THE EFFECT OF CARBOHYDRATE-LOADING AND CARBOHYDRATE INGESTION ON FUEL SUBSTRATE KINETICS DURING PROLONGED CYCLING

Thesis submitted for the degree of Doctor of Philosophy (Medical Physiology)

by

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Dedicated to Sandy

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DECLARATION

I, Andrew Norman Bosch, do hereby declare that the experiments presented in this thesis were conceived and executed by myself and apart from the normal guidance from my supervisors, I received no assistance.

Neither the substance, nor any part of this thesis, has been submitted in the past, or is being, or is to be submitted to the University of Cape Town for any other degree, or to any other University.

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ABSTRACT

It has been well established that both carbohydrate-loading before and carbohydrate ingestion during exercise can enhance endurance performance by supplying carbohydrate for oxidation. However, the precise mechanism(s) underlying the proposed ergogenic effects of these procedures remain to be established. The studies in this thesis were therefore designed to examine the effects of carbohydrate-loading and carbohydrate ingestion on fuel substrate kinetics.

The first study assessed the effects of carbohydrate-loading on hepatic glucose turnover, plasma glucose oxidation and muscle glycogen utilisation in 15 male endurance-trained cyclists who rode for 180 min at 70% of maximum oxygen consumption ($VO_{2\ max}$) in either a carbohydrate-loaded (CLP) or non-loaded (NLP) state. Total carbohydrate oxidation during exercise was similar in the CLP and NLP subjects (492 ± 77 vs 448 ± 43 g, respectively; mean ± SEM), as were oxidation of plasma glucose (plasma glucose oxidation) (103 ± 19 vs 99 ± 7 g, respectively) and total splanchnic (endogenous plus exogenous) glucose appearance (Ra) (110 ± 15 vs 127 ± 16 g, respectively). Plasma glucose concentrations were lower in NLP than CLP subjects towards the end of exercise. CLP subjects had higher muscle glycogen concentrations at the start and throughout the trial than NLP subjects (194 ± 4 vs 124 ± 7 mmol/kg ww; $p < 0.05$) and total muscle glycogen utilisation was greater in CLP than NLP subjects (134 ± 11 vs 95 ± 12 mmol/kg ww; $p < 0.05$). Whereas high rates of muscle glycogen breakdown were maintained throughout the trial in CLP subjects, rates of muscle glycogenolysis decreased to 26 mmol/kg ww/hr after 60 min of exercise in NLP subjects ($p < 0.05$), when their muscle glycogen concentrations had declined to ~70 mmol/kg ww. Comparable rates of plasma glucose and overall carbohydrate oxidation in CLP and NLP subjects, despite a slowing of muscle glycogenolysis in the NLP group, could be explained by an accelerated breakdown of glycogen in the non-working muscles to redistribute carbohydrate to the working muscles for oxidation. Low muscle glycogen concentrations appeared to be consistent with fatigue in the NLP subjects, since a significantly greater number of NLP than CLP subjects became exhausted before completion of the trial (4/8 vs 0/7; $p < 0.05$). However, the plasma glucose concentrations of the NLP subjects who became exhausted were not significantly different from those who were able to complete the trial. Since rates of plasma glucose oxidation in CLP and NLP subjects were the same, these data suggest that high initial muscle glycogen levels increase endurance by postponing muscle glycogen depletion rather than by sparing hepatic glycogen.

Having established the effects of carbohydrate-loading on fuel substrate kinetics, the effects of carbohydrate ingestion were investigated in carbohydrate-loaded subjects since most athletes both carbohydrate-load and ingest carbohydrate during endurance events. Fourteen male endurance-trained cyclists rode for 180 min at 70% of $VO_{2\ max}$ after carbohydrate-loading (starting muscle glycogen 203 ± 7 mmol/kg ww) and ingested 500 ml/hr of either a 10% carbohydrate drink (CLC) or placebo (CLP) during the experiment. Ra, plasma glucose oxidation and muscle glycogen utilisation were determined. Total carbohydrate oxidation was

similar in CLC and CLP subjects. Ra increased significantly during the trial ($p < 0.05$) in both groups, reaching a plateau in the CLC subjects after 75 min and was significantly greater in the CLC than CLP subjects at the end of exercise (102 ± 13 vs 77 ± 12 μmol/min/kg fat free mass (FFM), respectively). Plasma glucose oxidation also increased significantly during the trial to a plateau in the CLC subjects, and was significantly ($p < 0.05$) higher in CLC than CLP subjects at the end of exercise (107 ± 8 vs 76 ± 15 μmol/min/kg FFM (1.24 ± 0.1 vs 0.88 ± 0.15 g/min)). However, mean rate of endogenous glucose appearance (Ra_{end}) was significantly ($p < 0.05$) lower in the CLC than CLP subjects throughout exercise (35 ± 7 vs 54 ± 6 μmol/min/kg FFM), as was the oxidation of endogenous plasma glucose, which remained almost constant in CLC subjects, and reached values at the end of exercise of 43 ± 8 and 73 ± 13 μmol/min/kg FFM in the CLC and CLP groups, respectively. Of the 0.83 g/min rate of carbohydrate ingestion, a mean of 0.77 ± 0.03 g/min was oxidised. Muscle glycogen utilisation was identical in both groups, and was higher during the first hour of exercise. All subjects completed the trial. It was concluded that in carbohydrate-loaded subjects, ingestion of 500 ml/hr of a 10% carbohydrate solution during prolonged exercise: i) had a marked hepatic glycogen sparing effect or caused a reduction in gluconeogenesis, or both, that should ultimately extend exercise time to exhaustion by delaying the onset of hypoglycaemia, ii) increased plasma glucose oxidation, iii) provided carbohydrate at a rate closely matched by the rate of oxidation of the ingested carbohydrate, and iv) did not have a muscle glycogen sparing effect.

Since the subjects in Study 2 were carbohydrate-loaded, the kinetics specific to carbohydrate ingestion were not established. Thus Study 3 examined the effects of ingesting 500 ml/hr of either a 10% carbohydrate drink (NLC) or flavoured water placebo (NLP) on Ra, plasma glucose oxidation and muscle glycogen utilisation in 17 non carbohydrate-loaded male endurance trained cyclists who rode for 180 min at 70% of $VO_{2\ max}$. Mean muscle glycogen concentration at the start of exercise was 130 ± 6 mmol/kg ww. Total carbohydrate oxidation was similar in NLC and NLP subjects and declined during the trial. Ra increased significantly during the trial ($p < 0.05$) in both groups. Plasma glucose oxidation also increased significantly during the trial reaching a plateau in the NLP subjects, but was significantly ($p < 0.05$) higher in NLC than NLP subjects at the end of exercise (98 ± 14 vs 72 ± 10 μmol/min/kg FFM (1.34 ± 0.19 vs 0.93 ± 0.13 g/min)). However, mean Ra_{end} was significantly ($p < 0.05$) lower in the NLC than NLP subjects throughout exercise (35 ± 7 vs 54 ± 6 μmol/min/kg FFM), as was the oxidation of endogenous plasma glucose, which remained almost constant in NLC subjects and reached values at the end of exercise of 42 ± 13 and 72 ± 10 μmol/min/kg FFM in the NLC and NLP groups, respectively. Of the mean 0.83 g/min rate of carbohydrate ingestion throughout the trial, a mean of 0.83 ± 0.1 g/min was oxidised. Muscle glycogen disappearance was identical during the first 2 hr of exercise in both groups and continued at the same rate in NLP subjects. However, no nett muscle glycogen disappearance occurred during the final hour in NLC subjects. Four of the 8 NLP subjects became exhausted before the end of the trial and at exhaustion, both their muscle glycogen and plasma glucose concentrations were low. In contrast, 2 of the 9 NLC subjects who also could not complete the trial, had low muscle glycogen concentrations, but were euglycaemic. It was concluded that the ingestion of 500 ml/hr of a 10% carbohydrate solution during

prolonged exercise in non carbohydrate-loaded subjects: i) had a marked hepatic glycogen sparing effect, or caused a reduction in gluconeogenesis, or both, ii) maintained plasma glucose concentrations, iii) had a muscle glycogen sparing effect late in exercise, and iv) did not prevent the development of fatigue in 22% of the NLC subjects whose muscle glycogen concentrations became very low.

Finally, having established that carbohydrate-loading and carbohydrate ingestion exert independent effects on fuel substrate kinetics, the comparative kinetics of carbohydrate ingestion in the absence of carbohydrate-loading and carbohydrate-loading without carbohydrate ingestion, remained to be established. Thus fuel substrate kinetics were compared in trained cyclists (n=16) who rode for 180 min at 70% of $VO_{2\,max}$ when a 10% carbohydrate drink was ingested without prior carbohydrate-loading (NLC) or with ingestion of a flavoured water placebo after carbohydrate-loading (CLP). Muscle glycogen concentrations at the start of exercise were 194 ± 4 and 124 ± 8 mmol/kg ww in CLP and NLC, respectively. Total carbohydrate oxidation was similar. Ra and plasma glucose oxidation increased significantly over time ($p < 0.05$), with values in NLC subjects at the end of exercise being significantly higher than CLP subjects (104 ± 17 vs 79 ± 9 and 115 ± 16 vs 74 ± 11 μmol/min/kg FFM, respectively). However, Ra_{end} was lower ($p < 0.05$) in NLC than CLP (40 ± 10 vs 79 ± 9 μmol/min/kg FFM), as was endogenous plasma glucose oxidation (42±13 vs 75±11 μmol/min/kg FFM). Muscle glycogen utilisation was identical in the first hour, but declined in NLC subjects thereafter. Two NLC subjects with the lowest muscle glycogen concentration were unable to complete the trial despite carbohydrate ingestion. It was concluded that: i) carbohydrate ingestion had an endogenous substrate sparing effect, ii) carbohydrate ingestion resulted in an increase in plasma glucose oxidation, and iii) carbohydrate ingestion did not prolong exercise time to exhaustion if glycogen levels became very low.

In summary, the results of these studies suggest that: i) carbohydrate ingestion has a hepatic glycogen sparing effect regardless of muscle glycogen concentration at the start of exercise, ii) carbohydrate-loading prolongs exercise time to exhaustion, primarily by delaying muscle glycogen depletion. These substrate kinetics operate independently, iii) carbohydrate ingestion results in an increase in plasma glucose oxidation, iv) carbohydrate ingestion may not prolong exercise time to exhaustion if glycogen concentrations become very low, and v) carbohydrate ingestion does not spare muscle glycogen when glycogen concentrations at the start of exercise are high, but muscle glycogen sparing occurs in non-loaded subjects when carbohydrate is ingested.

PUBLICATIONS

The work described in this thesis has been (or will be) published in the following journal articles:

Full papers:

Bosch, A. N., S. C. Dennis, and T. D. Noakes. Influence of carbohydrate-loading on fuel substrate turnover and oxidation during prolonged exercise. Journal of Applied Physiology 74: 1921-1927, 1993.

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Bosch A. N., S. C. Dennis, and T. D. Noakes. Comparison of effects of carbohydrate-loading vs carbohydrate ingestion on fuel substrate turnover and oxidation during prolonged exercise. Metabolism, 1994 (In Review).

Bosch, A. N., S. C. Dennis, and T. D. Noakes. Influence of carbohydrate ingestion on fuel substrate turnover and oxidation in non carbohydrate-loaded subjects. Pflügers Archives, 1994 (In Review).

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Bosch, A. N. Carbohydrate-substrate kinetics during prolonged exercise. South African Journal of Science 84: 599, 1988.

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Bosch, A. N., S. C. Dennis, and T. D. Noakes.. Fuel substrate kinetics during prolonged cycling. Medicine and Science in Sports and Exercise (Supplement) 21: 44, 1989.

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Influence of carbohydrate-loading on fuel substrate kinetics during prolonged exercise. South African Physiological Society Annual Meeting (Cape Town, South Africa, September, 1992).

Bosch, A. N., S. C. Dennis, and T. D. Noakes.. Influence of carbohydrate-loading on fuel substrate turnover and oxidation during prolonged exercise (South African Medical Journal, January 1993).

Influence of carbohydrate-loading on fuel substrate turnover and oxidation during prolonged exercise (South African Medical Association Congress, Cape Town, South Africa, March, 1993).

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CHAPTER 1

INTRODUCTION AND AIMS

It has been well established that both carbohydrate-loading before (129, 180, 273, 289, 306) and carbohydrate ingestion during exercise (28, 69, 89, 91, 138, 189) can enhance endurance performance by supplying carbohydrate for oxidation.

