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POST-EXERCISE KETOSIS

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SUMMARY

1. The effect of exercise on blood ketone body concentrations was studied in trained athletes and in sedentary subjects pedalling a bicycle ergometer.

2. Although the untrained subjects had higher heart rates and blood lactate concentrations at the same work load as the athletes, neither group developed ketonaemia even after intense or prolonged exercise.

3. Older subjects developed post-exercise ketonaemia, reaching maximum about 3 hr after exercise.

4. A high-carbohydrate diet before the exercise could prevent the onset of post-exercise ketonaemia and a low-carbohydrate diet enhanced it. The highest post-exercise blood ketone levels were recorded in marathon runners after a 'glycogen-stripping' regimen.

5. Concentrations of free fatty acids, glucose, growth hormone and insulin in blood after exercise followed different patterns from that of ketones.

6. Post-exercise ketosis, when it occurs in untrained subjects, may be due to a lower carbohydrate intake than that of athletes.

INTRODUCTION

Post-exercise ketosis was first described by Forssner (1909), who noted that his urinary excretion of ketone bodies always increased after a brisk walk of 4 km in 36 min; the ketonuria persisted for several days. Preti (1911) observed the same phenomenon in a patient with a 'minor stomach complaint' after climbing up and down stairs until exhausted. Courtice & Douglas (1936) found that, after walking 16 km at 7 km/hr, the urinary excretion of ketone bodies began to rise only on completion of the exercise and continued to rise for the 9 hr during which observations were continued. Johnson, Walton, Krebs & Williamson (1969) observed a marked ketonaemia during and after 90 min running in untrained subjects but not in trained athletes; the blood ketone body levels in the untrained subjects were still rising 90 min after the exercise, when observations were discontinued.

The purpose of this study was to discover whether the apparent immunity of trained athletes to post-exercise ketosis could be overcome by exercise of sufficient intensity and duration. Trained and untrained subjects were tested and blood ketone body and lactate concentrations were determined at intervals for at least 9 hr after the exercise. Three subjects were tested after carbohydrate restriction and their

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blood levels of glucose, free fatty acids, and hormones, as well as of ketone bodies, were measured.

METHODS

Seven groups of healthy men (students and staff of the University of Cape Town) participated in the investigation. Their ages, physical characteristics and the athletes' training are summarized in Table 1.

Except in the case of the two marathon runners, who preferred to run on a treadmill, exercise was performed on an electrically braked Lanooy bicycle ergometer. Heart rates were recorded electrocardiographically at 15 min intervals. All exercises were performed at the same time of day (early morning) to avoid the influence of circadian rhythms, and on the same day of successive weeks to minimize training effects and variations due to different daily patterns of activity. Ambient temperatures were in the range 18–21°C.

Some subjects performed the tests after fasting since the previous evening, others after a standard breakfast (see below). All fasted during the period of observation after the exercise but consumed sugar-free beverages *ad lib*. Each set of experiments included a control day in which the same dietary regimen was followed but no exercise was performed. In successive investigations the effects of intensity of exercise, duration of exercise, age of subject, and diet were studied.

TABLE 1. Ages, physical characteristics and activity of subjects

	Group	Age (yr) range	Ht. (cm) (mean ± S.D.)	Wt. (kg) (mean ± S.D.)	Training
Part 1	Sedentary $n=7$	19	177 ± 4·4	69·0 ± 5·2	—
	Trained $n=7$	18–23	181 ± 4·7	71·8 ± 8·1	Running 5 km or more at least 4 days per week
Part 2	Sedentary $n=6$	18–19	176 ± 5·7	66·0 ± 16·7	—
	Trained $n=3$	18–20	183 ± 4·2	71·7 ± 5·8	Running 5 km or more at least 4 days per week
Part 3	Older subjects $n=6$	30–51	181 ± 7·3	74·9 ± 13·1	—
Part 4	Older subject $n=1$	37	178	65·1	—
	Marathon runners $n=2$	24 29	176 187	67·6 77·2	Running at least 16 km daily

Part 1. Intensity of exercise

The influence of varying intensities of exercise on blood ketone body levels was studied in seven sedentary subjects and seven trained athletes. There were 5 test days and a control day, during all of which the subjects fasted from the previous evening. Exercise was performed at 7.30 a.m. for 15 min at constant work load. The work load was different (75 W, 100 W, 125 W or 150 W, selected in random order) on each of four of the test days. The work load for the fifth test day was determined for each subject by extrapolation from a plot of his heart rate during the last minute of exercise against work load, to predict the maximum load he would be able to endure for 15 min (i.e. the load expected to produce a heart rate of 200 beats per min).

