Eur J Appl Physiol (1988) 57:531—539

European Journal of
Applied
Physiology
and Occupational Physiology

The effects of diet on muscle pH and metabolism during high intensity exercise

P. L. Greenhaff, M. Gleeson, and R. J. Maughan

Department of Environmental and Occupational Medicine, University Medical School, Aberdeen AB9 2ZD, Scotland

Summary. Five healthy male subjects exercised for 3 min at a workload equivalent to 100% $\dot{V}_{O_{2max}}$ on two separate occasions. Each exercise test was performed on an electrically braked cycle ergometer after a four-day period of dietary manipulation. During each of these periods subjects consumed either a low carbohydrate ($3 \pm 0\%$, mean $\pm$SD), high fat ($73 \pm 2\%$), high protein ($24 \pm 3\%$) diet (FP) or a high carbohydrate ($82 \pm 1\%$), low fat ($8 \pm 1\%$), low protein ($10 \pm 1\%$) diet (CHO). The diets were isoenergetic and were assigned in a randomised manner. Muscle biopsy samples (Vastus lateralis) were taken at rest prior to dietary manipulation, immediately prior to exercise and immediately post-exercise for measurement of pH, glycogen, glucose 6-phosphate, fructose 1,6-diphosphate, triose phosphates, lactate and glutamine content. Blood acid-base status and selected metabolites were measured in arterialised venous samples at rest prior to dietary manipulation, immediately prior to exercise and at pre-determined intervals during the post-exercise period. There was no differences between the two treatments in blood acid-base status at rest prior to dietary manipulation; immediately prior to exercise plasma pH ($p < 0.01$), blood P_{CO_2} ($p < 0.01$), plasma bicarbonate ($p < 0.001$) and blood base-excess ($p < 0.001$) values were all lower on the FP treatment. There were no major differences in blood acid-base variables between the two diets during the post-exercise period. Compared with the CHO diet, the FP diet resulted in plasma alanine ($p < 0.05$), blood lactate ($p < 0.05$), and plasma glutamine ($p < 0.01$) levels being lower immediately prior to exercise; plasma free fatty acids (FFA; $p < 0.05$), glycerol ($p < 0.01$), urea ($p < 0.001$) and blood 3-hydroxybutyrate (3-OHB; $p < 0.01$) levels were all higher. After the FP diet blood alanine, lactate and plasma glutamine levels were lower for the whole or the majority of the post-exercise period, while the concentrations of plasma FFA, glycerol, urea and blood 3-OHB and glucose were higher. There was no difference between the diets in pre-exercise glucose and insulin levels and post-exercise insulin levels. There was no difference in muscle pH between the two diets immediately prior to exercise; the decline in muscle pH was 104% greater during exercise on the FP diet resulting in a significant difference in post-exercise pH ($p = 0.05$). The FP diet resulted in 23% decline in muscle glutamine levels, resulting in lower levels ($p < 0.05$) immediately prior to exercise. Exercise had no influence on muscle glutamine levels after the FP diet but produced a 17% decline on the CHO diet. Muscle glycogen content increased by 23% on the CHO diet, but was unchanged after the FP diet. This resulted in levels being significantly different prior to exercise ($p < 0.05$). The decline in muscle glycogen content during exercise was 50% greater on the CHO diet. There were no differences when comparing the two dietary treatments in any of the pre-exercise glycolytic intermediates measured. Immediately post-exercise glucose 6-phosphate levels were 22% higher and fructose 1,6-diphosphate levels were 130% lower on the FP diet. There were no differences between the two diets in muscle triose phosphate or lactate levels at any point of the study. The present study demonstrates that a FP diet can induce metabolic acidosis and may reduce pre-exercise muscle buffering capacity, which may then influence subsequent exercise performance. However, this appears not to influence the efflux of H^+ from muscle during and after high intensity exercise.

Offprint requests to: R. J. Maughan

Key words: High intensity exercise — Muscle metabolism — Diet — Acid-base status

Introduction

We have previously demonstrated that a low carbohydrate, high fat, high protein diet which meets energy requirements can produce a mild resting metabolic acidosis and can reduce the time to exhaustion during high intensity exercise (Greenhaff et al. 1987b). We postulated that the metabolic acidosis could reduce the rate of muscle glycolysis during high intensity exercise, possibly by reducing pre-exercise muscle buffering capacity or H^+ efflux during exercise thereby reducing the exercise time to exhaustion. It was also apparent from the same study that the variation in dietary composition may influence the pattern of substrate mobilisation prior to and during exercise. The present experiment was undertaken to investigate the effect of dietary manipulation on muscle metabolism during high intensity exercise in an attempt to identify some of the mechanisms that may contribute to fatigue during this type of exercise.

Materials and methods

Subjects. Five healthy male subjects gave their written consent to take part in the present experiment which was approved by the local Ethics Committee. Their physical characteristics were: age 31±6 years (mean ±SD); height 176±4 cm; body weight 71.2±7.9 kg; $\dot{V}_{O_{2max}}$ 54±6 ml · kg^{-1} · min^{-1}.

