see below) and the absorption read at 340 nm (Beckman Spectrophotometer Model 35). The above procedure was carried out over three successive days.

Reagent Mixture	Per Cuvette
Tris buffer 0.2M pH 7.5	1.0 ml
ATP 20 mM	0.1 ml
$MgCl_2 6H_2O$ 1M	0.1 ml
NADP 1% (TPN)	0.1 ml
Distilled Water	1.5 ml
	2.8 ml

200µl of reagent mix was also added to 200µl of 1 mM glucose standard and 200µl of distilled water (used as the blank) and their absorptions at 340 nm recorded.

10µl of HK/G6PDH suspension (Boehringer Cat. No. 127825) was added to each cuvette and after standing for 30 minutes, the absorption at 340 nm was again read.

The glycogen content was calculated from the following formula:

$$\frac{OD_E - OD_B}{0.414} \times \frac{\text{volume in cuvette (= 3 ml)}}{\text{wet mass of sample (g)}}$$

where OD_E is the difference in optical density between the enzyme value and the baseline value ; and OD_B is the difference in optical density between the Enzyme Blank and the baseline value . 0.414 is the optical density of the standard solution.

This gives the glycogen content in mmol glucose equivalents kg^{-1} wet weight.

The recovery was calculated by

$$\frac{\text{Std reading} \times 100}{0.414}$$

The recover during the experiments was greater than 92%.

L11. MEASUREMENT OF MUSCLE MALATE DEHYDROGENASE (MDH) ACTIVITY:

The muscle sample was weighed and diluted 1 in 30 with a homogenating buffer consisting of:

0.1 M KPO_4 at pH 7.4 containing 0.1% albumin.

The muscle in ice cold buffer was homogenated for 10 seconds. (Ultra TURRAX-TP 18/10 Janke and Knukel - Germany B-12, Branson Sonic Power Company) and sonicated in ice for 15 seconds. (Sonifer Model B-12, Branson Sonic Power Company). It was then centrifuged for 1 minute at 4°C (2000 r.p.m.) and the supernatant fluid decanted off for measuring.

The supernatant was further diluted 1 in 10 with additional homogenizing buffer.

Next 0.1 ml of the muscle homogenate was added to a cuvette with the reagent mixture consisting of:

2.7 ml 0.1 M KPO_4 at pH 7.4

0.1 ml 0.006 M NADH in the cold 5 mM KPO_4 pH 7.4

0.1 ml 0.001 M cis-oxaloacetic acid in ice cold 5 mM KPO_4, pH 7.4

The mixture was agitated and read to extinction at 340 nm (Beckman Spectrophotometer - Model 35). The milli absorbance per minute was calculated from the slope of the curve.

The MDH activity was calculated according to the equation:

$$\frac{\text{m.A.U.}}{6.2} \cdot \frac{Vt \text{ dilution (1)}}{V\ s} = \text{U/mg}$$

Vt - total volume in cuvette

Vs - sample volume in cuvette

U - the amount of enzyme causing the oxidation of one micromole of NADH per minute at 25°C and pH 7.4.

TABLES OF RESULTS

in Trained and Untrained rats eating a Normal Diet.

	BEFORE EXERCISE	END OF EXERCISE	30 min. AFTER EXERCISE	60 min. AFTER EXERCISE	90 min. AFTER EXERCISE	120 min. AFTER EXERCISE
UNTRAINED CONTROLS (n = 6)	0.40 ± 0.01	+ 0.43 ± 0.02	+ 0.35 ± 0.02	+ 0.30 ± 0.02	+ 0.38 ± 0.06	+ 0.40 ± 0.01
UNTRAINED EXPERIMENTAL (n = 6)	0.48 ± 0.04	0.89 ± 0.05	* 1.24 ± 0.13	* 1.31 ± 0.04	* 1.01 ± 0.1	* 0.80 ± 0.05
TRAINED CONTROLS (n = 6)	0.44 ± 0.04	c 0.50 ± 0.03	c 0.40 ± 0.01	c 0.48 ± 0.06	c 0.47 ± 0.06	c 0.52 ± 0.01
TRAINED EXPERIMENTAL (n = 6)	0.54 ± 0.06	0.77 ± 0.02	0.69 ± 0.1	0.69 ± 0.02	0.62 ± 0.05	0.58 ± 0.03

Values are means ± S.E.M.

* P<0.05, Trained Experimental vs Untrained Experimental; +P<0.05, Untrained Controls vs Untrained Experimental;
c P<0.05, Trained Controls vs Trained Experimental

treadmill at 0.2 m/s) in Trained and Untrained rats eating a Normal Diet.