Blood was taken from an arm vein at 7.30 a.m., 7.45 a.m., 9.30 a.m., 1.00 p.m. and 5.00 p.m. for determination of the lactate, acetoacetate and 3-hydroxybutyrate concentrations.

Part 2. Duration of exercise

The influence of duration of exercise on blood ketone body levels was investigated in six sedentary and three trained subjects. There were 3 test days and a control day, on all of which

the subjects fasted from the previous evening. Exercise was performed for 30, 60 or 90 min (in random order), always ending at 8.00 a.m. The untrained subjects exercised at a work load of 75 W on each occasion and the trained subjects at 100 W. These loads were chosen on the basis of the results of Part 1 (Fig. 1) so that both groups would be exercising at approximately the same heart rates.

Blood was taken before exercise, at 8.00 a.m., 9.30 a.m., 1.00 p.m. and 5.00 p.m. for analysis of lactate, acetoacetate and 3-hydroxybutyrate concentrations.

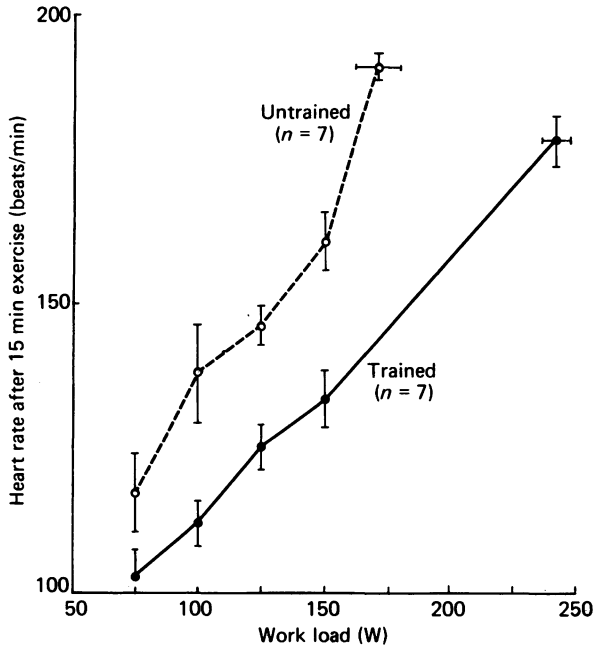

Fig. 1. Heart rate (mean ± S.E. of mean) of trained and untrained subjects during the last minute of 15 min cycling on a bicycle ergometer at different loads. Maximal work loads were determined for each subject by extrapolation of the plot of his heart rate during submaximal exercise, against work load, to predict the load which was expected to produce a heart rate of 200 beats/min after 15 min.

Part 3. Effect of age

Six older individuals were studied to investigate whether age was a factor in the development of post-exercise ketosis. Exercise was performed at 75 W for 90 min (7.30 a.m. to 9.00 a.m.). Since one of the subjects in a pilot study had developed symptoms suggestive of a hypoglycaemic attack 1–2 hr after exercise, all subjects ate a standard breakfast (two slices of bread with butter, one egg, some cheese and a sugarless beverage) at 6.30 a.m. before exercise. Courtice & Douglas (1936) found that such a breakfast did not influence the development of post-exercise ketosis.

Blood was taken by venipuncture at 7.30 a.m., 9.00 a.m., 10.00 a.m., 11.00 a.m., 12 noon, 1.00 p.m. and 5.00 p.m. for analysis of lactate, acetoacetate and 3-hydroxybutyrate concentrations.

Part 4. Effect of diet

The 37-year-old subject from Part 3 and two well-trained marathon runners (whose recent times for the 42 km race were 2 hr 54 min and 2hr 35 min, respectively) were studied to ascertain the influence of diet on post-exercise ketosis. The 37-year-old sedentary subject exercised at 100 W for 2 hr (from 7.00 a.m. to 9.00 a.m.) on 6 test days. Prior to the first test he had been

on his usual diet, which included about 250 g carbohydrate per day. For 2 days prior to the second and third tests he had added sugar (sucrose) to his tea and coffee, amounting to about 80 g per day. The fourth and fifth tests were each preceded by 2 days of carbohydrate restriction (total carbohydrate intake of about 80 g per day). The sixth test was carried out after returning to his usual diet.