Experimental protocol. Each subject exercised for 3 min at a workload equivalent to 100% $\dot{V}_{O_{2max}}$ on two occasions separated by two weeks. Each 3 min exercise test was performed on an electrically braked cycle ergometer with subjects having fasted overnight. Four weeks before the first high intensity exercise test subjects weighed and recorded their 'normal' dietary intake for 1 week. The records kept by subjects were used to assess their 'normal' energy intake and dietary composition using a computerised version of the food composition tables of McCance and Widdowson (1960). The results were used to design two dietary regimens which were consumed for the four days prior to each high intensity test. These were a high carbohydrate, low fat, low protein diet (CHO) and a low carbohydrate, high fat, high protein diet (FP) (Table 1). The two diets which were isoenergetic with each subject's 'normal' diet, were prepared and consumed within the department and were assigned in a randomised manner. No alcohol or caffeine-containing drinks were allowed during the study period. All subjects consumed at least 568 ml (1 pint) of low energy drink with each meal to ensure adequate fluid intake. Physical activity of any type was restricted to a minimum for the four days prior to each high intensity exercise test and subjects were asked to refrain from strenuous physical activity for the week preceding and for the whole of the experimental period. Nude body weight was recorded on the morning prior to dietary manipulation and immediately prior to exercise.

Table 1. Daily energy intake and dietary composition of the two dietary treatments. Each diet was designed from the records kept by subjects while consuming their 'normal' diet. Values are mean ±SD

	Low carbohydrate High fat High protein	High carbohydrate Low fat Low protein
Energy intake (MJ)	12.5±3.4	12.6±3.1
Carbohydrate (%)	3 ±0	82 ±1
Fat (%)	73 ±2	8 ±1
Protein (%)	24 ±3	10 ±1

On the morning prior to dietary manipulation, immediately prior to exercise and immediately post-exercise a needle biopsy sample was obtained from the vastus lateralis muscle of one leg. All samples were obtained after an overnight fast. At the same pre-exercise time points and at 2, 4, 6, 10 and 15 min post-exercise, 5 ml anaerobically drawn arterialised venous blood was obtained from a 21 g venous cannula inserted into a superficial vein on the dorsal surface of a heated hand (Forster et al. 1972). The cannula was kept patent between samples on the day of exercise by flushing with isotonic saline.

Muscle treatment and analysis. After removal from the muscle the biopsy needle was immediately plunged into liquid nitrogen and stirred vigorously to accelerate cooling. Within 30 min the muscle sample was removed from the biopsy needle and was divided into two portions under liquid nitrogen. One portion was stored at −55°C until analysed for muscle pH with a Radiometer BMS3 Mk2 (Copenhagen, Denmark) blood gas analyser using the muscle homogenate technique described by Spriet et al. (1987a). The other muscle portion was freeze dried and stored dessicated at −55°C. At a later date each freeze dried muscle sample was powdered using an agate pestle and mortar, care being taken to remove any visible connective tissue and blood. Using the method of Harris et al. (1974) each powdered muscle sample underwent extraction and neutralisation; the supernatant was then assayed enzymatically for glutamine (using a modification of the fluorometric technique of Lund 1985), glucose 6-phosphate (G6-P), fructose 1,6-diphosphate (F1,6DP), triose phosphates (TP) and lactate (using the fluorometric methods described by Maughan, unpublished doctoral thesis, 1978). Glycogen was determined on the neutralised extract and the muscle pellet left over from the extraction procedure using the acid hydrolysis technique described by Jansson (1981).

Blood treatment and analysis. The treatment of blood and the determination of blood acid-base status, blood glucose, lactate, alanine, 3-hydroxybutyrate (3-OHB) and plasma free fatty acid (FFA) and glycerol concentrations were as described previously (Greenhaff et al. 1987a). In addition blood was used for the determination of packed cell volume (conventional micro-haematocrit method) and haemoglobin concentration (colorimetric method, Sigma Chemical Company Ltd, Dorset, UK) and plasma was used for the measurement of urea (enzymatic colorimetric method, Boehringer Corporation Ltd, Lewes, UK), total protein (colorimetric method, Sigma Chemical Company Ltd, Dorset, UK), albumin (colorimetric method, Sigma Chemical Company Ltd, Dorset, UK), insulin

(radio-immunassay, Amersham, UK) and glutamine (using a modification of the fluorometric technique of Lund 1985). Plasma sodium was measured by flame photometry (410C clinical flame photometer, Ciba-Corning, Halstead, UK). Plasma chloride was measured using a titrimetric chloride meter (EEL 920, Ciba-Corning, Halstead, UK). Changes in plasma volume were calculated from packed cell volume and haemoglobin values (Dill and Costill 1974).

Statistical analysis was by the Student's paired *t*-test. Statistical significance was declared at $p<0.05$ or in the case of some muscle measurements at $p\leqslant 0.05$ due to the sample size being reduced. Values in tables and text refer to mean ±SD; for clarity graphs are represented as mean ±SE.

Results

Body weight

There was no difference in body weight between the value recorded prior to dietary manipulation (72.0±7.9 kg) and the value recorded immediately prior to exercise (71.4±8.2 kg) on the CHO diet. However over the four days of the FP diet there was a reduction in mean body weight amounting to 1.5 kg ($p<0.01$; 71.4±7.8 kg to 69.9±7.8 kg).

Blood acid-base status

As expected there were no differences in the acid-base measurements made on the morning prior to dietary manipulation when comparing the two experimental treatments (Figs. 1, 2). The FP diet resulted in plasma pH ($p<0.01$), blood P_{CO_2} ($p<0.01$), plasma bicarbonate ($p<0.001$) and blood base excess ($p<0.001$) values being significantly lower at rest prior to exercise when compared with their corresponding values on the CHO diet (Figs. 1, 2). There were no differences in post-exercise plasma pH measurements apart from at 4 min post-exercise when plasma pH was significantly lower after the FP diet (Fig. 1). Blood P_{CO_2} was lower on the FP diet at 6, 10, and 15 min post-exercise (Fig. 1); plasma bicarbonate was lower at 2 and 4 min post-exercise (Fig. 2). There were no differences in post-exercise base excess measurements (Fig. 2). There were no differences between the two dietary treatments when comparing the peak post-exercise decline in pH, P_{CO_2}, bicarbonate and base-excess.