The carbohydrate-loading diet was first popularised in the late 1960's after it had been found that muscle glycogen concentration could be increased by a high (70%) carbohydrate diet for three or more days before exercise (3) and that muscle glycogen stores, elevated by this technique, seemed to enhance performance during subsequent endurance exercise (28, 129, 180, 189, 273, 289, 306). However, the precise mechanism(s) underlying the proposed ergogenic effect of this dietary intervention remain to be established (75).

One possibility is that the higher muscle glycogen stores after carbohydrate-loading may delay the onset of fatigue resulting from muscle glycogen depletion during exercise (89). However, that explanation would depend on the extent to which rates of muscle and hepatic glycogen utilisation during exercise are influenced by a higher pre-exercise muscle glycogen content. A number of studies have shown that rates of muscle glycogen utilisation during exercise are increased after carbohydrate-loading (28, 180, 289, 306), in which case muscle glycogen depletion may not be delayed.

Alternatively, the proposed ergogenic effect of carbohydrate-loading could be due to a slowing of the rate of hepatic glycogen depletion, since an increased availability of muscle glycogen would be expected to reduce the dependence of the muscles on oxidation of plasma glucose (plasma glucose oxidation). However, a sparing of hepatic glycogen, again, would depend on how the rate of hepatic glycogenolysis is influenced by the pre-exercise hepatic glycogen content, since rates of hepatic glycogenolysis have also been reported to be accelerated by a high hepatic glycogen content after carbohydrate-loading (335).

An important limitation of previous studies is that the interaction between simultaneously increased hepatic and muscle glycogen content on fuel substrate kinetics has not been studied. Accordingly, the aim of the first study in this thesis was to quantitate the effects of pre-exercise carbohydrate-loading on muscle glycogen utilisation and hepatic glycogen to glucose turnover and oxidation in athletes ingesting only water during prolonged exercise.

Besides the ergogenic effect of carbohydrate-loading described above, there is also an ergogenic effect of carbohydrate ingestion during prolonged exercise that was first demonstrated by Christensen and Hansen in 1939 (64), and has subsequently been repeatedly confirmed (69, 71, 91, 138, 172, 235, 248, 252, 256, 350,

362). Based on the studies that have *not* shown an improvement in performance, it appears that more than 4.2 g (260) to 24 g (247) of carbohydrate per hour has to be ingested and the duration of exercise has to be greater than 1 hr (260) to show an ergogenic effect from carbohydrate ingestion.

Under such circumstances, with the exception of fructose, the oxidation rates of different types of carbohydrate (98, 135, 231, 232, 251) and their effects on performance (254) have been found to be very similar. The effect of ingesting from 50 to 400 g of carbohydrate during the exercise period on plasma glucose oxidation rate has also been studied. These studies have shown that the oxidation rates of exogenous carbohydrate tend to increase when larger amounts of carbohydrate are consumed. Thus, after ingestion of 50 - 70g of carbohydrate, oxidation rates of 0.4 - 0.5 g/min have been reported (98, 179) and after ingestion of 100 g of carbohydrate, rates of 0.4 g/min (114), 0.5 g/min (267), 0.6 g/min (135, 179) and 1.0 g/min (201) have been reported. After ingestion of 140 - 400 g of carbohydrate, oxidation rates have been found to be 0.8 g/min at 45 - 50 % of $VO_{2\ max}$ (231, 267).

Studies such as those of Massicotte et al. (232) and Pirnay et al. (277) have shown that less endogenous carbohydrate is oxidised when carbohydrate is ingested during exercise. These data were calculated from differences between total carbohydrate and exogenous carbohydrate oxidation. However, the effects of carbohydrate ingestion on rate of appearance of exogenous and endogenous carbohydrate, and the rates of oxidation of plasma glucose originating from exogenous and endogenous sources, could not be ascertained by this approach. By using tracer techniques, Radziuk (280) partly answered the question of the effects of ingesting carbohydrate on rate of appearance of endogenous glucose (Ra_{end}), but these studies were in non-exercising subjects and provided no information on plasma glucose oxidation rates and the influence of the exogenous carbohydrate on muscle glycogen utilisation.

Thus, despite all these studies, the precise effects of carbohydrate ingestion on total splanchnic (endogenous plus exogenous) glucose appearance (Ra) and Ra_{end} , plasma glucose oxidation, and muscle glycogenolysis have not been studied simultaneously. The effect of carbohydrate ingestion during exercise on the kinetics of hepatic glucose turnover, exogenous and endogenous glucose oxidation and muscle glycogen utilisation in carbohydrate-loaded cyclists when either a carbohydrate drink or a placebo is ingested during prolonged exercise, remains to be determined. Thus, one of the aims of the second study in this thesis was to determine whether carbohydrate ingestion altered either muscle glycogen utilisation or hepatic glucose turnover in a way that might explain the established ergogenic effect of carbohydrate ingestion during exercise.

However, the effect of carbohydrate ingestion on fuel substrate kinetics when the muscles are not glycogen loaded may be different from the kinetics found after carbohydrate-loading. Therefore, the aim of the third study was to determine the effect of carbohydrate ingestion during prolonged exercise on the kinetics of

glucose turnover, exogenous and endogenous glucose oxidation and muscle glycogen utilisation in cyclists who had not carbohydrate-loaded prior to exercise.

The options available to athletes to provide adequate carbohydrate during competition are to either, i) carbohydrate-load before and ingest carbohydrate during the event, ii) carbohydrate-load before the event and ingest water during the event, or iii) since some athletes may prefer not to carbohydrate-load, dispense with loading and merely ingest carbohydrate during the event. Thus, the aim of the final study was to determine what differences may exist in glucose turnover, exogenous and endogenous glucose oxidation, and muscle glycogen utilisation when carbohydrate-loading is done before exercise and water ingested during exercise, as opposed to carbohydrate ingestion during exercise without prior carbohydrate-loading.

CHAPTER 2

REVIEW OF LITERATURE

Early studies

In 1924 the first evidence was presented by Levine et al. (215) that hypoglycaemia may occur during marathon running and adversely affect race performance. This came from a study of Boston Marathon runners of that year. Low blood glucose concentrations in 6 of the runners were measured at the finish of the race and as a result it was suggested that the runners should eat a high carbohydrate diet for the last 24 hours before the race the following year and that carbohydrate (sweets) should be ingested during the race. This proved to be effective, as the runners who took part in the experiment did not develop hypoglycaemia and their race times improved.

Eight years after the Levine et al. (215) study, Dill et al. (101) presented laboratory evidence that carbohydrate ingestion during exercise can influence performance. These researchers trained two dogs to run on a motorised treadmill and found that when they were given only water to drink while running they became fatigued after 2 - 4 hr. But if given a glucose solution they could continue for 17 - 23 hr before becoming fatigued. In the trial in which water was ingested, the dogs became hypoglycaemic, but this did not occur in the glucose feeding trial.

Two other studies completed shortly after this (38, 64) also demonstrated that the ingestion of carbohydrate during exercise could delay the onset of fatigue. Subjects who had become exhausted during prolonged exercise were able to continue after ingesting sufficient glucose to return blood glucose concentrations to euglycaemic levels.

An important finding in one of the above studies (64), that was not commented on at the time, was that the respiratory exchange ratio (RER) increased in one of the two subjects after glucose ingestion. In retrospect, these were probably the first data to show that ingested carbohydrate not only reverses hypoglycaemia, but provides a source of carbohydrate for oxidation by the muscle during exercise. However, the increase in RER after carbohydrate ingestion was only reported and discussed in the literature 50 years later, by Benade et al. (24).

In 1967, the use of the needle biopsy technique for the sampling of muscle glycogen provided important new data on the role of diet, muscle glycogen concentration and fatigue during prolonged exercise (3, 28, 29, 157).

Muscle and hepatic glycogen concentrations

The concentration of glycogen in the leg muscles of untrained people eating a normal diet varies from about 80 - 120 mmol/kg of wet muscle (ww) (20, 36, 47, 157, 286), whereas average muscle glycogen concentrations of athletes who ingest a diet high in carbohydrate and are in training, are somewhat higher,

around 130 mmol/kg ww (299). Values of around 144 - 200 mmol/kg ww are usually found in trained athletes who have not exercised for 24 - 48 hr and who have consumed a high carbohydrate diet (82, 130, 262, 305, 306, 316, 317, 349, 363).

Liver glycogen concentration is normally higher than that of muscle and is about 300 mmol/kg ww when a normal diet is consumed (160, 259) and 500 mmol/kg ww when the diet is high in carbohydrate (70%) (259). However, hepatic glycogen levels can fall rapidly as a result of its utilisation for maintaining blood glucose concentration. A liver weighing about 1,8 kg has a total glycogen store of about 160 g when a high carbohydrate diet is eaten. The brain alone has a daily glucose requirement of about 125 g, which is therefore almost sufficient to deplete the hepatic glycogen stores within 24 hr. Since hepatic glycogen stores fall about 9 g/hr, hepatic glycogen depletion can occur after a fast of only 18 hr (261). In contrast, either eating a low carbohydrate, high protein-fat diet, or fasting for 6 - 24 hr, results in little change in muscle glycogen concentrations (157, 219) and five days of this dietary regimen causes muscle glycogen levels to fall by only about 30 - 50% if only normal daily activities are performed. This occurs predominantly in the active muscles (157, 158).

Muscle and hepatic glycogen concentrations and exercise performance

Diet can affect both muscle glycogen content and exercise performance. Possibly the best known study is that of Bergstrom et al. (28) in which initial muscle glycogen concentrations were manipulated through various combinations of diet and exercise. Muscle glycogen concentration was measured before subjects cycled to exhaustion at 75% of $VO_{2\ max}$ on a cycle ergometer. Average glycogen concentration was 97 mmol/kg ww and time to exhaustion was 114 min. For the next 3 days subjects ate a high fat-protein diet, after which their muscle glycogen concentrations were measured at 35 mmol/kg ww and exercise time to exhaustion was reduced to 57 min. The dietary regimen was then changed to a high carbohydrate one for the next 3 days. Muscle glycogen concentrations increased to 184 mmol/kg ww and exercise time to 167 min. Thus, not only did Bergstrom et al. (28) show that initial glycogen levels influenced subsequent exercise time to exhaustion, but also that muscle glycogen concentration could be influenced by dietary manipulation. It was not long before this procedure of first depleting muscle glycogen stores and then eating a large volume of carbohydrate (~600 g of carbohydrate daily) was used by endurance athletes in an effort to enhance performance. However, some researchers have shown that carbohydrate-loading by first depleting muscle glycogen does not always result in higher muscle glycogen levels (117).

It has only recently been established by Costill et al. (82) and Sherman et al. (305) that the depletion phase of eating only protein and fat is unnecessary in well trained athletes. Simply eating a high carbohydrate diet for 3 days (500-600 g/day), combined with a reduction in training, results in similar amounts of glycogen being stored to that obtained when the original loading regimen (28) is followed.

A number of papers were published in 1967 dealing with muscle glycogen content and exercise performance (3, 28, 29, 157), showing that fatigue coincided with muscle glycogen depletion. It was

therefore concluded that exhaustion during prolonged exercise was due *only* to muscle glycogen depletion (3, 28, 29, 157, 325). Many other studies have also demonstrated that muscle glycogen depletion is the cause of fatigue (19, 20, 37, 46, 47, 81, 132, 136, 159, 162, 174, 211, 212, 263, 264, 270, 298, 299, 317, 364, 365).

In the Ahlborg et al. (3) study cited above, untrained subjects cycled at 62% of the workload that elicited a heart rate of 170 bpm. A correlation of 0.68 was determined between initial muscle glycogen concentration and endurance time. Similarly, Hermansen et al. (150) studied both trained and untrained subjects who cycled to exhaustion at 77% of $VO_{2\ max}$. They, too, concluded that initial muscle glycogen levels may determine exercise performance. In another study, Blom et al. (37) reported that during exercise at 65 - 75% of $VO_{2\ max}$, time to fatigue correlated with pre-exercise muscle glycogen content and exhaustion coincided with depletion of muscle glycogen stores. Even at low exercise intensity, muscle glycogen depletion was implicated in exhaustion, since a large number of type I muscle fibres were found to be depleted of muscle glycogen after 60 min of exercise at 43% of $VO_{2\ max}$ (37).

Karlsson and Saltin (189) are the only researchers who have tested the glycogen-depletion theory in the field. Using well trained subjects, they found that after following the carbohydrate-loading regimen of Bergstrom et al. (28), subjects ran a faster time in a 30 km road race than when eating a normal diet. Of particular interest was the finding that loading did not result in a faster initial running speed. Rather, it allowed the athletes to maintain their initial speed for longer. The time in the race at which the runners slowed down correlated with their starting muscle glycogen concentrations. However, over a distance of 21 km, there was no improvement in running performance as a result of starting with elevated muscle glycogen concentrations (306).