The two marathon runners were studied on 2 test days and on a control day on which they had a standard breakfast at 6.30 a.m. as in Part 3. Exercise consisted of running at 12–13 km per hour for 2 hr (from 7.00 a.m. to 9.00 a.m.) on a level treadmill. Prior to the first test they were on their normal diet (approximately 500 g carbohydrate per day) but on the 2 days preceding the second test they ate a protein–fat diet consisting of meat, fish, eggs and cheese, while continuing their training. This so-called 'glycogen stripping' regimen has been suggested by Ahlborg, Bergström, Brohult, Ekelund, Hultman & Maschio (1967) as the most effective way of depleting the muscle glycogen stores.

Blood was taken from all three subjects at hourly intervals from 7.00 a.m. to 2.00 p.m. and again at 4.30 p.m. for determination of acetoacetate, 3-hydroxybutyrate, glucose, free fatty acids, immunoreactive insulin, and human growth hormone concentrations. Blood lactate was not measured.

Analytical methods

A portion of the venous blood was deproteinized in ice-cold 0·6 *N*-perchloric acid and the precipitated protein removed by centrifugation. The concentration of L-lactate in the supernatant solution was determined with the Boehringer Mannheim Biochemica Test Combination (Cat. No. 15972). The concentrations of D-3-hydroxybutyrate and acetoacetate were determined by the method of Williamson, Mellanby & Krebs (1962) using 3-hydroxybutyric dehydrogenase obtained from the Sigma Chemical Company (Cat. No. H. 6126).

The remaining venous blood was allowed to clot, and serum glucose concentrations were measured by the Boehringer Mannheim enzymatic colorimeter test, GOD-PAP (Cat. No. 166391). Serum free fatty acid levels were measured by the method of Dole & Meinertz (1960).

Serum immunoreactive insulin concentrations were measured with the Sorin Biomedica insulin radioimmunoassay kit (Ref. INSIK-1), and serum human growth hormone concentrations with the Sorin Biomedica HGH K radioimmunoassay kit (^{125}I). The data were analysed on a Hewlett-Packard 9815A Computer using Student's *t* test for paired (intragroup) and unpaired (intergroup) comparisons.

RESULTS

Part 1. Intensity of exercise

Mean ketone body concentrations in blood (acetoacetate + 3-hydroxybutyrate) showed no significant difference between trained and untrained subjects at rest or after different degrees of exercise and there was no significant difference between test days and control days (Table 2). The values were all within the range of normal daily variation (Wildenhoff, 1972).

Heart rates were higher in the untrained than in the trained subjects both at submaximal and at maximal work loads (Fig. 1). The mean maximum heart rate was 193 (± S.D. 6) beats per min for the untrained and 179 (± S.D. 10) for the trained. Blood lactate levels after exercise were significantly higher in the untrained subjects than in the trained at some submaximal but not at maximal heart rates (Fig. 2).

Part 2. Duration of exercise

Mean post-exercise blood ketone body concentrations did not, in trained or in untrained subjects, differ significantly from control day values and there was no significant difference between the two groups (Table 3).

The mean heart rate of untrained subjects on a work load of 75 W was 129 (± S.D.

15) beats per min and that of the trained group on 100 W was 109 (± S.D. 8). Blood lactate concentrations after exercise were not significantly different from resting levels in either group (Table 4). The difference between resting blood lactate levels of trained and of untrained subjects was statistically significant ($P < 0.05$) but the differences after exercise were not.

TABLE 2. Fasting total blood ketone body concentrations (m-mole/l.) in seven untrained (Roman type) and seven trained (*italics*) subjects after 15 min of exercise between 7.30 a.m. and 7.45 a.m. (mean ± S.D.).