Blood and plasma constituents

The FP diet resulted in blood alanine ($p<0.05$, Fig. 3), blood lactate ($p<0.05$; Fig. 3) and plasma

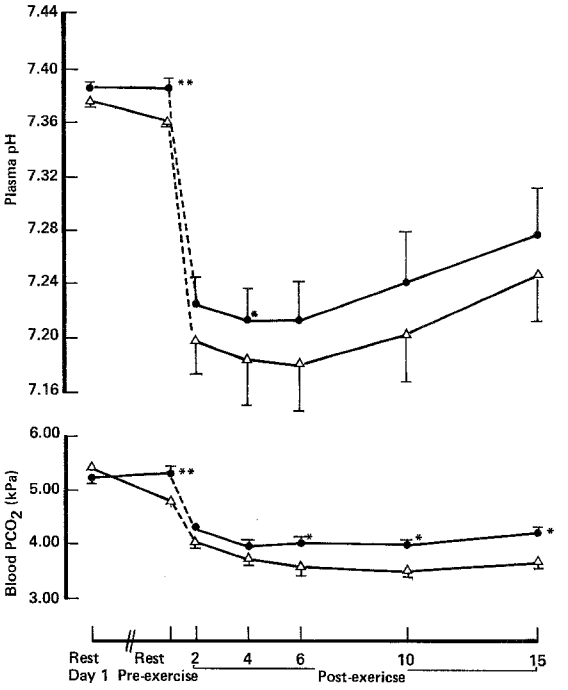

Fig. 1. Plasma pH and blood P_{CO_2} (mean ±SE) at rest prior to dietary manipulation (d1), at rest immediately prior to exercise (d5) and at 2, 4, 6, 10 and 15 min post-exercise on a low carbohydrate, high fat, high protein diet (△) and a high carbohydrate, low fat, low protein diet (●). Significant difference between corresponding time points on each diet is as follows: $*=p<0.05$; $**=p<0.01$; $***=p<0.001$

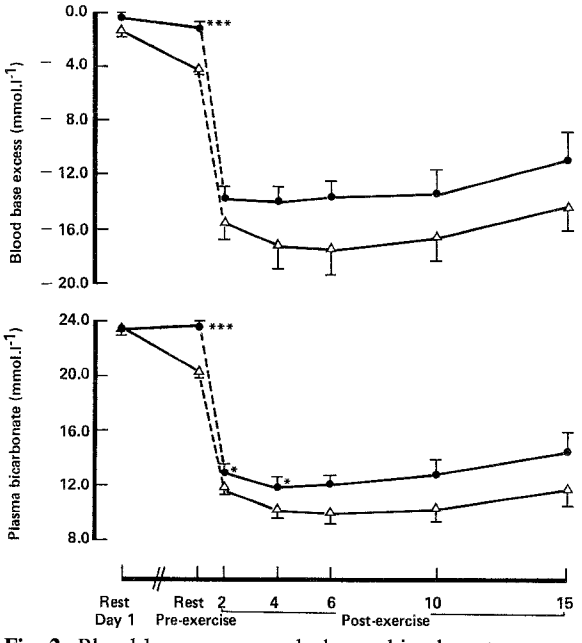

Fig. 2. Blood base excess and plasma bicarbonate concentrations (mean ±SE) at rest prior to dietary manipulation (d1), at rest immediately prior to exercise (d5) and at 2, 4, 6, 10 and 15 min post-exercise on a low carbohydrate, high fat, high protein diet (△) and a high carbohydrate, low fat, low protein diet (●). Significant difference between corresponding time points on each diet is as follows: $*=p<0.05$; $**=p<0.01$; $**=p<0.001$

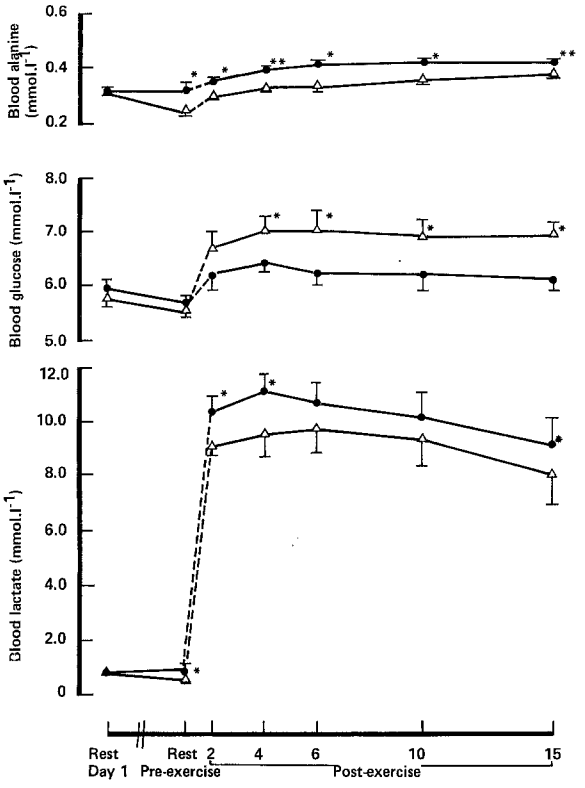

Fig. 3. Blood alanine, glucose and lactate concentrations (mean ±SE) at rest prior to dietary manipulation (d1), at rest immediately prior to exercise (d5) and at 2, 4, 6, 10 and 15 min post-exercise on a low carbohydrate, high fat, high protein diet (△) and a high carbohydrate, low fat, low protein diet (●). Significant difference between corresponding time points on each diet is as follows: $*=p<0.05$; $**=p<0.01$; $***=p<0.001$