A study by Galbo et al. in 1979 (123) also investigated the relationship between starting muscle glycogen concentrations and fatigue in runners. Subjects started exercise with either high or low muscle glycogen content as a result of ingesting either a high or low carbohydrate diet for 4 days prior to the experiment. Time to fatigue was longer in the athletes with high initial muscle glycogen content. At exhaustion, glucose was infused to restore plasma glucose to pre-exercise levels. This eliminated symptoms of hypoglycaemia, but did not influence performance time, suggesting that muscle glycogen depletion and not hypoglycaemia was responsible for exhaustion in these subjects.

The effects of moderate- or high-carbohydrate diets on muscle glycogen content and performance was studied in runners and cyclists over 7 consecutive days of training by Sherman et al. (307). Muscle glycogen content of cyclists and runners was maintained with the high-carbohydrate diet but was reduced 30 - 36% with the moderate-carbohydrate diet. There were no differences in times to exhaustion at 80% of $VO_{2\ max}$ on day 7. Thus, consuming a moderate-carbohydrate diet over 7 days of intense training reduced muscle glycogen concentration, but had no apparent deleterious effect on training capability or high-intensity exercise performance. Conversely, a high-carbohydrate diet maintained muscle glycogen concentrations, but had no apparent benefit on training capability or high-intensity exercise performance (307). Thus, these

authors (307) concluded that muscle glycogen concentration may not be an absolute requirement for exercise to be performed at a fairly high intensity. One study in particular did not show a difference in running time to fatigue (77 min) at 75 - 80% of $VO_{2\ max}$ between carbohydrate-loaded and non-loaded groups of well trained runners (221). However, glycogen concentrations at exhaustion in that study were quite high (125 and 100 mmol/kg ww, respectively, in the two groups) and since the subjects were still euglycaemic, fatigue was probably due to factors other than glycogen depletion and hypoglycaemia at this moderately high intensity. Costill et al. (83) also found that trained runners became fatigued even though muscle glycogen concentration at exhaustion was 63 mmol/kg ww in the vastus lateralis and 86 mmol/kg ww in the soleus.

Other studies, however, have shown that exercise at higher intensities (100% of $VO_{2\ max}$) may be affected by muscle glycogen content at the start of exercise. An increase in time to exhaustion after carbohydrate-loading and decreased time to exhaustion with glycogen depletion, compared to exercise which commenced with normal muscle glycogen levels, has been shown by Maughan et al. (236) at 100% of $VO_{2\ max}$. However, Greenhaff et al. (133) found that exercise time to exhaustion during high intensity exercise in carbohydrate-loaded subjects did not differ from that of subjects who consumed a normal diet, but that fatigue occurred earlier in subjects who consumed a low carbohydrate diet.

Power output at maximal exercise intensity has been found to be reduced by 14% in subjects whose muscles are glycogen depleted. At any given power output, oxygen uptake, heart rate, and ventilation were higher and RER lower, in glycogen depleted subjects (146). Likewise, at workloads corresponding to 15% of maximal voluntary contraction, glycogen depletion was found to limit exercise capacity (161). In a study by Jacobs et al. (174), two groups of subjects were tested after one group had run a marathon and the other, a laboratory group, had cycled at 70% of $VO_{2\ max}$ for 30 min, run for 75 min and performed repeated bouts of sprint cycling and rapid, maximal contractions of the quadriceps. A strength test was performed before and within 1 - 2 hr of completion of the exercise protocols. In the laboratory group, glycogen depletion occurred in both type I and II fibre types, but in the marathon group, mostly the type I fibres were depleted. Depletion of glycogen in both type I and type II muscle fibres caused a reduction in the maximal force generated during a single contraction (174), as well as a significant increase in fatigue during 50 contractions. However, in the marathon group where only the type I fibres were depleted, no impairment in maximal strength was apparent (174), but a significant increase in fatigue occurred during 50 contractions. Since these measurements were made an hour after completion of exercise, other factors may have been responsible for the reduced force generation and earlier onset of fatigue. However, these findings are similar to those of Young and Davies (365), who measured isometric force of the triceps surae following prolonged exercise at 75% of $VO_{2\ max}$ in subjects with low and high muscle glycogen contents. Before exercise there were no differences in maximal twitch tension, maximal tetanic tension at various frequencies and maximum voluntary contraction between the groups. However, the loss of force during a subsequent 2 min electrically-evoked fatigue test was greater in the low glycogen group than in the high glycogen group.

After prolonged exercise, muscle weakness was found in both high and low glycogen groups, but was more pronounced in the low glycogen group.

Although there may be a strong relationship between pre-exercise muscle glycogen content and endurance performance, this may be true only for people who habitually consume a high carbohydrate diet. Phinney et al. (274) showed that when subjects consumed a high fat diet for 4 weeks, they were able to exercise at 63% of $VO_{2\ max}$ for as long on that diet as they were able to do when eating their normal diet, even though their muscle glycogen concentrations were much lower at the start of exercise when consuming the high fat diet (76 mmol/kg ww) than their normal diet (143 mmol/kg ww). Thus, in this experiment, no relationship was found between pre-exercise muscle glycogen levels and endurance, but significantly less muscle glycogen was used during exercise when on the high fat diet. This was due to an improved ability to oxidise fat as a fuel after adaptation to a high fat diet, and a resultant decline in the rate of oxidation of muscle glycogen. Muscle glycogen concentrations at exhaustion were not particularly low, being 53 and 56 mmol/kg ww after the normal and high fat diets, respectively. Neither were blood glucose concentrations low. Thus the reason that these subjects stopped exercise is not clear.

Glycogen utilisation was also less after a low carbohydrate (high fat) diet than after a high carbohydrate diet after exercise at 65% of $VO_{2\ max}$ in the study of Jansson and Kaijser (183). This may be because during exercise at 50% (237) and 65% (181) of $VO_{2\ max}$, plasma free fatty acids (FFA) and glycerol concentrations have been reported to be higher than normal after a low carbohydrate diet. Similar findings have also been shown in studies with rats (246).

Not all studies, however, have shown that muscle glycogen depletion is the cause of fatigue in prolonged exercise (37, 65, 89, 115, 150). Particularly, one challenge to the "glycogen depletion causes exhaustion" theory came from Coyle et al.(89) who showed that exercise could be continued even when muscle glycogen content was low, provided that the blood glucose concentration remained high. Cyclists ingested either a glucose polymer solution or water placebo while cycling at 70% of $VO_{2\ max}$. Those subjects ingesting the carbohydrate solution were able to exercise for an hour longer than subjects ingesting the placebo, even though their muscle glycogen concentrations during that hour were as low as those of the subjects in the first trial who could not continue and who were exhausted, supposedly as a result of muscle glycogen depletion alone. Thus, Coyle et al. (89) concluded that it could not have been muscle glycogen depletion that stopped the subjects from continuing to exercise, but rather an inadequate supply of plasma glucose for oxidation. Ingestion of glucose polymer appears to have provided glucose at a rate high enough for the muscle to maintain an adequate rate of carbohydrate oxidation late in exercise. The exogenous carbohydrate prevented the development of hypoglycaemia and provided an adequate source of carbohydrate as fuel substrate for oxidation. The final conclusion of the study was that as long as the muscle was provided with sufficient glucose to oxidise, exercise could be continued when normally this would not be possible because hypoglycaemia terminated exercise prematurely. However, if the data are examined carefully, it becomes apparent that what Coyle et al. (89) termed low muscle glycogen concentrations are not really all *that* low.

The muscle glycogen concentrations they measured at exhaustion were around 40 mmol/kg ww, whereas it has previously been reported that 17 - 28 mmol/kg ww is the concentration consistent with exhaustion (189). Thus, it is likely that the ergogenic effect of the carbohydrate was mainly due to the maintenance of euglycaemia in the over-night fasted subjects of Coyle et al. during the additional hour of exercise.

Numerous other studies have also indicated that hypoglycaemia is a cause of fatigue (55, 65, 72, 86, 211, 238) or have shown an increased time to fatigue when euglycaemia is either maintained by ingestion of carbohydrate (70, 71, 81, 85, 87, 89, 91, 93, 94) or restored by ingestion of carbohydrate (69) or infusion of glucose (69).

The ingestion of carbohydrate during exercise has resulted in a decrease in ratings of perceived exertion (RPE) in some (51, 294, 350), but not all studies (111, 112, 116, 172, 253). Of particular interest is the study by Murray et al. (253) in which subjects improved exercise performance but showed no decrease in RPE after carbohydrate ingestion. In another study, glucose was infused intermittently into overnight-fasted subjects exercising for 220 min at 50% of $VO_{2\ max}$. During the first 120 min when hepatic and muscle glycogen stores were presumably adequate, RPE was not influenced by the infusion of glucose, but later in exercise when glycogen levels were probably becoming depleted, the glucose infusion lowered RPE values (327). However, these findings could also be explained by a maintenance of blood glucose concentration by hepatic glycogenolysis during the initial exercise period, but once hepatic glycogen was depleted, blood glucose concentration may have declined during the periods of sham infusion, leading to increased RPE, unrelated to muscle glycogen concentration.

In a laboratory-based performance trial, Widrick et al. (349) examined whether the ergogenic benefits of carbohydrate ingestion are affected by pre-exercise muscle glycogen levels. Cyclists performed four time-trials on a laboratory ergometer over a distance of 70 km, with either high or low pre-exercise muscle glycogen levels (HG and LG, respectively) and ingested either carbohydrate (CHO) or water (W) during the trial. The carbohydrate drink provided ~58 g CHO/hr. No differences in power output or speed were observed in the HG-CHO, HG-W, and LG-CHO trials over the final 14% of the time-trial, but both variables were significantly lower during the LG-W trial (349). No fuel substrate kinetics such as rates of hepatic glucose appearance were determined.

Influence of carbohydrate ingestion and FFA concentrations on patterns of muscle glycogen utilisation

In a review article in 1984, Coyle and Coggan (88) stated that carbohydrate ingestion delays fatigue by slowing the rate of muscle glycogen depletion. By 1986, however, the same authors showed that trained cyclists who exercised at 71% of $VO_{2\ max}$ to fatigue whilst ingesting either water placebo or a glucose polymer solution, could exercise for an hour longer before becoming fatigued when polymer rather than water was ingested, although there was no difference in the rate of muscle glycogen utilisation (89). Hargreaves and Briggs (137) also found no muscle glycogen sparing during exercise at 70% of $VO_{2\ max}$ when 120 g of carbohydrate was ingested during 2 hr of exercise. In an earlier trial by Hargreaves et al.

(140) in which the carbohydrate was ingested 45 min prior to exercise, muscle glycogen utilisation actually *increased* as a result of glucose ingestion prior to exercise. This was probably due to the fact that the subjects became hypoglycaemic during the first 20 min of exercise, thus increasing the dependence of the muscle on glycogen. Neufer et al. (256) also failed to show any muscle glycogen sparing effect during 45 min of exercise after the ingestion of 45 g of glucose prior to exercise at 77% of $VO_{2\ max}$, but in this study, the 45 min exercise period may have been too short to demonstrate any possible glycogen sparing effect.

A number of other researchers (78, 91, 98, 116, 256, 262, 316) have failed to demonstrate a muscle glycogen sparing effect during exercise with ingestion of different types of carbohydrate. Ingestion of glucose or fructose 1 hr before exercise at 60% of $VO_{2\ max}$ did not result in a sparing of muscle glycogen in the study by Decombaz et al. (98), but as in the study of Neufer et al. (256), exercise lasted for only 45 min. Likewise, no muscle glycogen sparing occurred in a study by Hargreaves et al. (139) in which subjects exercised at 75% of $VO_{2\ max}$ to fatigue after ingestion of 75 g of glucose, fructose or placebo. Neither were there any differences in exercise time to fatigue, which was about 90 min. The failure to show muscle glycogen sparing in this study may have been due to the moderately high exercise intensity and short time to fatigue.

Some studies have even failed to show a glycogen sparing effect with glucose infusion (69, 92). However, Hultman et al. (158) reported a muscle glycogen sparing effect at very high blood glucose concentrations (28 mmol/l), attained with intravenous glucose infusion. At more modest rates of glucose infusion in exercising humans, Galbo et al. (121) also found that glycogen utilisation rates in the active muscles tended to decrease, whereas total carbohydrate oxidation rate remained unchanged. Other studies have also shown that infusion of glucose results in muscle glycogen sparing or resynthesis (166) in dogs, and an increased exercise time to fatigue in rats (17).