Exercise load	7.30 a.m.	7.45 a.m.	9.30 a.m.	1.00 p.m.	5.00 p.m.
Control	0·06 ± 0·04	0·04 ± 0·01	0·03 ± 0·01	0·07 ± 0·05	0·12 ± 0·06
	0·05 ± 0·04	*0·04 ± 0·01*	*0·04 ± 0·02*	*0·05 ± 0·03*	*0·07 ± 0·04*
75 W	0·04 ± 0·02	0·03 ± 0·02	0·03 ± 0·02	0·04 ± 0·04	0·21 ± 0·14
	0·06 ± 0·05	*0·06 ± 0·04*	*0·04 ± 0·03*	*0·06 ± 0·04*	*0·09 ± 0·07*
100 W	0·05 ± 0·03	0·03 ± 0·01	0·04 ± 0·03	0·11 ± 0·11	0·22 ± 0·16
	0·04 ± 0·02	*0·03 ± 0·02*	*0·03 ± 0·02*	*0·07 ± 0·06*	*0·14 ± 0·09*
125 W	0·06 ± 0·06	0·05 ± 0·02	0·05 ± 0·04	0·07 ± 0·03	0·22 ± 0·12
	0·07 ± 0·07	*0·05 ± 0·03*	*0·05 ± 0·03*	*0·08 ± 0·07*	*0·15 ± 0·11*
150 W	0·04 ± 0·02	0·04 ± 0·02	0·04 ± 0·02	0·10 ± 0·06	0·11 ± 0·08
	0·07 ± 0·05	*0·05 ± 0·02*	*0·05 ± 0·04*	*0·10 ± 0·08*	*0·11 ± 0·12*
Maximal load	0·10 ± 0·08	0·06 ± 0·03	0·09 ± 0·07	0·12 ± 0·08	0·24 ± 0·21
	0·05 ± 0·02	*0·06 ± 0·01*	*0·04 ± 0·01*	*0·09 ± 0·05*	*0·07 ± 0·04*

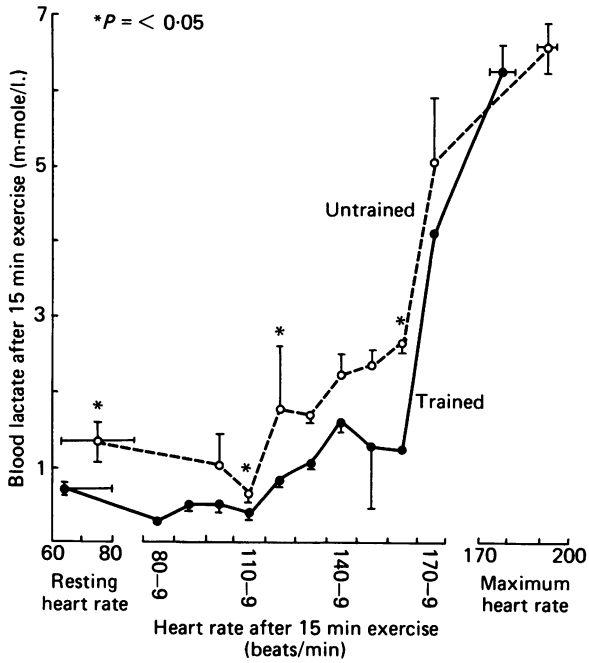

Fig. 2. Blood lactate levels (mean ± S.E. of mean) of trained and untrained subjects at rest and after submaximal and maximal exercise.

TABLE 3. Fasting total blood ketone body concentrations (m-moles/l.) in six untrained (Roman type) and three trained (*italics*) subjects after exercise of varying duration, ending at 8.00 a.m. (mean ± S.D.)

Duration of exercise	Before exercise	8.00 a.m.	9.30 a.m.	1.00 p.m.	5.00 p.m.
Control	0·05 ± 0·02	0·08 ± 0·02	0·05 ± 0·01	0·08 ± 0·05	0·08 ± 0·02
	0·05 ± 0·02	*0·04 ± 0·02*	*0·03 ± 0·02*	*0·07 ± 0·06*	*0·04 ± 0·02*
30 min	0·05 ± 0·03	0·08 ± 0·05	0·07 ± 0·04	0·12 ± 0·06	0·08 ± 0·06
	0·05 ± 0·03	*0·05 ± 0·03*	*0·03 ± 0·01*	*0·08 ± 0·04*	*0·08 ± 0·03*
60 min	0·05 ± 0·02	0·07 ± 0·05	0·10 ± 0·06	0·15 ± 0·18	0·14 ± 0·13
	0·04 ± 0·01	*0·04 ± 0·01*	*0·04 ± 0·01*	*0·05 ± 0·02*	*0·05 ± 0·02*
90 min	0·05 ± 0·01	0·09 ± 0·06	0·11 ± 0·11	0·10 ± 0·10	0·13 ± 0·17
	0·05 ± 0·03	*0·08 ± 0·05*	*0·18 ± 0·20*	*0·15 ± 0·16*	*0·15 ± 0·19*