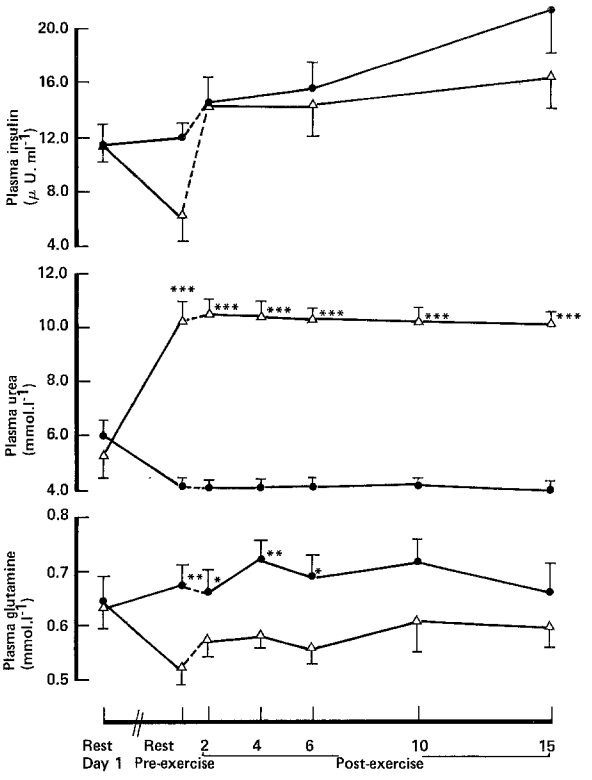

Fig. 4. Plasma insulin, urea and glutamine concentrations (mean ±SE) at rest prior to dietary manipulation (d1), at rest immediately prior to exercise (d5) and at 2, 4, 6, 10 and 15 min post-exercise on a low carbohydrate, high fat, high protein diet (△) and a high carbohydrate, low fat, low protein diet (●). Significant difference between corresponding time points on each diet is as follows: $*=p<0.05$; $**=p<0.01$; $***=p<0.001$

glutamine ($p<0.01$; Fig. 4) concentrations being lower immediately prior to exercise when compared with their corresponding values on the CHO diet. The opposite response occurred in the cases of plasma FFA ($p<0.05$; Fig. 5), plasma glycerol ($p<0.01$; Fig. 5), blood 3-OHB ($p<0.01$; Fig. 5) and plasma urea ($p<0.001$; Fig. 4). Although mean pre-exercise insulin levels appeared to be lower after the FP diet when compared with the CHO diet no statistical difference was apparent (Fig. 4). In addition, there were no differences between the two diets when considering pre-exercise glucose levels (Fig. 3). Plasma albumin concentration was higher immediately prior to exercise after the FP diet when compared with the CHO diet (Table 2; $p<0.05$); plasma total protein concentration was not different ($p=0.06$). However, plasma protein levels did increase on the FP diet ($p<0.05$); no such increase was seen over the four days of the CHO diet. After the FP diet blood alanine concentration (Fig. 3) was lower than the values recorded on the CHO diet for the whole of the post-exercise period; blood lactate concentration was lower at 2, 4, and 15 min post-exercise (Fig. 3); plasma glutamine concentration was lower at 2, 4, and 6 min post-exercise (Fig. 4). The concentration of plasma FFA, plasma glycerol, blood 3-OHB (Fig. 5) and plasma urea (Fig. 4) were all higher for the whole of the post-exercise period after the FP diet when compared with the CHO diet; the concentration of blood glucose was higher at 4, 6, 10 and 15 min post-exercise (Fig. 3). There were no differences in post-exercise plasma insulin levels between the two diets (Fig. 4).

Plasma volume and electrolyte changes

The calculated decrease in plasma volume over the four days of the FP diet (2.3 ± 6.3%) was not different from the value calculated for the CHO