A sparing of muscle glycogen without glucose infusion has been shown by Levine et al. (214) who studied runners who ran for 30 min at 75% of $VO_{2\ max}$ after ingesting 300 ml of either water or a solution containing 75 g fructose or glucose. They found that the extent of muscle glycogen depletion with fructose ingestion was lower than in the control group or the group who ingested glucose.

Subjects also ingested fructose in the study of Bjorkman et al. (34). In that study, 53 g/hr of fructose, glucose, or water was ingested while cycling at 68% of $VO_{2\ max}$. In subjects who ingested glucose during exercise, muscle glycogenolysis was only 56% of that in subjects who ingested water or fructose. In a study of Hargreaves et al. (366), cyclists who ingested 43 g of sucrose in solid form with 400 ml of water at the start of exercise and then every hour for 4 hr during an intermittent cycling protocol, showed 20% less muscle glycogen utilisation compared to a control group. During a sprint ride to exhaustion at 100% of $VO_{2\ max}$ at the end of the trial, the group that ingested carbohydrate was able to exercise for 45% longer (138). Erickson et al. (107) also showed a reduction in muscle glycogen use during 90 min of cycling at 65 - 70% of $VO_{2\ max}$ when 1 g/kg glucose was ingested during exercise. Muscle glycogen sparing after

carbohydrate ingestion has also been shown by Bergstrom and Hultman (29), Yaspelkis et al. (364), and Kuipers et al. (204). However, in none of these studies (29, 107, 138, 204, 364) were muscle glycogen concentrations high at the start of exercise.

In the study of Kuipers et al. (204), subjects exercised at 40% of $VO_{2\ max}$ for 3 hr or rested for 3 hr after first undergoing glycogen-depleting exercise to exhaustion. During the 3 hr of exercise or rest, 2 litres of a 25% malto-dextrin solution was ingested. Glycogen repletion occurred in type IIa and type IIb fibres of the vastus lateralis during exercise. Thus carbohydrate ingestion during exercise, as well as providing a substrate for energy metabolism, can also be utilised for glycogen synthesis in the inactive skeletal muscle fibres. No sparing of glycogen after ingestion of varying (0 - 24 g/hr) amounts of carbohydrate was found by Mitchell et al. (247), but the amount of carbohydrate ingested may have been too little to show any glycogen sparing effect.

Contrary to some of the findings in humans (98, 140, 256), ingestion of glucose before exercise resulted in muscle glycogen sparing in rats (16). However, glycogen depletion, rather than glucose ingestion *per se*, stimulates glycogen resynthesis in muscle during exercise in rats (203). In a study by Constable et al. (78), glucose feeding in rats that started exercise in a glycogen depleted state resulted in an increase in muscle glycogen content during 90 min of exercise. However, there was no hepatic glycogen sparing. In another rat study (163) in which labelled glucose was infused 10 min before the end of 90 min of exercise, it was found that the glucose was incorporated into muscle and hepatic glycogen. Addington and Grunewald (1) reported a greater decline in hepatic glycogen content in rats after ingestion of fructose prior to exercise, and a smaller decline in muscle glycogen content, compared to the decline after glucose and sucrose ingestion. Muscle glycogen sparing has also been shown after fructose infusion in dogs (199).

The possible glycogen sparing effect of non-carbohydrate fuel substrates have also been investigated. Decombaz et al. (97) fed subjects either 1 MJ of medium chain triglycerides or malto-dextrin 1 hr before exercise at 60% of $VO_{2\ max}$ and found no difference in the contribution of carbohydrate to energy utilisation and no sparing of muscle glycogen. Likewise, ingestion of medium or long chain triglycerides immediately before a 2 hr run in trained rats was found to have no hepatic or muscle glycogen sparing effect (16), and neither has ingestion of glycerol 30 min before exercise (245).

Increased plasma FFA concentrations have also failed to show a glycogen sparing effect in some studies (216, 283), but this is controversial. In some studies, elevated plasma FFA concentrations after heparin (80) or intralipid (105) infusion or caffeine ingestion to increase plasma FFA concentrations (107), spared muscle glycogen during exercise in human subjects, in some studies by up to 40% (80). Other studies have also shown that increased oxidation of FFA spares muscle glycogen (41, 200, 270, 288, 340) and increases exercise time to exhaustion (41, 270).

Studies of the metabolic effects of muscle and hepatic glycogen depletion and fatigue

The mechanism whereby muscle glycogen depletion causes fatigue has been investigated by Broberg and Sahlin (46, 47) who have shown that submaximal exercise to exhaustion results in a breakdown of the total adenine nucleotide pool (ATP + ADP + AMP) in the working muscle through deamination of AMP to IMP and NH_3^+ ions. They concluded that the higher average rate of AMP deamination when muscle glycogen is depleted may be due to a relative impairment of ATP resynthesis caused by the low muscle glycogen levels. This has also been shown by other authors (159, 162, 263). However, no significant decrease in muscle ATP content at fatigue has been shown in other studies (19, 341). More recently, Broberg and Sahlin (298) have shown that deamination of AMP is enhanced during exercise when the capacity to re-phosphorylate ADP is impaired. In human muscle, the formation of IMP (20, 299, 317) and NH_3^+ ions (46, 263, 298, 316) during exercise is greater when muscle glycogen is depleted or when muscle glycogenolysis is reduced. This is also true for exercise at submaximal intensities (46).

Muscle glycogen depletion has other detrimental effects. Exercise for 60 min at 60% of $VO_{2\ max}$ after either carbohydrate-loading or depletion (213) caused serum and sweat urea nitrogen concentration to increase up to 154-fold in glycogen depleted subjects compared to a 66-fold increase in carbohydrate-loaded subjects. This corresponds to a protein breakdown of 13.7 g/hr, or 10.4% of the total caloric cost in the glycogen depleted group (213) and contradicts the data of Wagenmakers et al. (342), which showed that amino acids and protein did not contribute substantially as an energy source during exercise.

Another study of the effect of starting muscle glycogen content on amino acid metabolism was performed on trained cyclists after either glycogen depletion or carbohydrate-loading (341). Whereas subjects were able to complete 2 hr of exercise at 70 - 75% of maximal workload after carbohydrate-loading, the intensity had to be reduced in glycogen depleted subjects. Plasma NH_3^+ ion concentrations increased more rapidly and plasma alanine, glutamate and glutamine concentrations were lower in glycogen depleted subjects. In the latter subjects, there was an increase in the concentration of active branched-chain 2-oxoacid dehydrogenase (BC) complex in muscle, indicating an increased oxidation of branch chain amino acids (BCAA). No activation of BC occurred in the carbohydrate-loaded subjects and an inverse correlation between the activity of the BC complex and the glycogen content in the post-exercise muscle biopsy samples was found. Thus carbohydrate-loading abolished the increases in BCAA oxidation during exercise and part of the NH_3^+ ion production during prolonged exercise. The conclusion drawn was that acceleration of the BCAA aminotransferase reaction may drain the Krebs cycle (341). Krebs cycle intermediates have been shown in other studies to be reduced with muscle glycogen depletion and fatigue (299), but increased in muscle with a high glycogen content (317).

The importance of muscle glycogen in determining exercise time to exhaustion is illustrated in a study by Van Baak et al. (331), in which β blockers were administered to inhibit glycogenolysis in subjects who exercised at 70% of $VO_{2\ max}$. Exercise time to exhaustion was reduced by 40% by the β blocker. Infusion

of intralipid and heparin to provide an alternative source of FFA did not improve (restore) exercise time to exhaustion. Thus if glycogenolysis is inhibited, availability of FFA is not a major factor determining exercise time to exhaustion. Conversely, Pernow and Saltin (273) in human studies, and Stankiewicz-Chorozucha and Gorski (321) working with rats, used nicotinic acid to block FFA release from fat cells, thus limiting the contribution of FFA as a fuel substrate. Accelerated utilisation of muscle glycogen occurred and caused more rapid fatigue. Liver glycogen utilisation has also been shown to increase when plasma FFA concentration are reduced (200, 288, 321).

Low muscle glycogen and plasma glucose concentrations are not the only possible metabolic causes of exhaustion. According to Hultman et al. (159, 162), muscle contraction requires energy in at least three different ATPase reactions and can consequently be inhibited when ATP hydrolysis is decreased, that is, when ATP content is too low or when the reaction products (ADP, Pi and H^+) reach inhibiting levels, or when muscle pH has decreased to values inhibiting actomyosin ATPase activity. Low pH also decreases Ca^{2+} release from the sarcoplasmic reticulum and affinity of troponin for Ca^{2+}. The negative effect of either of these processes on fatigue has been demonstrated by numerous researchers (54, 149, 211, 212, 319, 320, 326).

Effect of muscle glycogen status on glucoregulatory hormones and other substrates

The effects of a low carbohydrate diet and muscle glycogen depletion on substrate metabolism and hormonal changes during exercise were investigated in a study by Helie et al. (147). They infused glucose intravenously at a constant rate during 75 min of exercise at 60% of $VO_{2\ max}$ and found that in glycogen depleted subjects, epinephrine and FFA concentrations were higher and RER values and insulin concentrations lower, than in non-depleted subjects. Others have also shown that plasma norepinephrine concentrations after exercise are higher in subjects ingesting a low carbohydrate diet (181, 207, 300) and negatively correlated to muscle glycogen concentrations (300).

Jacobs et al. (175) examined the influence of a high carbohydrate diet on skeletal muscle lipoprotein lipase (LPL) activity and muscle glycogen levels by comparison with a short-term adaptation to a fat and protein diet. After the fat-protein diet, glycogen concentration was 55% of that of a mixed diet, and LPL activity increased by 21%. After the high carbohydrate diet, glycogen levels increased by 82% and LPL activity decreased by 55% compared to the mixed diet. The changes in LPL activity after the carbohydrate diet were related to the changes in muscle glycogen concentration. It was concluded that the uptake of fat from the circulation may be regulated by the cytoplasmic substrate availability in the muscles (175).

Carbohydrate-loading has also been shown to decrease plasma FFA (123, 181, 207), glycerol (181, 207), and cortisol (207) concentrations and increase insulin concentrations (181, 207), during cycling at 64 - 70% of $VO_{2\ max}$. Carbohydrate oxidation, glycogen utilisation (123, 129, 289), glucose and lactate concentrations (123, 181, 289) and rate of appearance of glucose from liver were higher (123, 181) in carbohydrate-loaded subjects than in glycogen depleted subjects. A study by Lavoie et al. (206) in which one

leg was glycogen-loaded and the other glycogen depleted, showed that when subjects exercised at 70% of $VO_{2\ max}$ for 20 min using either the depleted or the loaded leg and then continued for another 20 min with the other leg, plasma FFA and glycerol concentrations decreased and plasma lactate concentrations increased when switching from the depleted to the loaded leg. The opposite response was observed when the subjects switched from the loaded to the depleted leg.

Plasma insulin concentrations decreased during exercise in subjects with both high and low muscle glycogen content (123), but the decrease was greater in subjects with low, than with high glycogen concentrations in a study by Jansson et al. (181). In contrast, glucagon (123), epinephrine (123, 181), growth hormone (123) and cortisol (123) concentrations were higher in subjects with low muscle glycogen content. However, infusion of glucose (123) decreased epinephrine and glucagon concentrations to pre-exercise levels. Infusion of insulin with the glucose (123) did not change the plasma levels of these hormones more than glucose infusion alone, but decreased FFA concentrations.

Effects of training on fuel substrate oxidation

Trained subjects oxidise more fat and less carbohydrate than untrained subjects when performing submaximal work at the same absolute intensity (15, 18). This increased capacity to utilise energy from fat conserves crucial muscle and hepatic glycogen stores and can contribute to increased endurance (15). The extent of the increase in fat oxidation with training (154, 155, 196) may depend on the percentage of type I and II muscle fibres, since type I fibres have a greater capacity to oxidise FFA than type II fibres (148).

Others have also noted that training results in less utilisation of muscle glycogen, a smaller increase in plasma lactate concentration (18, 154) and less utilisation of blood glucose (73, 154) during exercise at the same workload. Decreases in the rate of plasma glucose oxidation accounted for approximately half of the total reduction in carbohydrate oxidation during the last 30 min of a 2 hr exercise period in trained compared to untrained subjects (73). Training also increased exogenous glucose oxidation, resulting in sparing of endogenous carbohydrate in human subjects, but did not effect the response of plasma glucose, insulin or FFA concentrations to glucose ingestion in the study of Krzentowski et al. (202). In trained subjects, plasma glucose turnover is less than untrained subjects at the same absolute intensity (73), but in trained rats, glucose turnover is increased at a high exercise intensity (50).