TABLE 4. Blood lactate levels (mean ± S.D., m-mole/l.) before and after exercise

		After exercise		
Subjects	Before exercise	lasting 30 min	lasting 60 min	lasting 90 min
Untrained $n = 6$	1·28 ± 1·12	1·64 ± 1·20	1·24 ± 1·20	0·90 ± 0·44
Trained $n = 3$	0·81 ± 0·59	0·89 ± 0·46	0·71 ± 0·40	0·68 ± 0·36
P	< 0·05	n.s.	n.s.	n.s.

Part 3. Effect of age

There was wide variation in the post-exercise blood ketone body concentrations of the older subjects (Fig. 3). Three of the subjects, aged 37, 38 and 51 years, attained levels well above those found after the same exercise in younger subjects. The mean heart rate during exercise of these subjects was 127 (± S.D. 11) beats per min, and that of the remaining subjects, aged 30, 38 and 41 years, was 125 (± S.D. 14) beats per min. There was no significant difference between the heart rate response to exercise of this group and that of the young sedentary subjects of Part 2. Post-exercise ketonaemia reached a peak about 3 hr after exercise, followed by a fall which occurred in spite of continued fasting.

Blood lactate levels in these subjects were 0·64 (± S.D. 0·26) m-mole/l. at rest and 1·16 (± S.D. 0·37) m-mole/l. after 1½ hr exercise. This difference is statistically significant ($P < 0·005$).

Part 4. Effect of diet

Ketone bodies

On a normal diet post-exercise ketone body concentrations in the 37-year-old subject rose to peak values between noon and 1.00 p.m., as had been the case in Part 3. The actual peak ketone body concentrations attained (1·26 and 0·41 m-mole/l. on the first and sixth test days, respectively) were lower than after the shorter and

less intense exercise of Part 3 (1·72 m-mole/l.), suggesting the possibility that habituation had influenced the results. A biphasic response was seen in the post-exercise ketone body concentrations after carbohydrate restriction, with an early peak at about 1–2 hr after exercise, followed by a trough at 1.00 p.m., and a second more sustained rise to 2·18 and 1·64 m-mole/l. at 4.30 p.m. on the first and second carbohydrate restriction test days, respectively. After a high carbohydrate diet, however, blood ketone body concentrations, in this subject, were indistinguishable from control day values (Fig. 4).

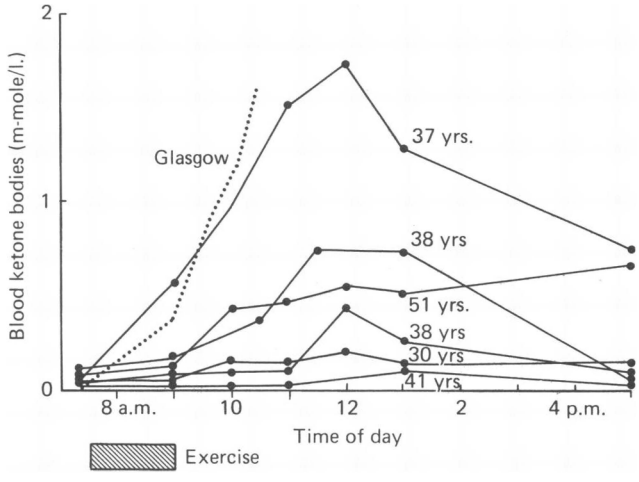

Fig. 3. Fasting blood ketone body levels in six older subjects after cycling for 90 min at 75 W. Mean blood ketone body levels of eighteen untrained subjects (aged 20–23 years) who ran for 90 min on an outdoor track in Glasgow (Johnson *et al.* 1969) are indicated for comparison. Control day ketone body levels, which were similar to those in Tables 2 and 3, have been omitted for the sake of clarity.