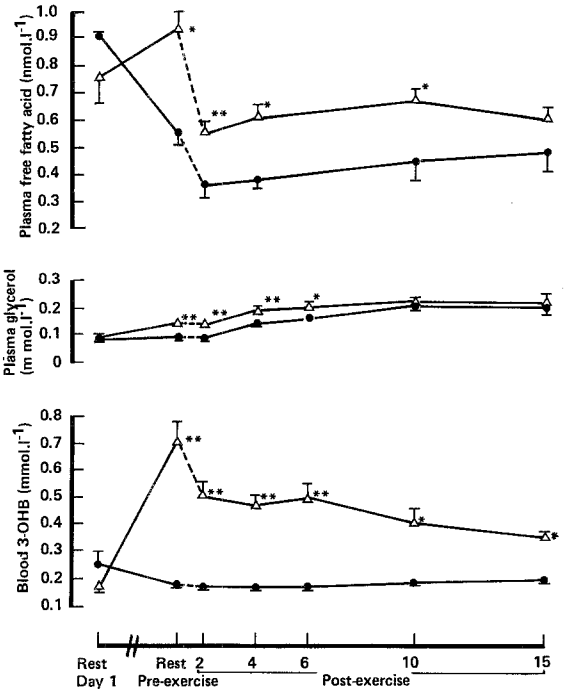

Fig. 5. Plasma free fatty acid, glycerol and 3-hydroxybutyrate (3-OHB) concentrations (mean ±SE) at rest prior to dietary manipulation (d1), at rest immediately prior to exercise (d5) and at 2, 4, 6, 10 and 15 min post-exercise on a low carbohydrate, high fat, high protein diet (△) and a high carbohydrate, low fat, low protein diet (●). Significant difference between corresponding time points on each diet is as follows: $*=p<0.05$; $**=p<0.01$; $***=p<0.001$

diet (2.1±6.8%). The two dietary treatments had no influence on plasma sodium and chloride levels (Table 2).

Muscle measurements

Muscle pH (Fig. 6) was higher on the morning prior to the commencement of the CHO diet

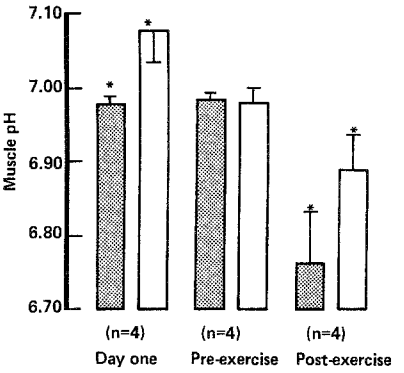

Fig. 6. Muscle pH (mean ±SE) at rest prior to dietary manipulation (d1), at rest immediately prior to exercise (d5) and immediately post-exercise on a low carbohydrate, high fat, high protein diet (■) and a high carbohydrate, low fat, low protein diet (□). * indicates significant difference between corresponding values ($p \leq 0.05$)

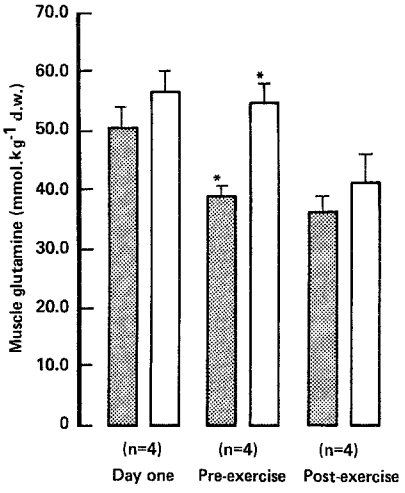

Fig. 7. Muscle glutamine content (mean ±SE) at rest prior to dietary manipulation (d1), at rest immediately prior to exercise (d5) and immediately post-exercise on a low carbohydrate, high fat, high protein diet (■) and a high carbohydrate, low fat, low protein diet (□). * indicates significant difference between corresponding values ($p<0.05$)

Table 2. Plasma concentrations of sodium (mmol · l⁻¹), chloride (mmol · l⁻¹) albumin (g · l⁻¹) and protein (g · l⁻¹) measured at rest prior to dietary manipulation and immediately prior to exercise on the two dietary treatments. Values are mean ±SD. * indicates significant difference between corresponding values ($p<0.05$)

	Low carbohydrate, High fat, High protein		High carbohydrate, Low fat Low protein	
	Pre-diet	Pre-Exercise	Pre-diet	Pre-Exercise
Plasma Sodium (mmol l⁻¹)	136 ±3	138 ±4	137 ±4	138 ±6
Plasma Chloride (mmol l⁻¹)	104 ±5	98 ±3	101 ±5	104 ±3
Plasma Albumin (g l⁻¹)	42.9±1.4	44.2*±1.2	42.6±1.8	43.2*±1.1
Plasma Protein (g l⁻¹)	82.7±4.1	86.7±1.7	82.1±5.9	82.3±5.0

Table 3. The values (mmol · kg^{-1} d.w.) recorded on each dietary treatment for muscle glycogen, glucose 6 phosphate (G6-P), fructose 1,6 diphosphate (F1,6-DP), triose phosphates (TP) and lactate. Muscle biopsy samples were taken at rest prior to dietary manipulation, at rest immediately prior to exercise and immediately post-exercise. Values are mean ±SD. * indicates significant difference between corresponding values ($p \leq 0.05$)

	Low carbohydrate, High fat, High protein			High carbohydrate, Low fat Low protein		
	Pre-Diet	Pre-Exercise	Post-Exercise	Pre-Diet	Pre-Exercise	Post-Exercise
Total Glyogen (mmol kg^{-1})	321.7 ±32.4 ($n=4$)	299.5* ±23.9 ($n=4$)	198.8* ±24.3 ($n=4$)	309.4 ±96.3 ($n=4$)	381.4* ±23.2 ($n=4$)	230.3* ± 9.8 ($n=4$)
G6-P (mmol kg^{-1})	0.83± 0.48 ($n=4$)	0.91± 0.50 ($n=4$)	11.03* ± 2.55 ($n=3$)	0.72± 0.51 ($n=4$)	0.74± 0.35 ($n=4$)	9.03* ± 1.81 ($n=3$)
F1,6-DP (mmol kg^{-1})	0.06± 0.03 ($n=3$)	0.06± 0.01 ($n=3$)	0.13 ± 0.04 ($n=3$)	0.14± 0.12 ($n=3$)	0.03± 0.01 ($n=3$)	0.30 ± 0.12 ($n=3$)
TP (mmol kg^{-1})	0.11± 0.03 ($n=4$)	0.10± 0.03 ($n=4$)	0.17 ± 0.06 ($n=3$)	0.13± 0.01 ($n=4$)	0.12± 0.01 ($n=4$)	0.21 ± 0.01 ($n=4$)
Lactate (mmol kg^{-1})	4.5 ± 2.0 ($n=4$)	4.4 ± 2.5 ($n=4$)	125.3 ±15.2 ($n=4$)	3.6 ± 1.1 ($n=4$)	5.5 ± 1.2 ($n=4$)	104.4 ±28.1 ($n=4$)