As previously discussed, training results in greater muscle (18, 255, 328) and hepatic glycogen stores (18, 328). The higher muscle glycogen levels of trained athletes can be partly explained by changes that may occur in eating patterns as people become fitter. The trend is to eat a higher carbohydrate diet as fitness increases, causing an increase in muscle glycogen stores that would occur regardless of any training effects. The effect of dietary changes alone, however, can be established from studies by Hultman (157) and Jardine et al. (184) in which untrained people eating a high (70%) carbohydrate diet had muscle glycogen levels of up to 100 mmol/kg ww. Thus it appears that the remaining increase (from 100 to 130 mmol/kg ww) in athletes occurs as a consequence of training. The mechanism for this may be explained by the finding in

rats that training increases glycogen synthase and phosphorylase activities 60 - 150% , and glycogen content in the soleus by 50 - 70% , the increase being proportional to the degree of training (176).

Control of muscle glycogenolysis

In non-exercising skeletal muscle, glycogenolysis can occur independently of contractile activity (41, 240, 243, 354) when epinephrine levels rise (4), but both epinephrine (41, 60, 143, 223) and contractile activity (41, 60, 143, 144, 223) regulate glycogenolysis in working muscle.

Issekutz (167) proposed that glycogenolysis in working muscle is controlled by two mechanisms: an intracellular control operating at the beginning of exercise and a hormonal control involving epinephrine and β adrenergic receptors later in exercise, after demonstrating that during the first 15 min of exercise the rate of appearance of lactate increased rapidly in both control dogs and those receiving a propanolol infusion, but that with the continuation of exercise the rate of lactate appearance was lower in animals receiving a propanolol infusion than in controls.

Further evidence for the role of epinephrine in muscle glycogenolysis has been shown by Richter et al. (290) in a study in which muscle glycogenolysis was reduced by adrenomedullation in dogs, but not by sympathectomy. In a follow-up study, Richter et al. (293) showed that the inhibition of glycogenolysis in exercising muscle with adrenomedullation was not accompanied by a compensatory increase in the rate of breakdown of triglyceride and protein in muscle or liver, suggesting that all these processes are stimulated by epinephrine. However, infusion of epinephrine into running rats has been shown to stimulate muscle glycogenolysis but not hepatic glycogenolysis (14). Similarly, another study with running rats by Jansson et al. (182) showed that physiological concentrations of epinephrine may enhance muscle glycogenolysis during submaximal exercise. The incremental addition of epinephrine in the presence of insulin in an isolated incubated rat skeletal muscle preparation caused a 15-fold increase in glycogenolytic rate (59), while insulin alone only caused a 7-fold increase in glycogenolysis (59).

In contrast, high circulating FFA concentrations decrease rates of hepatic and muscle glycogen depletion in exercising rats (200, 288). Similarly, Dohm et al. (102) demonstrated that rats that had higher FFA concentrations as a result of fasting, were able to run longer and with a lower blood glucose concentration than fed rats. It was suggested that the improved endurance was due to a lower rate of muscle glycogen utilisation in the fasted rats. Conversely, blocking FFA release resulted in an increase in muscle glycogen utilisation in both humans (129, 273) and rats (321). Liver glycogen utilisation also increases when the plasma FFA concentration is reduced (200, 288, 321). Thus FFA concentrations plays an important role in regulating glycogen utilisation.

Allosteric regulators may be important controllers in skeletal muscle glycogenolysis. This was indicated in a rat hind limb perfusion experiment in sympathectomised or adrenomedullated rats receiving epinephrine treatment either at rest or during electrically induced muscle contractions (205). When epinephrine or

norepinephrine deficiency, or both, was induced, muscle contraction resulted in a decrease in glycogen content (-63%) despite a decrease in the ratio of glycogen phosphorylase a to phosphorylase b, and an increase in the ratio of glycogen synthase I to glycogen synthase D. Under these conditions, epinephrine treatment further reduced muscle glycogen content while blunting the changes in the activity ratio of the rate-limiting enzymes, indicating that catecholamines do not play a primary role in regulating skeletal muscle glycogen breakdown during acute exercise. A dissociation between the percentage of glycogen phosphorylase in the 'a' form and glycogenolytic rate has also been demonstrated in other studies (60, 127) and indicates that glycogenolysis is regulated by both allosteric effectors and enzyme interconversion. Activity of phosphorylase a is dependent on the concentration of Pi in muscle (60, 62, 143) and is inhibited by a low pH (61). With increasing exercise intensity, the percentage of phosphorylase in the 'a' form increases. However, with increasing duration of exercise, the rate of glycogenolysis decreases even though the percentage of phosphorylase in the 'a' form may remain constant (127).

The effect of different pre-contraction muscle glycogen concentrations on rate of muscle glycogenolysis, glucose uptake and transport, free glucose and glucose-6-phosphate concentrations have been studied by Hespel and Richter (151) in perfused resting and contracting rat skeletal muscle. A positive correlation was found between pre-contraction muscle glycogen concentration and rates of muscle glycogenolysis. A number of other studies have also found that higher muscle glycogen levels at the start of exercise result in a greater rate of muscle glycogen utilisation during exercise (28, 129, 143, 206, 289, 306). Glycogen concentration may therefore be an important determinant of glycogen phosphorylase activity in contracting skeletal muscle, as phosphorylase a activity is approximately twice as high in carbohydrate-loaded rat muscles than in glycogen depleted muscles (152). However, Ren (286) found no relationship between phosphorylase a activity and glycogen content of muscle in exercising humans when muscle glycogen concentration was fairly low (36 - 81 mmol/kg ww) and exercise intensity was high.

Because they found no difference in the rates of muscle glycogen utilisation or lactate production between glycogen-loaded and depleted subjects during exercise at 95% of $VO_{2\ max}$, Spencer and Katz (316) proposed that, although the accumulation of fructose-6-phosphate, which activates phosphofructokinase (PFK), was decreased with muscle glycogen depletion, glycogenolysis was maintained due to the accumulation of AMP and ADP, which also activate PFK.

In a study in which one leg was carbohydrate-loaded and the other not, Bangsbo et al. (20) found that there was no difference in glycogen utilisation between the loaded leg and non-loaded leg during supramaximal exercise. In contrast, at a lower exercise intensity of 75% of $VO_{2\ max}$, subjects with high muscle glycogen concentrations utilised more muscle glycogen than when the same subjects exercised without prior carbohydrate-loading (2.4 vs 1.1 mmol/min/kg ww) (317). However, when muscle glycogen levels fall below 80 mmol/kg ww there is no longer an influence from initial muscle glycogen concentration on the rate of utilisation, at least during short, intense contraction (286).

Similar findings were obtained by Yan et al. (363) in subjects who cycled to fatigue at 75% of $VO_{2\ max}$ after prior glycogen depletion or carbohydrate-loading. In glycogen depleted subjects, muscle glycogen concentration decreased by 36 mmol/kg ww during 35 min of exercise and glycogen synthase fractional activity (GSF) was higher throughout exercise, whereas in the carbohydrate-loaded subjects, muscle glycogen concentration decreased by 70 mmol/kg ww during the same period and in these latter subjects, the change in GSF in muscle was positively related to the exercise duration and negatively related to the glycogen content at the end of exercise.

As muscle glycogen stores become depleted, the contribution of muscle glycogen to carbohydrate oxidation during exercise at 70% of $VO_{2\ max}$ decreases (316). The decrease in glycogenolysis during prolonged exercise coincides with an inhibition of glycogen phosphorylase activation which is associated with decreased muscle glycogen stores (152), but other, as yet unidentified factors associated with prolonged exercise also play a role in the decrease in glycogenolysis (77).

Rates of muscle glycogen utilisation during cycling are influenced by both exercise intensity and pedalling frequency. Cycling at 60% of $VO_{2\ max}$ for 2 hr results in a 77% decline in muscle glycogen concentration, with only minor elevation of muscle and blood lactate concentrations, whereas ten 1 min supramaximal work bouts results in a 52% decrease in glycogen concentration and substantially elevated muscle and blood lactate concentrations. These differences may be due to glycogen depletion during the submaximal exercise occurring mainly in the slow twitch muscle fibres, whereas during supramaximal exercise the type IIb fibres become the most glycogen depleted (329). Vollestadt et al. (336) showed that, after 60 min of exercise at 43% of $VO_{2\ max}$, almost all type I fibres and about 20% of type IIa fibres showed depletion. After the same exercise period at 61% of $VO_{2\ max}$, almost all type I fibres were depleted and about 65% of type IIa fibres. At 91% of $VO_{2\ max}$, all type I and IIa fibres and about 50% of type IIb fibres were depleted. Pedalling frequency may affect the rate of muscle glycogen utilisation in only the type II muscle fibres, which was found to be higher at lower pedal frequencies, probably due to the higher muscle force required (9).

Glycogenolysis in non-exercising muscle

Decrements in muscle glycogen content have been found to be similar in both non-exercising and exercising muscles after 90 min of exercise in rats (241). In both non-exercising and exercising muscles, lactate concentrations increased, but there were only slight changes in levels of glycolytic intermediates (241). The sum of the accumulated glycolytic intermediates and lactate in the non-exercising muscles accounted for 15-28% of the glycogen degraded, indicating that the lactate produced from glycogenolysis in non-exercising muscle is not retained within the muscle (241). Ahlborg (4) showed that the stimulus for this glycogenolysis in non-working muscle during exercise is an increase in epinephrine concentration.

The formation of lactate under fully aerobic conditions is thought to represent an important mechanism whereby different tissues share a common carbon source for oxidation and other processes such as gluconeogenesis (49). During high intensity cycling, 60% of the muscle glycogen utilised reappears as

lactate within the working muscle, 20 - 25% is found as other glycolytic intermediates, and 4 - 13% is oxidised (242). Lactate released to blood accounts for approximately 10% of all lactate produced (242). During sustained, steady-state exercise, most (75%+) of the lactate formed is oxidised (48). Only approximately 20% is converted to glucose (48). During supine cycling exercise at 43% of $VO_{2\ max}$, it has been reported (323) that 20% of glucose utilisation goes to lactate formation, 20% of blood lactate appearance is from blood glucose, and approximately 20% of the carbohydrate oxidised enters the circulating lactate pool before it is completely oxidised. Lactate extraction of approximately 50% occurs during net lactate release from active skeletal muscle. Simultaneous production and utilisation of lactate has also been shown in exercising dog muscle by Issekutz et al. (169) and by Stanley et al. (322) in human muscle. Substrate supply was shown by Issekutz et al. (169) to be the major factor controlling the rate of lactate oxidation and its use in gluconeogenesis. Thus oxidation of lactate is quantitatively the most important means of disposing of lactate, whether in exercising or non-exercising muscle.

The lactate gradient between muscle and blood may be an important factor dictating whether lactate is taken up or released by muscle, independent of whether the muscle is active or not (239). Gollnick et al. (129) found that during two-legged cycling with one leg glycogen depleted and the other with normal glycogen concentration, the low glycogen leg extracted lactate from the blood, whereas the normal leg released lactate.

After glycogen depletion, lactate production increases later in exercise (278) and after carbohydrate-loading, lactate concentration is higher during exercise (317). In both subjects with normal glycogen, and glycogen depleted subjects, there is a high correlation between plasma lactate and catecholamine concentrations during exercise (278).

Factors influencing glucose uptake by muscles

Leg glucose uptake during exercise can be substantial. Using the arterio-venous difference technique, Martin et al. (226) showed that leg glucose uptake provided approximately 23% of energy after a fat-protein diet, 30% after a mixed diet and 46% after a high carbohydrate diet during the first 30 min of exercise at 70% of $VO_{2\ max}$, but these differences were not significant. Jansson and Kaijser (183) have reported a slightly higher rate of plasma glucose uptake in human subjects who followed a low (5%) carbohydrate diet before exercise, compared to subjects who followed a 70% carbohydrate diet. Again, these differences were not significant.

Glucose uptake during contraction has been found to be approximately 50% higher in glycogen depleted muscle than in control muscle; in glycogen loaded muscle uptake was 30% lower than in control muscle (151). Muscle cell membrane glucose transport, as measured by the rate of accumulation of ^{14}C-3-O-methylglucose in the contracting muscles, was 25% lower in glycogen loaded than in glycogen depleted muscles at the onset as well as at the end of a 15 min contraction period. It was concluded that the rate of glucose uptake in contracting skeletal muscle is dependent on the pre-contraction muscle glycogen

concentration and that regulating mechanisms include limitations of cell membrane glucose transport as well as of glucose metabolism, as indicated by an accumulation of free glucose and glucose-6-phosphate in muscle in which glycogen concentration is high (151). Gollnick et al. (129) and Hargreaves et al. (142) have shown that leg glucose uptake is positively correlated with the percentage of glycogen depleted muscle fibres and negatively correlated with the intramuscular glucose-6-phosphate concentration.