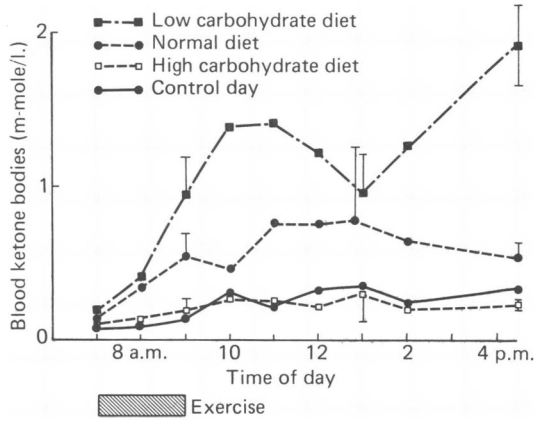

Fig. 4. Mean fasting blood ketone body levels in the 37-year-old subject from Fig. 3, after 120 min cycling at 100 W, following various dietary regimens on the 2 days preceding the exercise. Each test was done in duplicate and the range of the readings is indicated at 9 a.m., 1 p.m. and 4.30 p.m.

Post-exercise ketone body concentrations after a normal diet in the marathon runners did not differ from control day levels. 'Glycogen-stripping' produced a biphasic post-exercise ketone body response similar to that observed in the sedentary subject after carbohydrate restriction. These post-exercise ketone body concentrations were, however, considerably higher than any of the others observed, reaching 3·88 m-mole/l. in one of the subjects (Fig. 5).

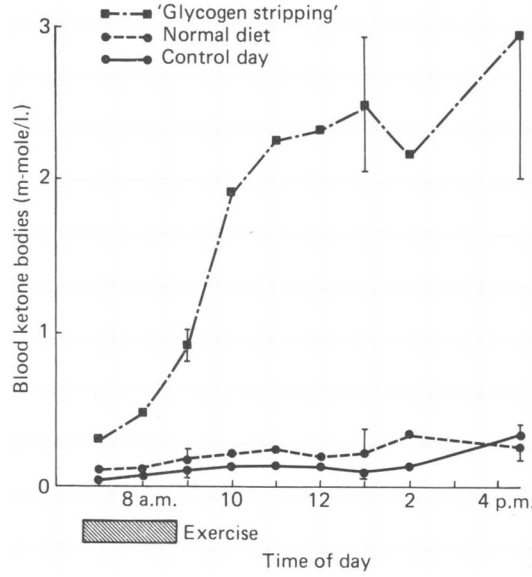

Fig. 5. Mean fasting blood ketone body levels in two highly trained marathon runners after running at 12–13 km/hr for 120 min, following a normal diet and 'glycogen stripping'. The 'glycogen-stripping' regimen consisted of eating a protein–fat diet for 2 days while continuing normal training. The range of the readings ($n = 2$) is indicated at 9 a.m., 1 p.m. and 4.30 p.m.

Free fatty acids

Serum free fatty acid concentrations rose to a peak during exercise in all three subjects and fell, 1–2 hr after exercise, to a plateau which lasted as long as the period of observation (Figs. 6 and 7). The concentrations at the end of the exercise were related to the previous carbohydrate intake but not to the level of training.

Glucose

In the sedentary subject blood glucose levels were apparently unaffected by diet or by exercise (Fig. 6). In the marathon runners there was a marked increase in blood glucose during exercise (Fig. 7). After glycogen stripping this was followed by a fall before the end of the exercise, which continued during the recovery period to reach levels significantly lower than on control days ($P < 0{\cdot}001$) at 4.30 p.m.

Insulin

Immunoreactive insulin concentrations in serum fell during exercise in all three subjects and remained lower than on control days for some hours after exercise (Figs. 6 and 7).