($p < 0.05$) when compared with the corresponding value prior to the FP diet. Immediately prior to exercise no difference between dietary conditions was evident. As expected, muscle pH fell during the two 3 min exercise tests. The decline was 104% greater on the FP diet resulting in a significant difference in muscle pH between the two diets immediately post-exercise ($p = 0.05$). There was no difference between experimental treatments in muscle glutamine content prior to dietary manipulation (Fig. 7); by day 5 a 23% decline in muscle glutamine content had occurred on the FP diet resulting in levels being significantly different between diets immediately prior to exercise ($p < 0.05$). Exercise had little influence upon muscle glutamine content on the FP diet but produced a decline of 17% on the CHO diet, resulting in levels being very similar post-exercise.

Table 3 shows the values recorded for muscle glycogen, G6-P, F1,6-DP, TP and lactate content during the present study. There was no difference between experimental treatments in muscle glycogen content on the morning prior to dietary manipulation. By day 5 (immediately prior to exercise) muscle glycogen content had increased by 23% on the CHO diet while the four days on the FP diet did not influence muscle glycogen content. This resulted in muscle glycogen levels being significantly different between diets prior to exercise ($p < 0.05$). The mean decrease in muscle glycogen content during the 3 min of exercise was 50% greater on the CHO diet, but levels were still higher than those of the FP diet immediately post-exercise ($p < 0.05$). There were no differences in the content of G6-P prior to dietary manipulation or immediately prior to exercise when comparing the two treatments. Immediately post-exercise G6-P levels were 22% higher on the FP diet resulting in a significant difference between the two diets ($p = 0.05$). The content of F1,6-DP did not differ between the two experimental treatments prior to dietary manipulation or immediately prior to exercise. Immediately post-exercise there is a suggestion that F1,6-DP levels were higher after the CHO diet than after the FP diet; however, due to the small sample size ($n = 3$), the difference between the two diets was not statistically significant. There was no difference in TP content at any point when comparing the two dietary treatments. The 3 min of exercise produced a dramatic increase in muscle lactate content; despite this increase no difference in content was found prior to or post-exercise when comparing the two diets.

Dicussion

Body weight changes

Despite the level of physical activity being kept at a minimum and the energy content of each diet being the same during the present experiment, the FP diet was accompanied by a reduction in body weight although body weight remained constant on the CHO diet. Table 3 indicates that the decline in body weight cannot be attributed to a loss of water associated with a reduction in muscle

glycogen content. A fall in body weight of a similar magnitude to the reduction in body weight observed in the present experiment was recorded when a group of subjects walked 37 km per day for each of 4 consecutive days while on a low CHO diet which gave a slight positive energy balance; again, however, body weight was maintained when an isoenergetic high CHO diet was given while the same exercise level was maintained for a 4 day period (Maughan et al. 1987). However, the composition of the diets used in the present study was almost identical to that used in an earlier experiment (Greenhaff et al. 1987b) where no decline in body weight was observed. During the present experiment subjects were asked to drink at least 568 ml of low energy drink with every meal to maintain hydration. In the absence of measurements of urine output it is not known whether there was a differential diuresis on the two diets. There appeared to be no difference between the two diets in the changes in plasma volume and electrolytes over the four day period.

Pre-exercise acid-base balance

We have previously demonstrated that a high protein, low CHO diet can influence resting acid-base status (Greenhaff et al. 1987b). The results of the present study support the supposition that the metabolic acidosis arising from a FP diet (Figs. 1, 2) is not a consequence of a variation in the level of plasma strong inorganic ions (Table 2) but probably results from increases in the concentration of circulating organic strong ions (FFA, 3-OHB; Fig. 5) and non volatile weak acids (plasma proteins; Table 2). The elevated dietary protein intake on a FP diet will also increase the production of hydrogen ions derived from the catabolism of dietary amino acids by the liver (Newsholme and Leech 1983) and will add further to the diet-induced acidosis. The increased degradation of dietary protein during the FP diet of the present study is characterised by the dramatic increase in plasma urea levels (Fig. 4) and will also result in an increase in urea excretion (Folin 1905) bringing about the removal of ammonia. Under normal circumstances skeletal muscle uses ammonia to synthesise the amino acid alanine which is then released by the muscle. In the liver the alanine is then broken down providing ammonia for urea synthesis and a carbon source for gluconeogenesis (Newsholme and Leech 1983; Felig 1977). However, during acidosis the rate of ammonia incorporation into glutamine and the release of glutamine by skeletal muscle are increased. The reverse reaction occurs in the kidneys to release ammonia which combinines with hydrogen ions in the kidney tubules to help maintain acid-base balance (Goldstein 1980), leaving a carbon source for glucose formation. The reduced muscle (Fig. 7) and plasma (Fig. 4) glutamine levels during the FP diet of the present study may represent an increased renal removal of glutamine in an attempt to maintain acid-base balance and blood glucose concentration.