The entry of glucose into muscle cells is achieved primarily via a carrier-mediated system consisting of protein transport molecules (21). GLUT-1 transporter isoform is normally found in the sarcolemmal membrane and is thought to be involved in glucose transport at rest (21). With insulin stimulation, glucose transport is accelerated by translocating GLUT-4 transporters from an intracellular pool to the T-tubule system and sarcolemmal membranes. Activation of transporters may also be involved, but the evidence is not conclusive. When insulin binds to its receptor, it autophosphorylates tyrosine and serine residues on the β-subunit of the receptor (21). The tyrosine residues are thought to activate tyrosine kinases, which in turn phosphorylate and possibly thereby activate as yet unknown second messengers. Insulin receptor antibodies, however, have been reported to increase glucose transport without increasing kinase activity (21), implying that another second messenger may be involved, as insulin has been shown to increase diacylglycerol and protein kinase C activity in target cells (110).

Although the number of GLUT-4 transporters in the sarcolemma increases with exercise, neither insulin nor its receptor is involved (21). After an initial acute phase, which may involve calcium as the activator, a secondary phase of increased insulin sensitivity can last for up to a day after exercise. The mechanism responsible for the increased insulin sensitivity with exercise is unknown. Regular exercise training also increases insulin sensitivity, which can be documented several days after the final bout of exercise, and again the mechanism is unknown (21).

Exercise (contractile activity) (99, 243, 292, 366) and increases in insulin concentration (99, 106, 131, 228, 243, 292, 360), both increase glucose uptake by non-exercising and exercising muscles. An increase in exercise intensity also increases oxidation of plasma glucose by muscle (296). Conversely, insulinopaenia attenuates the increases in muscle glucose uptake and oxidation that occurs with exercise by approximately 50% , independent of changes in circulating metabolic substrate levels. Substantial increases in muscle glucose uptake and oxidation are, however, still present with exercise even in the absence of detectable insulin levels (347). Insulin-independent glucose uptake occurs during exercise in dogs, but normal insulin concentrations are required for a full response (33). In another study with dogs, Zinker et al. (366) demonstrated that exercise increased the V_{max} but did not change the K_m for leg glucose uptake, and that glucose uptake increased with increasing plasma glucose concentrations when insulin was maintained at basal levels. The effects of exercise and glucose concentration on glucose uptake were synergistic.

Recent findings (66) indicate that glucose uptake by contracting hindlimb muscles of the rat is stimulated by epinephrine acting through alpha-adrenergic mechanisms. Since hepatic glucose output may be increased markedly by activation of alpha-adrenergic receptors during exercise and matched by the increase

in muscle glucose uptake so that the blood glucose concentration is maintained relatively constant, it is now proposed by Clark et al. (66) that a general co-ordination of glucose metabolism may operate via alpha-adrenergic receptor mechanisms.

Control of plasma glucose concentration

As discussed previously, many studies have shown an association between hypoglycaemia and fatigue (55, 69, 70, 71, 72, 81, 85, 86, 87, 89, 91, 93, 94, 211, 238) and maintenance of plasma glucose concentrations within a narrow range is crucial even at rest (125). The liver is the most important source of plasma glucose (125) and considering the importance of the control of glucose homeostasis it is not surprising that a variety of mechanisms exist for regulating hepatic glycogenolysis during exercise. These include decreased circulating insulin:glucagon ratio (165), increased epinephrine or norepinephrine concentrations, or both (33, 195, 224, 225, 272, 290, 314), sympathetic neural activation (311, 312, 313, 333, 337, 343), and autoregulation by the liver in response to a change in blood glucose concentration (185, 257).

According to Wolfe et al. (360), there must be a reduction in insulin concentration or an increase in glucagon concentration, or both, during exercise if hypoglycaemia is to be prevented and plasma glucose homeostasis maintained (360). This was demonstrated in an experiment to determine whether plasma glucose homeostasis can be maintained during exercise at 40% of $VO_{2\ max}$ when changes in plasma insulin and glucagon concentrations are prevented. Hormonal control was achieved by the infusion of somatostatin, insulin, and glucagon. Two different rates of replacement of insulin and glucagon were used. In one group, the hormone levels were clamped at approximately resting portal venous concentrations, and in the other group, at peripheral venous concentrations. Without hormonal control, plasma glucose homeostasis was maintained during exercise because the increase in glucose uptake was balanced by a corresponding increase in hepatic glucose production. When changes in insulin and glucagon were prevented by hormonal control as described, plasma glucose concentration fell and epinephrine increased, particularly in the high (portal concentration) insulin group. Glucose uptake increased to a greater extent than when hormonal control was not used, and glucose production did not increase sufficiently to compensate for the increased glucose uptake. The increase in glucose uptake in the hormonal control groups was associated with an increased rate of glucose oxidation. When euglycaemia was maintained by glucose infusion in the hormonal control subjects, the modest increase in glucose production that otherwise occurred, was prevented.

Increase in hepatic glucose appearance (Ra) in exercising dogs has been found to be directly correlated with the glucagon : insulin molar ratio (244, 346). Glucagon stimulates hepatic glycogenolysis in rats (122, 291) and controls approximately 70% (346) to 80% (dogs) (338) of the increase in Ra during exercise. Insulinopenia facilitates the increase in Ra, independent of the increase due to glucagon action (347). In contrast to the findings of Wasserman et al. (346) and Wolfe et al. (360), Vranic et al. (339) reported that during high intensity exercise in dogs, regulation of hepatic glucose production during exercise is multifactorial and that the glucagon : insulin molar ratio was not an essential regulator of glucose

metabolism during exercise. They hypothesised that increases in blood flow and capillary surface area might increase the amount of insulin delivered to the muscle even when serum insulin concentrations are reduced during exercise (339).

During exercise, and particularly under fasted conditions, plasma epinephrine concentrations were shown by Born et al. (42) to increase gluconeogenesis from lactate in rats, a finding confirmed by Cherington et al. (63) in dogs. Gluconeogenesis in rats accounts for a substantial fraction of total Ra (330). In a study with rats injected with a gluconeogenic inhibitor, mercaptopicolinic acid (MPA), Ra increased 45% during exercise in sham-injected rats but only 35% for the first 15 min in the MPA-treated rats, and then decreased to resting values. Concomitantly, plasma glucose concentration decreased 35% and blood lactate concentration increased 160% (330).

Plasma epinephrine concentrations increase in response to both hypoglycaemia and to exercise, but the response to these two stimuli can be dissociated, suggesting that different control mechanisms exist (315) and that, to some extent, the release of counter-regulatory hormones during exercise is regulated by glucose-independent mechanisms, although these responses may be augmented by concurrent hypoglycaemia.

To determine the role of adrenal medullary hormones in controlling the rate of hepatic glycogenolysis during exercise, adrenodemedullated and sham-operated rats were studied during running exercise (56). Adrenomedullation had no effect on the rate of hepatic glycogenolysis, or on hepatic adenosine 3',5'-cyclic monophosphate (cAMP), plasma glucagon, or plasma free fatty acid concentrations, but caused a reduction in glycogenolysis in type II muscle fibres during exercise. Adrenomedullation also prevented the normal exercise-induced increase in blood glucose and lactate concentrations and decline in plasma insulin concentrations. It was concluded that, during submaximal exercise, the principal targets for epinephrine released from the adrenal medulla appear to be receptors in the pancreatic beta-cells and skeletal muscle and not the liver. In a similar experiment, infusion of epinephrine into the adrenomedullated rats corrected hypoglycaemia that had occurred, restored plasma lactate concentrations to normal, and stimulated glycogenolysis in contracting type I and non - contracting type II muscle fibres (356).

In a subsequent study (358), fasted adrenodemedullated (ADM) and sham-operated rats (SHAM) were run on a rodent treadmill for 30 min or until exhaustion. ADM rats were infused with saline, epinephrine, glucose, or lactate during the exercise bouts. ADM saline-infused rats showed markedly reduced endurance, hypoglycaemia, elevated plasma insulin and reduced blood lactate concentrations, and reduced muscle glycogenolysis compared with exercising SHAM's. Epinephrine infusion corrected all these metabolic abnormalities, while glucose infusion restored endurance run times and blood glucose concentrations to normal without correcting the deficiencies in blood lactate concentrations and muscle glycogenolysis. It was concluded that, in the fasted exercising rat, the actions of epinephrine, in addition to provision of gluconeogenic substrate, are essential for preventing hypoglycaemia and allowing the rat to run for long periods of time (358).

The same authors (357) later presented data showing that hepatic fructose-2,6-bisphosphate concentrations decreased in rats as exercise intensity and duration increased. Changes in fructose-2,6-bisphosphate concentrations occurred before a detectable decline in hepatic glycogen content and in the absence of any significant change in blood glucose concentrations. The rapid decline in fructose-2,6-bisphosphate concentrations probably plays a role in decreasing hepatic glycolysis, thereby ensuring that glucose-6-phosphate derived from glycogenolysis is diverted to blood glucose. Fructose-2,6-bisphosphate was also shown to play a role in providing lactate from non-exercising muscle as a substrate for hepatic gluconeogenesis during prolonged exercise (355), by increasing muscle glycogenolysis in inactive type IIb fibres, and decreasing glycogenolysis in liver during exercise in fasted rats. Glucose-1,6-bisphosphate also increased in the type IIb fibres. The increases in fructose-2,6-bisphosphate and glucose-1,6-bisphosphate concentrations may thus be important in accelerating glycolysis and enhancing lactate production in muscles that are not glycogen depleted during long-term exercise. This effect was subsequently shown to be mediated by epinephrine (354).

Similarly, in a study by Sonne and Galbo (314), the rates of hepatic glycogenolysis and glucose production in rats were not influenced by hepatic denervation, but were halved by adrenomedullation, indicating that hepatic glycogenolysis and glucose production are not influenced by autonomic nerve supply to the liver, but are enhanced by circulating epinephrine. Hepatic innervation is also not essential to increase Ra (348) during moderate intensity exercise in dogs.

As in the Sonne and Galbo study (314), hepatic glycogenolysis was decreased in adrenomedullated rats but not in sham-operated rats in the study of Richter et al. (290). In the adrenomedullated rats, plasma insulin concentrations at exhaustion were higher and glucagon levels lower than in sham-operated rats (290). Marker et al. (224) showed in a study utilising α and β adrenergic blockade together with somatostatin, insulin and glucagon infusion, that the catecholamines play a minor role when changes in insulin and glucagon can occur, but become critical to the prevention of hypoglycaemia during exercise when changes in insulin and glucagon do not occur, as in the study of Wolfe et al. (360) described previously. Kjaer et al. (195) found that in exercise in humans, high physiological concentrations of epinephrine can increase Ra and decrease FFA and glycerol concentrations. It was also shown that hepatic sympathetic nerve activity was not a major stimulus for Ra during exercise. Others have also suggested that the catecholamines are the controllers of Ra (33, 225). However, some studies have found no correlation between epinephrine (37, 223, 244, 291, 346) and norepinephrine (244, 346) concentrations and hepatic glycogenolysis.

Studies by Lavoie et al. (208, 209) with rats supported the concept of the presence of hepatic glucoreceptors, which are sensitive to changes in hepatic glycogen concentration. During exercise, muscle glycogen utilisation was greater in rats with hepatic vagotomy than in sham-operated rats.

Evidence for the participation of hepatic glucoreceptors in the control of sympathetic activity during exercise has been provided by Bernal et al. (30). They found that intraportal infusion of glucose in small amounts into dogs, although not increasing plasma glucose or insulin levels, resulted in decreased FFA

mobilisation from adipose tissue. However, the infusion resulted in a reduction of the plasma norepinephrine response to exercise, indicating that this response is independent of the inhibitory effect of insulin on sympathetic activity, as plasma glucose levels did not increase (199).

The influence of hepatic glycogen levels on Ra during exercise was investigated by Vissing et al. (335). During treadmill running for 35 min, Ra and plasma glucose concentrations increased more and hepatic glycogenolysis was higher in fasted-refed compared with control rats, even though the stimuli for Ra were higher in control rats, as the plasma concentrations of insulin and glucose were lower. Plasma concentrations of glucagon and the catecholamines also tended to be higher in control compared with fasted-refed rats, indicating that hepatic glycogenolysis during exercise is directly related to hepatic glycogen content. The smaller endocrine glycogenolytic signal in face of higher plasma glucose concentrations in fasted-refed compared with control rats indicates metabolic feedback control of glucose mobilisation during exercise. However, the higher exercise-induced increase in Ra, plasma glucose concentrations and hepatic glycogenolysis in fasted-refed compared with control rats indicates that metabolic feedback mechanisms may not always accurately match Ra to the metabolic needs of working muscles.