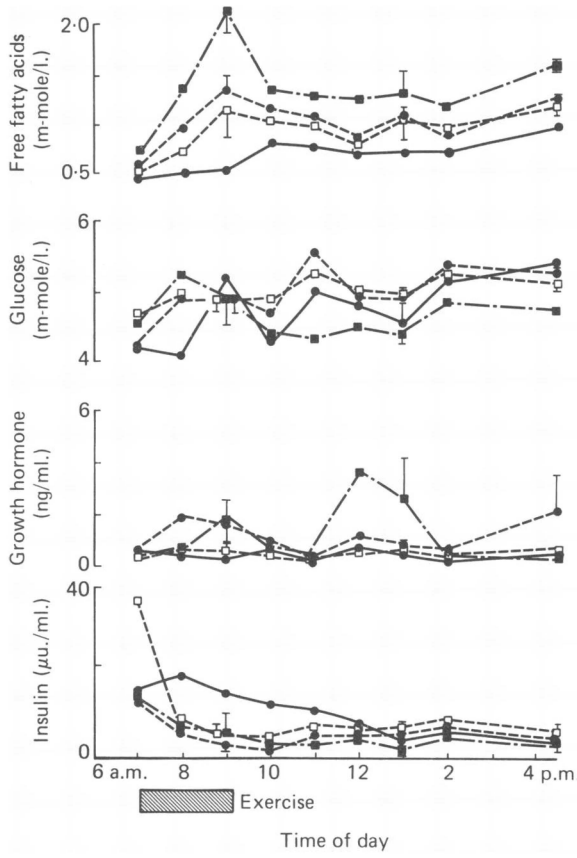

Fig. 6. Mean fasting serum free fatty acids, glucose, growth hormone and immunoreactive insulin levels in the 37-year-old subject from Fig. 4 after 120 min of cycling at 100 W. Experimental conditions and symbols are the same as in Fig. 4.

Growth hormone

The concentrations of human growth hormone in serum rose significantly ($P < 0{\cdot}025$) in all three subjects, except in the 37-year-old subject after a high-carbohydrate diet (Fig. 6). The highest levels occurred at the end of exercise following glycogen stripping in the marathon runners (Fig. 7). Pre-exercise levels were regained within an hour of finishing the exercise; thereafter the pattern followed that of the control day, usually with a peak between noon and 2 p.m.

DISCUSSION

Our findings that young untrained subjects in Cape Town did not develop ketonaemia, even after maximal exercise, contrasts with studies in Glasgow (Johnson *et al.*, 1969; Rennie, Jennett & Johnson, 1974), in which untrained subjects showed marked post-exercise ketonaemia, though with wide individual variation. The difference is unlikely to be due to differences in physical fitness between the national groups since the fitness index of young men in Cape Town and in Glasgow (as estimated by the

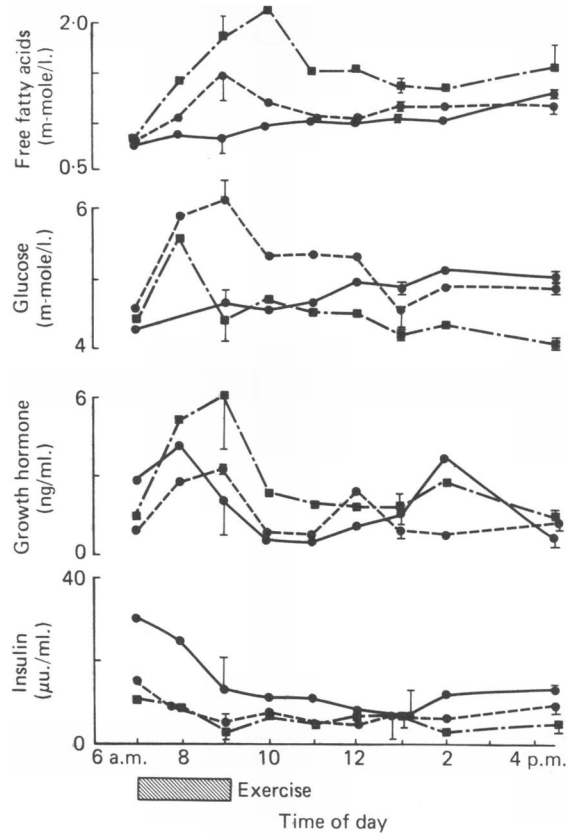

Fig. 7. Mean fasting serum free fatty acids, glucose, growth hormone and immunoreactive insulin levels in two marathon runners after running at 12–13 km/hr for 120 min. Experimental conditions and symbols are the same as in Fig. 5.