Post-exercise blood acid-base status

The similarities between the two dietary treatments in the peak post-exercise decline in pH, P_{CO_2}, bicarbonate and base excess suggests that the pre-exercise metabolic acidosis of the FP diet did not influence the rate of H^+ efflux from the exercising muscle during the high intensity exercise. Moreover, the lower blood lactate concentration at 2, 4, and 15 min post-exercise on the FP diet implies a reduced removal of lactate from muscle or an increased post-exercise uptake of lactate by inactive muscle or liver.

Muscle acid-base changes

The majority of subjects commented that they found the 3 min exercise test more strenous after the FP diet. Muscle pH after exercise on this diet was reduced to 6.76 ± 0.13 which is similar to the value reported by Sahlin et al. (1976) after exhaustive exercise on an electrically braked cycle ergometer. The larger increase in G6-P after the FP diet of the present study suggests a relative inhibition of phosphofructokinase (PFK) activity during exercise compared with the CHO diet. However, although a decrease in muscle pH, resulting in an inhibition of PFK activity, has been suggested as a limiting factor to the performance of high intensity exercise (Sahlin 1978), previous research, using a variety of experimental models, has pointed to a hydrogen ion induced inhibition of activation of contractile proteins by calcium ions (Donaldson et al. 1978; Spriet et al. 1987b) or a depletion of muscle phosphocreatine stores (Katz et al. 1986) as major limitations to performance. The finding that the metabolic acidosis of the FP diet of the present study did not influence

pre-exercise muscle pH when compared with the CHO diet but did lead to a greater decline (104%) in muscle pH during exercise is in agreement with the data of Hultman et al. (1985). They demonstrated that metabolic acidosis (induced by oral ingestion of NH_4Cl) will reduce pre-exercise buffering capacity, thereby producing a larger decrease in muscle pH after 75 s of electrical stimulation. However, unlike the present experiment they found no evidence to suggest that an inhibition of muscle glycolytic flux or lactate efflux had occurred during exercise in the acidotic condition, and pointed to a breakdown in the contractile mechanism as a possible cause of fatigue. The greater decrease in muscle pH after exercise on the FP diet in the present experiment suggests muscle buffering capacity was reduced prior to exercise. The larger fall in pH during exercise on the FP treatment may have been responsible for a reduction in PFK activity (Sahlin 1978) thereby resulting in the larger accumulation (22%) of G6-P and a reduction in the rate of glycolysis during exercise on this diet (as indicated by the mean decrease in muscle glycogen content being 50% less on the FP diet). The finding that blood glucose levels were higher for nearly the whole of the post-exercise period after the FP diet gives support to this proposal: the greater elevation of G6-P levels post-exercise on the FP diet will have produced a larger inhibition in hexokinase (HK) activity bringing about an inhibition of further G6-P production and muscle glucose uptake (Newsholme and Start 1973). In addition, it is known that 8–10% of the muscle glycogen degraded during glycogenolysis forms free glucose (Field 1960); this corresponds to about 10 $mmol \cdot kg^{-1}$ of glucose units during the 3 min of exercise on the FP treatment of the present study. This free glucose accumulation in the face of an already reduced HK activity may have led to the diffusion of glucose from the muscle (Wahren et al. 1971; Lavoie et al. 1987). However, if one accepts that the larger fall in pH of the FP diet may have reduced the rates of glycogenolysis and glycolysis it then becomes difficult to explain the similarity in post-exercise muscle lactate levels when comparing the two treatments. The lower post-exercise blood lactate of the FP treatment could indicate that removal of lactate (but not H^+) after exercise on this treatment was reduced. This would then account for the similarity in post-exercise muscle lactate levels between the treatments. Alternatively, the metabolic fate of the lactate produced may be different during exercise on each dietary treatment.

Substrate availability and utilisation

The finding that muscle glycogen levels were still high after 3 min of exercise on the FP diet is in support of the suggestion that muscle glycogen availability is not a limiting factor to performance during high intensity exercise (Hermansen 1981). However, it is possible that selective substrate depletion in type IIb muscle fibres could be responsible for fatigue (Tesch 1980). The four day FP diet had no influence on muscle glycogen content. This is in agreement with the results of Hultman (1967) which showed muscle glycogen content was not influenced unless dietary CHO intake was reduced for longer than six days. Gollnick et al. (1981) and Richter and Galbo (1986) demonstrated that the intracellular availability of glycogen may influence substrate utilisation during exercise. The difference in mean pre-exercise muscle glycogen content between the two diets of the present study was 80 $mmol \cdot glucose\ units \cdot kg^{-1}$ and arose not from a decrease in glycogen content on the FP diet, but an increase on the CHO diet. Whether this difference is large enough to influence substrate utilisation is not known. The finding that the absolute decline in muscle glycogen content during exercise was 50% greater on the CHO diet when compared with the FP diet supports the suggestion that muscle glycogen content may influence the pattern of substrate utilisation during exercise. However, the finding that there was no difference in the post-exercise muscle lactate levels between the two treatments and that the changes in muscle G6-P and pH were larger during exercise after the FP diet when compared with the CHO diet does not support the suggestion. If muscle glycogen levels were influencing the pattern of substrate utilisation one might have expected the enhanced CHO metabolism during exercise after the CHO diet to have produced the largest changes in G6-P, lactate and pH. However, as stated previously, it is possible that differences between dietary treatments in the rate of muscle lactate efflux or the metabolic fate of lactate could explain the similarity in muscle lactate levels. The lower post-exercise blood lactate levels after the FP diet would appear to indicate that this may be the case.