Sonne et al. (313) have also shown that Ra depends on hepatic glycogen content. Subjects with low hepatic glycogen levels have been shown to have increased FFA (32, 210), glycerol (210) and plasma catecholamine concentrations and lower insulin concentrations (32, 210) compared with subjects who started exercise with normal hepatic glycogen content. Thus hepatic glycogen content may be involved in the regulatory mechanism. However, in the Lavoie et al. (210) study, the pre-experimental period of arm exercise and low-carbohydrate diet to reduce hepatic glycogen content, in spite of an increase in leg muscle glycogen content, means that those results must be interpreted with caution, since the arm exercise that preceded leg exercise, with the intention to deplete hepatic glycogen, may have resulted in glycogenolysis in the non-working leg muscle to provide substrate precursors for gluconeogenesis. The result is that the leg muscles may also have been glycogen depleted by the time that leg exercise started. Glycogen concentrations were not measured in this study and if this occurred, the results may be related to leg muscle glycogen depletion and the conclusions would need to be modified. However, in the study of Bjorkman et al. (32), in which subjects were fasted for 60 hr before exercise at 60% of $VO_{2\ max}$, Ra was found to be lower in the 60 hr compared to the overnight fasted subjects and gluconeogenesis accounted for approximately 80% of Ra in the 60 hr fasted subjects.

Various studies (185, 199, 224, 257, 352, 360) have suggested that Ra is regulated by feedback inhibition mediated by the blood glucose concentration. The hypothesis that Ra during exercise is subject to feedback control via glucose concentration within a control range that is determined by the circulating insulin concentration was investigated by Jenkins et al. (186). They found that a simple difference controller exists where Ra is proportional to the deviation of plasma glucose from a defined set point. Insulin affects Ra and regulates the steady-state glucose level by altering the sensitivity of this control system.

The possibility that feedforward control of hepatic glycogenolysis during exercise is modified by negative feedback when blood glucose concentration is elevated, was investigated by Winder et al. (352). With glucose infusion, hepatic glycogen content decreased less compared with saline-infused rats during 40 min of treadmill running. Hepatic adenosine 3',5'-cyclic monophosphate (cAMP) concentration was significantly lower in the glucose-infused rats during the exercise bout, which concurs with another study by Winder et al. (353) in which hepatic cAMP was found to increase only after hepatic glycogen was depleted. The concentration of hepatic fructose-2,6-bisphosphate remained elevated throughout the exercise bout in glucose-infused rats (352), but decreased markedly in saline-infused rats. Plasma insulin concentrations were higher and plasma glucagon concentrations were lower, in glucose-infused rats than in saline-infused rats during exercise. Thus, early in exercise, hepatic glycogenolysis proceeded in the glucose-infused rats despite the fact that glucose and insulin concentrations were markedly elevated and hepatic cAMP was unchanged from resting values, suggesting the existence of a cAMP-independent feedforward system for activation of hepatic glycogenolysis that can over-ride classical negative feedback mechanisms during exercise.

In another effort to determine whether metabolic feedback mechanisms or central command from the central nervous system (CNS) regulate Ra during exercise, fasted and fed rats were treated with phlorizin, a drug which induces hypoglycaemia by increasing glucose clearance (333). Initial exercise-induced increases in Ra were similar in treated and control rats in the fed and fasted state. After 5 min of exercise, Ra remained similar in treated and control rats in the fasted state, whereas in the fed state Ra increased almost twice as much in treated compared with control rats. In both fed and fasted rats, muscle glycogenolysis and lipolysis were higher with phlorizin treatment. The results suggest a central command regulation of Ra from CNS motor centres and that this primary setting may be modulated by metabolic feedback mechanisms as previously suggested (311, 312, 313).

The increase in Ra is also related to exercise intensity (225, 311, 312), duration of exercise (74, 225) and carbohydrate ingestion (6, 7). The latter studies showed an increase in Ra, but a decrease in gluconeogenesis when carbohydrate was ingested before or during low intensity exercise. At low exercise intensity, hepatic glucose production in rats has been found to be sensitive to metabolic feedback inhibition, whereas feedforward control may play a role at high workloads (334). As found in the studies of Sonne and Galbo (311, 312), Ra was controlled predominantly by feedforward mechanisms and feedback mechanisms were added if plasma glucose concentrations decreased (313). It appears that during exercise, mobilisation of hepatic glycogen is a primary event and not secondary to increased muscular demand (311).

Carbohydrate ingestion

The physiological and biochemical effects of the ingestion of various forms of carbohydrate, either before or during prolonged exercise, has received much experimental investigation. Most of the attention has centred on answering three major questions: the time at which the carbohydrate should be ingested, the amount that should be ingested, and the ideal type and form (solid or liquid) of carbohydrate to ingest. The effect of

exercise duration, and the percentage of $VO_{2\ max}$ at which exercise is performed on the utilisation of exogenous carbohydrate has also been investigated. Mostly, the parameters measured have been exercise time to exhaustion, the respiratory exchange ratio (RER), blood glucose concentration, ratings of perceived exertion, changes in muscle glycogen content to establish a possible sparing effect (discussed earlier in this review), the effect on the metabolism of other endogenous substrates such as hepatic glycogen and fat, and the rates of oxidation of the exogenous carbohydrate.

i) Effect on time to fatigue

Many studies have demonstrated an increase in exercise time to fatigue after carbohydrate ingestion (34, 69, 71, 89, 91, 126, 138, 170, 172, 235, 248, 252, 256, 350, 362), but, perhaps surprisingly, others have shown no ergogenic effect of carbohydrate ingestion (10, 34, 111, 116, 139, 193, 294, 301). One of these latter studies (34) showed an increase in time to fatigue when glucose was ingested, but no improvement after ingestion of fructose. Likewise, a study by Flynn et al. (116) showed that ingestion of different combinations of fructose and malto-dextrin, glucose and malto-dextrin, and malto-dextrin alone, did not increase time to fatigue compared to ingestion of water. This could be due to the subjects in the study starting exercise with muscle glycogen concentrations around 185 mmol/kg ww as a result of having carbohydrate-loaded prior to the experiment. With such a large supply of endogenous carbohydrate at the start of exercise, there would be less likelihood of any differences in time to fatigue with or without carbohydrate ingestion. Not unexpectedly, there was no evidence of muscle glycogen sparing in the subjects who ingested carbohydrate. Hargreaves et al. (139) also reported that when either glucose or fructose was ingested before exercise there was no improvement in exercise time to fatigue. The failure to demonstrate an improvement in performance in this study may be attributable to a relatively high exercise intensity (75% of $VO_{2\ max}$), since the subjects became exhausted after only 90 min and were neither hypoglycaemic nor severely glycogen depleted at this time, indicating that factors unrelated to carbohydrate availability caused their exhaustion. There is no clear explanation as to why the other studies did not show an improvement in exercise time to fatigue (10, 34, 111, 193, 294, 301).

ii) Effect on RER and carbohydrate oxidation

RER is based on the assumption that VO_2 and VCO_2 reflect gas exchange at the tissues (119). The RER is the ratio of CO_2 production to O_2 consumption. This has become a widely used measure of fuel substrate oxidation. Carbohydrate ingestion has generally been found to increase RER (5, 40, 69, 71, 80, 89, 98, 138, 193, 294, 350). Not surprisingly, therefore, total carbohydrate oxidation is also increased after carbohydrate ingestion (5, 80, 126, 172, 193, 308), reaching values of up to 2 g/min during the later stages of exercise (70). However, in some studies (10, 52, 91, 116), RER did not increase after carbohydrate ingestion. In the case of the study of Flynn et al. (116) discussed previously, the lack of an increase in RER may, again, be related to the high initial muscle glycogen concentrations of the subjects.

The oxidation of exogenous carbohydrate in glycogen depleted subjects appears to be identical to that of subjects with normal glycogen concentrations (284). The effect of carbohydrate (glucose) ingestion before or

during high intensity exercise (80% of $VO_{2\ max}$) was investigated by Bonen et al. (40) in a study in which subjects exercised in a glycogen depleted state, in an attempt to increase their dependence on the exogenous carbohydrate. As a result of the glycogen depletion, RER during exercise was low (0.83) in the group who did not ingest carbohydrate and blood glucose concentrations remained low throughout exercise. Subjects who ingested 1.5 g/kg of glucose 15 min before or during exercise had RER values which were much higher, ranging between 0.90 and 0.92. In the group which ingested carbohydrate during exercise, insulin concentrations remained low and blood glucose concentrations increased during exercise. However, in the group which ingested glucose before exercise, insulin and glucose concentrations initially increased, but even though insulin returned to basal concentrations after 10 min, blood glucose concentrations declined throughout the 30 min of exercise. Thus during high intensity exercise, glucose ingestion increased carbohydrate utilisation and the differences in the pre-exercise insulin concentrations appeared to exert a persistent effect on glucose utilisation throughout the 30 min of exercise. This agrees with findings that the contribution of blood glucose to total carbohydrate oxidation is higher after the ingestion of carbohydrate or infusion of glucose, reported values ranging from 50 to 100% (5, 69, 74). Part of this increase may be due to the increase in glucose uptake by leg muscles reported by Ahlborg and Felig (6, 7). Of the increase in blood glucose oxidation, the contribution from exogenous carbohydrate can be substantial (Table 2.1).

Several factors may affect the rate of oxidation of ingested carbohydrate. Table 2.1 provides a summary with which to evaluate the effect of ingesting different amounts of carbohydrate, the rate of ingestion, the time of ingestion, exercise duration and intensity, and type of carbohydrate ingested, on the rate of oxidation of the exogenous carbohydrate. To account for differences in oxidation rate that may be due to different amounts of carbohydrate ingested in different experiments, mean and peak rates of oxidation were calculated and expressed as μg of ingested carbohydrate oxidised per gram of carbohydrate ingested. The mean rate of ingestion (g/min) was also calculated.

A number of studies have measured the oxidation of exogenous carbohydrate by using naturally enriched ^{13}C-labelled glucose. In some of these studies the rate of oxidation may have been overestimated by approximately 9% because of the release of ^{13}C from glycogen and fat stores during exercise, and which have not always been accounted for in experiments (230).

iii) Effect of time of ingestion

In a number of studies, subjects have ingested carbohydrate prior to the commencement of exercise. These have ranged from 5 min (256), 20 min (91), 30 min (1, 10, 100, 112, 156, 245), 40 min (5), 45 min (80, 126, 140, 197, 214), 60 min (97, 98, 134, 135, 284, 308), 180 min (177) to 240 min before exercise (304). Of these studies, a number measured oxidation of the ingested carbohydrate (97, 98, 135, 177, 201, 277, 284). The rates measured are shown in Table 2.1 and are similar to the rates obtained for the oxidation of glucose ingested *during* exercise (Table 2.1).

Table 2.1 Rates of oxidation of exogenous carbohydrate (CHO) from various studies.