	BEFORE EXERCISE	END OF EXERCISE	30 min. AFTER EXERCISE	60 min. AFTER EXERCISE	90 min. AFTER EXERCISE	120 min. AFTER EXERCISE
UNTRAINED CONTROLS (n = 6)	316 ± 10	+ 321 ± 10	+ 313 ± 10	+ 314 ± 5	+ 293 ± 8	+ 306 ± 5
UNTRAINED EXPERIMENTAL (n = 6)	300 ± 8	166 ± 3	135 ± 8	85 ± 5	87 ± 3	167 ± 5
TRAINED CONTROLS (n = 6)	370 ± 7	+ 386 ± 8	+ 354 ± 5	+ 354 ± 8	+ 358 ± 7	+ 353 ± 2
TRAINED EXPERIMENTAL (n = 6)	* 413 ± 15	* 225 ± 8	* 244 ± 13	* 241 ± 1	* 245 ± 8	* 261 ± 4

Values are means ± S.E.M.

* $P<0.05$, Trained Experimental vs Untrained Experimental; +$P<0.05$, Untrained Controls vs Untrained Experimental;
c $P<0.05$, Trained Controls vs Trained Experimental

a level treadmill at 0.2 m/s) in Trained and Untrained rats eating a Normal Diet.

	BEFORE EXERCISE	END OF EXERCISE	30 min. AFTER EXERCISE	60 min. AFTER EXERCISE	90 min. AFTER EXERCISE	120 min. AFTER EXERCISE
UNTRAINED CONTROLS (n = 6)	43 ± 1	+ 50 ± 2	+ 44 ± 2	+ 45 ± 2	+ 40 ± 2	+ 42 ± 2
UNTRAINED EXPERIMENTAL (n = 6)	39 ± 1	13 ± 1	28 ± 1	23 ± 1	32 ± 1	34 ± 2
TRAINED CONTROLS (n = 6)	46 ± 2	c 46 ± 2	c 47 ± 2	c 55 ± 2	c 50 ± 2	c 49 ± 2
TRAINED EXPERIMENTAL (n = 6)	* 54 ± 1	* 20 ± 2	* 30 ± 1	* 31 ± 1	* 39 ± 1	* 39 ± 2

Values are means ± S.E.M.

* $P<0.05$, Trained Experimental vs Untrained Experimental; +$P<0.05$, Untrained Controls vs Untrained Experimental;
c $P<0.05$, Trained Controls vs Trained Experimental

in Trained and Untrained rats eating a Low Carbohydrate Diet.

	BEFORE EXERCISE	END OF EXERCISE	30 min. AFTER EXERCISE	60 min. AFTER EXERCISE	90 min. AFTER EXERCISE	120 min. AFTER EXERCISE
UNTRAINED CONTROLS (n = 6)	0.40 ± 0.06	+ 0.34 ± 0.07	+ 0.40 ± 0.08	+ 0.44 ± 0.08	+ 0.39 ± 0.07	+ 0.46 ± 0.07
UNTRAINED EXPERIMENTAL (n = 6)	0.49 ± 0.06	* 0.72 ± 0.14	* 0.92 ± 0.2	* 0.95 ± 0.2	* 1.10 ± 0.2	* 1.14 ± 0.25
TRAINED CONTROLS (n = 6)	0.56 ± 0.08	+ 0.48 ± 0.08	+ 0.35 ± 0.07	+ 0.38 ± 0.07	+ 0.46 ± 0.07	+ 0.40 ± 0.08
TRAINED EXPERIMENTAL (n = 6)	0.62 ± 0.11	0.54 ± 0.09	0.70 ± 0.15	0.80 ± 0.15	0.70 ± 0.13	0.78 ± 0.17

Values are means ± S.E.M.

* $P<0.05$, Trained Experimental vs Untrained Experimental; + $P<0.05$, Untrained Controls vs Untrained Experimental;
c $P<0.05$, Trained Controls vs Trained Experimental

Table 5: Muscle Glycogen concentration (μmol glucosyl units/g wet mass) before and after exercise (60 min. run on a level treadmill at 0.2 m/s) in Trained and Untrained rats eating a Low Carbohydrate Diet.

	BEFORE EXERCISE	END OF EXERCISE	30 min. AFTER EXERCISE	60 min. AFTER EXERCISE	90 min. AFTER EXERCISE	120 min. AFTER EXERCISE
UNTRAINED CONTROLS (n = 6)	27 ± 1	+ 30 ± 2	+ 29 ± 1	+ 26 ± 1	+ 22 ± 2	+ 32 ± 2
UNTRAINED EXPERIMENTAL (n = 6)	28 ± 1	10 ± 1	10 ± 1	10 ± 1	17 ± 1	15 ± 2
TRAINED CONTROLS (n = 6)	40 ± 1	c 36 ± 2	c 35 ± 4	c 32 ± 1	c 39 ± 1	c 39 ± 2
TRAINED EXPERIMENTAL (n = 6)	* 43 ± 2	* 28 ± 1	* 20 ± 1	* 19 ± 2	* 24 ± 2	* 29 ± 2

Values are means ± S.E.M.

* $P<0.05$, Trained Experimental vs Untrained Experimental; +$P<0.05$, Untrained Controls vs Untrained Experimental;
c $P<0.05$, Trained Controls vs Trained Experimental

a level treadmill at 0.2 m/s) in Trained and Untrained rats eating a Low Carbohydrate Diet.