Acknowledgements. M. Gleeson was supported by the Health Promotion Research Trust. R. J. Maughan is supported by Shell Expro (UK) Ltd. Thanks are due to Dr M. Cheetham, Mr J. Leiper, Mr K. Murray, Dr J. Robertson and Mr R. Summers for assistance at various stages of this work and to Mrs L. Alexander for preparation of the manuscript.

References

Dill DB, Costill DL (1974) Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 37:247–248

Donaldson SKB, Hermansen L, Bolles L (1978) Differential, direct effects of H^+ on Ca^{2+} — activated force of skinned fibres from the soleus, cardiac and adductor magnus muscles of rabbits. Pflügers Arch 376:55–65

Felig P (1977) Amino acid metabolism in exercise. In: Milvy P (ed) The marathon physiological, medical, epidemiological and psychological studies. Ann NY Acad Sci 301:56–63

Field (1960) Glycogen deposition diseases. In: The metabolic basis of inherited disease. McGraw-Hill, New York, pp 156–207

Folin (1905) Laws governing the chemical composition of urine. Am J Physiol 13:66–115

Forster HV, Dempsey JA, Thomson J, Vidruk R, DoPico GA (1972) Estimation of arterial P_{O_2}, P_{CO_2}, pH and lactate from arterialised venous blood. J Appl Physiol 32:134–137

Goldstein L (1980) Adaptation of renal ammonia production to metabolic acidosis. Physiologist 23:19–25

Gollnick PD, Pernow B, Essen B, Jansson E, Saltin B (1981) Availability of glycogen and plasma FFA for substrate utilisation in leg muscle of man during exercise. Clin Physiol 1:27–42

Greenhaff PL, Gleeson M, Maughan RJ (1987a) The effects of dietary manipulation on blood acid-base status and the performance of high intensity exercise. Eur J Appl Physiol 56:331–337

Greenhaff PL, Gleeson M, Maughan RJ (1987b) Diet induced metabolic acidosis and the performance of high intensity exercise in man. Eur J Appl Physiol (in Press)

Harris RC, Hultman E, Nordesjo LO (1974) Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. J Clin Lab Invest 33:109–120

Hermansen L (1981) Muscular fatigue during maximal exercise of short duration. Med Sport 13:45–52

Hultman E (1967) Physiological role of muscle glycogen in man with special reference to exercise. Circ Res [Suppl 1] 20–21:99–111

Hultman E, Del Canale S, Sjoholm H (1985) Effect of induced metabolic acidosis on intracellular pH, buffer capacity and contraction force of human skeletal muscle. Clin Sci 69:505–510

Jansson E (1981) Acid soluble and insoluble glycogen in human skeletal muscle. Acta Physiol Scand 113:337–340

Katz A, Sahlin K, Henriksson J (1986) Muscle ATP turnover rate during isometric contraction in humans. J Appl Physiol 60:1839–1842

Lavoie JM, Bonneau M-C, Roy J-Y, Brisson GR, Helie R (1987) Effects of dietary manipulations on blood glucose and hormonal responses following supramaximal exercise. Eur J Appl Physiol. 56:109–114

Lund P (1985) Glutamine. UV-method with glutaminase and glutamate dehydrogenase. In: Bergmeyer HU (ed) Methods of enzymatic analysis, vol VIII Metabolites 3: Lipids, Amino Acids and Related Compounds. VCF, Weinheim, pp 357–363

Maughan RJ, Greenhaff PL, Gleeson M, Fenn CE, Leiper JB (1987) The effect of dietary carbohydrate intake on the metabolic response to prolonged walking on consecutive days. Eur J Appl Physiol 56:538–591

McCance RA, Widdowson ED (1960) The composition of foods. MRC Special, Report Series No. 297, London HMSO

Newsholme EA, Start C (1973) Regulation in metabolism. J Wiley and Sons, Chichester

Newsholme EA, Leech AR (1983) Biochemistry for the medical sciences. J Wiley and Sons, Chichester

Richter EA, Galbo H (1986) High glycogen levels enhance glycogen breakdown in isolated contracting skeletal muscle. J Appl Physiol 61:827–831

Sahlin K (1978) Intracellular pH and energy metabolism in skeletal muscle of man. Acta Physiol Scand [Suppl 455] 1–56

Sahlin K, Harris RC, Nylind B, Hultman E (1976) Lactate content and pH in muscle samples obtained after dynamic exercise. Pflügers Arch 367:143–149

Spriet LL, Soderlund K, Hultman E (1987a) pH measurement in human skeletal muscle samples: effect of phosphagen hydrolysis. J Appl Physiol 61:1949–1954

Spriet LL, Soderlund K, Bergstrom M, Hultman E (1987b) Skeletal muscle glycogenolysis, glycolysis, and pH during electrical stimulation in men. J Appl Physiol 62:616–621

Tesch P (1980) Muscle fatigue in man with special reference to lactate accumulation. Acta Physiol Scand [Suppl 480] 1–40

Wahren J, Ahlborg G, Felig P, Jorfeldt L (1971) Glucose metabolism during exercise in man. In: Pernow B, Saltin B (eds) Muscle metabolism during exercise. Plenum Press, New York, pp 189–204

Accepted January 29, 1988