Harvard step test) is approximately the same (McGuinness & Sloan, 1971) and the unfitness of the untrained South Africans is shown by their high post-exercise heart rates and blood lactate concentrations. Post-exercise ketonaemia occurred in some of the older South African subjects but the response was very variable.

The highest levels of post-exercise ketonaemia were found in the two highly trained marathon runners after dietary carbohydrate restriction (Fig. 5). This, together with the results of the dietary manipulations in the 37-year-old sedentary subject, suggests that the carbohydrate status of the body is a critical factor in the development of post-exercise ketonaemia. The addition of 60–90 g sucrose (approximately 1000–1500 kJ dietary energy) to the daily food intake of the 37-year-old subject abolished the post-exercise ketonaemia which he developed on his usual diet; whereas a low-carbohydrate diet caused a post-exercise ketonaemia comparable to that reported by Johnson *et al.* (1969) (Fig. 4).

The differences in post-exercise blood ketone concentrations of athletes and non-athletes may be due as much to diet as to the metabolic effects of training. Forssner (1909), Preti (1911) and Courtice & Douglas (1936) all emphasized the role of diet in

the development of post-exercise ketosis. Forssner had been on a low-carbohydrate diet (55–60 g carbohydrate per day) for 14 days before commencing his exercise experiments. Preti's subject was on a 'high protein diet', and Courtice & Douglas found that Courtice (aged 24 years) developed ketonuria after exercise only if he had restricted his carbohydrate intake on the previous day. In other reports of post-exercise ketosis the subjects had also been subjected to some form of dietary restriction before exercising (Passmore & Johnson, 1958; Boninsega, Federspil & De Palo, 1974; Balasse, 1978). Thus it would appear that neither the intensity nor the duration of the exercise, nor the level of training are as important as the diet (and presumably the body's store of carbohydrate) in determining the blood ketone body levels after exercise.

A further finding in this investigation was, that when post-exercise ketonaemia did occur, the variations in blood ketone body concentrations were both quantitatively and qualitatively different from any of the other parameters studied. Although serum free fatty acid levels rose during exercise, reaching peak values on cessation of the work, or shortly thereafter, as described by other investigators (Johnson *et al.* 1969; Rennie & Johnson, 1974; Bloom, Johnson, Park, Rennie & Sulaiman, 1976), they had returned to pre-exercise levels when the ketone bodies were at their highest. Serum glucose concentrations also were unrelated to the behaviour of the ketone bodies. The blood hormones measured here, and those reported by others (Rennie & Johnson, 1974; Bloom *et al.* 1976) were similarly unrelated to the presence or degree of post-exercise ketonaemia. Thus it is clear that the blood ketone body concentration is not a simple correlate of the free fatty acid, glucose, lactate, insulin or human growth hormone levels in the blood, but must be regulated by an independent mechanism.

It has been shown that there is decreased utilization of ketone bodies after exercise (Johnson & Walton, 1972; Standl, Janka, Dexel & Kolb, 1976), which may be partly or wholly responsible for post-exercise ketonaemia when it occurs. It is not clear whether this diminished utilization of ketones is incidental to another regulatory mechanism (e.g. uptake of glucose by glycogen-depleted muscles) or is part of an integrated mechanism to maintain ketone body homeostasis, but it does suggest that the primary function of post-exercise ketonaemia is not to provide substitute fuel for tissues starved of carbohydrate.

Newsholme (1976) suggests that ketone bodies are important regulators of fuel-homeostasis, having antilipolytic, antiproteolytic and antiglycolytic actions during fuel crises involving carbohydrate metabolism (e.g. starvation, diabetes mellitus). Exercise may result in such a crisis if the glycogen stores in the liver or in the muscles are significantly depleted by the work undertaken. According to Newsholme's hypothesis the behaviour of the ketone bodies will then depend on the severity of the crisis and on whether the crisis can be resolved by simple redistribution of the body fuel stores, or requires a new intake of fuel by mouth. The monophasic post-exercise ketonaemia seen in Part 3 of this investigation would, in this view, be compatible with a local glycogen shortage, possibly in the liver, which could be corrected within hours by gluconeogenesis. The more prolonged, biphasic response seen after carbohydrate restriction in the subjects of Part 4 would indicate a more general depletion of body glycogen stores, which would not be corrected by redistribution of depot fuels alone.

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