	BEFORE EXERCISE	END OF EXERCISE	30 min. AFTER EXERCISE	60 min. AFTER EXERCISE	90 min. AFTER EXERCISE	120 min. AFTER EXERCISE
UNTRAINED CONTROLS (n = 6)	174 ± 14	+ 141 ± 5	+ 170 ± 9	+ 137 ± 26	+ 146 ± 16	+ 184 ± 18
UNTRAINED EXPERIMENTAL (n = 6)	150 ± 7	27 ± 1	31 ± 3	39 ± 3	30 ± 2	17 ± 2
TRAINED CONTROLS (n = 6)	219 ± 8	c 218 ± 18	c 195 ± 35	c 198 ± 22	c 195 ± 8	c 219 ± 15
TRAINED EXPERIMENTAL (n = 6)	* 236 ± 10	* 72 ± 12	* 61 ± 9	* 57 ± 7	* 116 ± 5	* 142 ± 15

Values are means ± S.E.M.

* P<0.05, Trained Experimental vs Untrained Experimental; +P<0.05, Untrained Controls vs Untrained Experimental;
c P<0.05, Trained Controls vs Trained Experimental

in Trained and Untrained rats eating a High Carbohydrate Diet.

	BEFORE EXERCISE	END OF EXERCISE	30 min. AFTER EXERCISE	60 min. AFTER EXERCISE	90 min. AFTER EXERCISE	120 min. AFTER EXERCISE
UNTRAINED CONTROLS (n = 6)	0.03 ± 0.01	+ 0.04 ± 0.01	+ 0.04 ± 0.01	+ 0.03 ± 0.01	+ 0.05 ± 0.02	+ 0.03 ± 0.01
UNTRAINED EXPERIMENTAL (n = 6)	0.08 ± 0.02	* 1.2 ± 0.18	* 1.35 ± 0.4	* 1.4 ± 0.4	* 1.3 ± 0.3	* 0.8 ± 0.23
TRAINED CONTROLS (n = 6)	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.02 ± 0.01	0.04 ± 0.02
TRAINED EXPERIMENTAL (n = 6)	0.04 ± 0.01	0.05 ± 0.02	0.07 ± 0.02	0.1 ± 0.02	0.05 ± 0.02	0.05 ± 0.01

Values are means ± S.E.M.

* P<0.05, Trained Experimental vs Untrained Experimental; +P<0.05, Untrained Controls vs Untrained Experimental;
c P<0.05, Trained Controls vs Trained Experimental

treadmill at 0.2 m/s) in Trained and Untrained rats eating a High Carbohydrate Diet.

	BEFORE EXERCISE	END OF EXERCISE	30 min. AFTER EXERCISE	60 min. AFTER EXERCISE	90 min. AFTER EXERCISE	120 min. AFTER EXERCISE
UNTRAINED CONTROLS (n = 6	455 ± 12	+ 462 ± 10	+ 428 ± 36	+ 447 ± 20	+ 429 ± 27	+ 433 ± 39
UNTRAINED EXPERIMENTAL (n = 6)	* 450 ± 8	* 270 ± 14	* 266 ± 12	* 288 ± 23	* 347 ± 36	* 367 ± 10
TRAINED CONTROLS (n = 6)	577 ± 19	c 602 ± 23	c 554 ± 17	c 541 ± 30	c 593 ± 38	c 530 ± 24
TRAINED EXPERIMENTAL (n = 6)	546 ± 10	348 ± 19	307 ± 18	313 ± 19	350 ± 10	446 ± 28

Values are means ± S.E.M.

* P<0.05, Trained Experimental vs Untrained Experimental; +P<0.05, Untrained Controls vs Untrained Experimental;
c P<0.05, Trained Controls vs Trained Experimental

Table 9. Muscle glycogen concentration (μmol glucosyl units/g wet mass) before and after exercise (60 min. run on a level treadmill at 0.2 m/s) in Trained and Untrained rats eating a High Carbohydrate Diet.

	BEFORE EXERCISE	END OF EXERCISE	30 min. AFTER EXERCISE	60 min. AFTER EXERCISE	90 min. AFTER EXERCISE	120 min. AFTER EXERCISE
UNTRAINED CONTROLS (n = 6)	61 ± 2	+ 63 ± 2	+ 65 ± 8	+ 66 ± 4	+ 62 ± 3	+ 70 ± 4
UNTRAINED EXPERIMENTAL (n = 6)	* 62 ± 3	* 44 ± 4	* 42 ± 4	* 43 ± 4	* 49 ± 4	* 59 ± 4
TRAINED CONTROLS (n = 6)	77 ± 6	c 82 ± 6	c 80 ± 5	c 90 ± 7	c 88 ± 6	c 77 ± 7
TRAINED EXPERIMENTAL (n = 6)	83 ± 5	48 ± 7	55 ± 5	52 ± 6	62 ± 4	68 ± 3

Values are means ± S.E.M.

* P<0.05, Trained Experimental vs Untrained Experimental; +P<0.05, Untrained Controls vs Untrained Experimental;
c P<0.05, Trained Controls vs Trained Experimental

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