Mean oxidation rate (g/min)	Mean [1] oxidation rate (μg/g/min)	Peak oxidation rate (g/min)	Peak [2] oxidation rate (μg/g/min)	Amount ingested (g)	Mean [3] ingestion rate (g/min)	Time of [4] ingestion	Exercise duration (min)	% VO_2 max	CHO type	Ref.
0.02	0.60	-	-	32	0.53	30 min	90	66	glucose	(79)
0.02	2.00	-	-	10	0.16	120 min	180	50	glucose	(332)
0.05	1.19	-	-	42	0.70	120 min	180	50	glucose	(332)
0.18	1.80	0.45	4.50	100	1.11	m	90	70	glucose	(251)
0.18	1.80	0.45	4.50	100	1.11	m	90	70	sucrose	(251)
0.22	2.93	-	-	75	1.25	60 min p	60	60	glucose	(98)
0.22	2.20	0.50	5.00	100	0.95	s	105	40	glucose	(202)
0.23	2.30	0.60	6.00	100	1.11	m	90	70	GP[5]	(251)
0.25	3.33	-	-	75	1.25	60 min p	60	60	fructose	(98)
0.27	5.40	-	-	50	0.42	s	120	61	fructose	(2)
0.27	2.70	0.60	6.00	100	1.11	m	90	70	GP	(251)
0.28	2.80	0.67	6.70	100	0.42	180 min p	240	45	glucose	(177)
0.31	6.20	-	-	50	0.42	s	120	61	glucose	(2)
0.32	2.13	-	-	150	0.83	m	180	45	fructose	(178)
0.33	3.30	0.43	4.30	100	0.83	60 min p	120	40	glucose	(284)
0.38	3.80	-	-	100	0.83	s	120	61	fructose	(2)
0.39	3.90	-	-	100	0.42	s	240	50	sucrose	(124)
0.40	4.00	0.65	6.50	100	0.83	s	120	50	glucose	(277)
0.40	4.00	0.63	6.30	100	0.42	s	240	50	glucose	(276)
0.44	3.14	0.64	4.57	140	0.78	m	180	50	fructose	(231)
0.45	4.50	0.53	5.30	100	0.83	m	120	53	fructose	(232)
0.45	4.50	-	-	100	0.83	60 min p	120	60	fructose	(135)
0.45	3.00	-	-	150	0.83	m	180	45	glucose	(178)
0.46	4.60	0.67	6.70	100	0.42	120 min	240	45	glucose	(201)
0.46	4.60	0.67	6.70	100	0.42	s	240	45	glucose	(201)
0.46	2.56	0.9	5.00	180	2.00	m	90	70	glucose	(145)
0.48	4.80	-	-	100	0.83	s	120	61	glucose	(2)
0.51	2.55	0.67	3.35	200	0.74	m	270	45	glucose	(267)
0.54	5.40	0.67	6.70	100	0.83	m	120	53	GP	(232)
0.56	5.60	-	-	100	0.83	60 min p	120	60	glucose	(135)
0.57	3.17	1.00	5.56	180	2.00	m	90	70	maltose	(145)
0.58	4.14	0.83	5.93	140	0.78	m	180	50	glucose	(231)
0.6	6.00	0.70	7.00	100	0.83	m	120	53	glucose	(232)
0.84	8.40	-	-	100	0.83	60 min p	120	60	starch	(135)
0.84	2.10	1.17	2.93	400	1.48	m	270	45	glucose	(267)

[1] and [2]: Oxidation rate in μg/g of CHO ingested/min; [3]: Rate as total CHO ingested per min of exercise time;

[4]: m=multiple feedings; s=single feeding at start of exercise; p=ingestion prior to start of exercise; [5]: glucose polymer.

Ingestion of a mixed snack food 30 min before intermittent cycling at 70% of $VO_{2\ max}$ has been shown to increase plasma glucose and insulin concentrations compared to a control group who ingested placebo (100). There were no differences in these measures between the groups, however, at the end of 50 min of exercise. Neither were there any differences in the time to fatigue or in muscle glycogen utilisation (100). There were also increases in blood glucose and insulin concentrations at the start of exercise in subjects who ingested 75g of glucose 45 min before exercise (80), and 200 g 50 min before or after 90 min of low intensity (30% of $VO_{2\ max}$) exercise (6, 7). Most of the subjects became hypoglycaemic later in exercise in the former study (80), and there was a 13% increase in the extent of muscle glycogen utilisation. A decrease in plasma glucose concentration soon after the start of exercise was also found after ingestion of glucose 30 min before exercise, together with a decrease in exercise time to fatigue at 80% of $VO_{2\ max}$, but not at 100% of $VO_{2\ max}$ (118). More recently, however, Costill and Hargreaves (81) reported in a review that ingestion of carbohydrate an hour before exercise, while resulting in some metabolic changes, does not necessarily impair exercise performance and may sometimes enhance performance. An increase in plasma insulin concentrations at the start of exercise and a decreased fat oxidation during exercise was found even if medium or long chain triglycerides were added to the carbohydrate fed an hour before exercise (171). In a study by Bonen et al. (39), ingestion of glucose 15 min before exercise at 80% of $VO_{2\ max}$ decreased blood glucose concentrations during the first 15 min of exercise, and the normal exercise-induced increase in growth hormone concentrations were less than in subjects who did not ingest glucose before exercise. Cortisol and, contrary to most of the aforementioned studies (6, 7, 80, 100, 171), insulin responses during exercise, were not influenced by the pre-exercise ingestion of glucose (39).

Oxidation of larger amounts of exogenous carbohydrate (100 g of glucose) ingested 3 hr before treadmill exercise at 45% of $VO_{2\ max}$ lasting 4 hr was measured by Jandrain et al. (177). Of the exogenous glucose, 11% was oxidised during the 3 hr before exercise and a large amount, 68%, was oxidised during the 4 hr of exercise. Mobilisation of FFA and glycerol was not impaired by the carbohydrate ingestion since concentrations of both increased during exercise.

An improvement in performance after ingestion of carbohydrate was shown in the study of Sherman et al. (304) in which up to 312 g of carbohydrate was ingested 4 hr before exercise. Blood glucose returned to basal concentrations 1 hr after feedings of varying amounts of carbohydrate, and insulin returned to basal concentrations 3 hr after ingestion of 45 or 156 g of carbohydrate, but were still 84% higher after 4 hr in the group that ingested 312 g. The latter was the only group that had higher insulin concentrations during exercise. These findings are similar to those of Coyle et al. (90) who also investigated the effect of a high carbohydrate meal 4 hr before exercise at 70% of $VO_{2\ max}$. The pre-exercise meal produced a transient elevation of plasma insulin and blood glucose concentrations which returned to basal levels prior to the initiation of exercise. During the first hour of exercise when subjects were fed, there was a 13 - 25% decline in blood glucose concentration, a suppression of the normal increase in plasma FFA and blood glycerol concentrations, and a 45% greater rate of carbohydrate oxidation compared with exercise when subjects

were fasted. However, after 105 min of exercise there were no significant differences in blood glucose concentrations, rate of carbohydrate oxidation, or muscle glycogen concentrations in subjects who had fasted or eaten (90).

Ingestion of carbohydrate even longer (>4 hr) before exercise was investigated by Montain et al. (250). They studied the effect of ingestion of 2 g/kg of carbohydrate 2, 4, 6, 8, and 12 hr before exercise in trained and untrained subjects cycling for 30 min at 70% of $VO_{2\ max}$. Carbohydrate oxidation was 13 - 15% higher during exercise when carbohydrate was ingested 2 and 4 hr before exercise compared to ingestion 8 and 12 hr before exercise. Plasma glucose concentrations decreased during exercise when carbohydrate was ingested 2 - 6 hr before exercise. Insulin concentrations at the start of exercise were higher and mean FFA concentrations during exercise lower after carbohydrate was ingested 2 hr before exercise. If carbohydrate was ingested more than 2 - 4 hr before exercise, insulin concentrations had returned to basal levels by the time exercise started.

To determine whether the effect of exogenous carbohydrate was the same or different when ingested either early or late in exercise, Krzentowski et al. (396) fed subjects 100 g of glucose after 15 min or 120 min of treadmill exercise at 45% of $VO_{2\ max}$. There was no difference between the two groups in the glucose that was oxidised in the 2 hr following ingestion. Thus time of ingestion appears to have no effect on the amount of exogenous carbohydrate oxidised. However, a study by Wright et al. (362) showed that time to fatigue at 70% of $VO_{2\ max}$ was longer when carbohydrate was ingested during exercise than prior to exercise, but longer still when carbohydrate was ingested both prior to exercise and during exercise.

iv) Effect of amount of carbohydrate ingested and concentration of the drink

A possible relationship between the amount of carbohydrate ingested during 2 hr of cycling at ~70% of $VO_{2\ max}$ and subsequent performance over a distance of 4.8 km was investigated by Murray et al. (253). Although subjects who ingested carbohydrate performed better than subjects who ingested water, there was no evidence for a dose response effect. Sherman et al. (308) and Mitchell et al. (248) also showed no dose response effect of carbohydrate ingestion on exercise performance. But in another study by Mitchell et al. (247), it was found that performance was better when a 12% carbohydrate drink was ingested compared to 6% or 18% carbohydrate. The failure to show an effect on performance when an 18% carbohydrate solution was ingested may be explained by the finding of Maughan et al. (234) that a 16.5% solution of glucose polymer resulted in a decreased plasma volume, suggesting that the high osmolality of the digestion products of the polymer may have resulted in water movement into the gut. At lower concentrations, glucose polymer solutions have been found to empty faster from the stomach than glucose and fructose (310).

Studies in which the effect of the amount of carbohydrate ingested on rate of oxidation of the ingested carbohydrate have been investigated have also shown conflicting responses. Thus, when either 200 or 400 g of glucose was ingested during 285 min of exercise (267), it was found that the oxidation rate of the

exogenous glucose averaged 0.51 g/min when a total of 200 g was ingested, but 0.84 g/min when 400 g was ingested. A peak rate of 1.17 g/min was reached during ingestion of the 400 g. However, no differences were found in the rates of oxidation in the study of Adopo et al. (2) when subjects were fed either 50 or 100 g of glucose, 50 or 100 g of fructose, or 50 g fructose with 50 g of glucose.

A higher rate of oxidation after ingestion during exercise of a 15% carbohydrate solution than a 7.5% solution was found in the study of Moodley et al. (251), but absolute amount of carbohydrate ingested increased as well as concentration. The effect of different concentrations of carbohydrate drinks on the rate of exogenous and total carbohydrate oxidation was determined by Jandrain et al. (179) by dissolving 50 g of glucose in different volumes of water. Drinks were ingested after 15 min of exercise. Total carbohydrate oxidation and rate of exogenous glucose oxidation were not significantly affected by the concentration of the ingested solution. Based on these results, it is unlikely that the increase in exogenous carbohydrate oxidation shown in the study of Moodley et al. (251) was due to the higher concentration of some of the drinks, but rather resulted from an increase in the absolute amount of carbohydrate ingested.

v) Effect of carbohydrate form and type

The oxidation rate of various types of carbohydrate have also been compared. In one such study, glucose, fructose or starch was ingested 60 min before exercise and it was found that there was no difference in the subsequent rate of oxidation between glucose and starch, but significantly less fructose was oxidised during exercise (135). While a slower rate of oxidation of fructose than glucose has been shown (178, 231, 233), no difference in the rate of oxidation of these two carbohydrates occurred in fasted subjects (233).

A comparison of glucose, fructose and glucose polymer ingested during exercise (232) showed no differences in oxidation rate between glucose and glucose polymer, but fructose oxidation was, again significantly lower. Whereas 75% of a glucose load ingested during 3 hours of exercise was metabolised, only 56% of an equivalent fructose load was oxidised during the same period of exercise. Furthermore, whereas exogenous glucose began to be oxidised as a fuel within 20 minutes of ingestion, ingested fructose first contributed to energy metabolism only 40 minutes after ingestion. The authors conclude that ingested fructose is less available for muscle metabolism than is glucose because the fructose must first be converted to glucose in the liver before it can be oxidised by muscle and the activity of this metabolic pathway in the liver is low. Thus fructose ingestion, which enters blood as glucose or lactate, was not more beneficial than was glucose ingestion during prolonged exercise. Endogenous carbohydrate utilisation was significantly lower during exercise when either of these three carbohydrates was ingested, than with water (232). The rates of oxidation of glucose and maltose ingested during exercise also appear to be the same, both contributing 50% of the total carbohydrate oxidation (145).

Performance was shown to be better with the ingestion of glucose and sucrose than with fructose in the study of Murray et al. (254) in which subjects ingested either of these carbohydrates.

The form in which exogenous carbohydrate is ingested, either solid or liquid, has also been investigated and no influence on RER (229), insulin concentration, time to fatigue (364), or muscle glycogen utilisation (364) has been found.

vi) Effect of exercise intensity

An increase in exercise intensity has been found to increase the amount of exogenous carbohydrate oxidised after ingestion of 100 g of the carbohydrate at the start of exercise (275). However, when the oxidation rates of ingested carbohydrate from many studies, at differing exercise intensities, are examined (Table 2.1), it appears that little relationship may exist between exercise intensity and the rate of oxidation of ingested carbohydrate.

vii) Effect on hepatic glycogenolysis

Carbohydrate feedings prevent the typical hormonal responses during exercise which are responsible for hepatic glucose release (220, 249), thus eliciting a possible hepatic glycogen sparing (249). Exogenous glucose in moderate amounts appears to replace hepatic glucose production without changes in mobilisation of muscle glycogen or fat (334). Jackson et al. (173) calculated that the initial splanchnic extraction accounted for 19% of a 90 g oral glucose load in resting humans, whereas Radziuk et al. (280) calculated, using tracer techniques, that 90% of an exogenous glucose load is absorbed and passes through the liver to appear in the systemic pool, suppressing endogenous glucose production by 66% (280). This would have an effect on the levels of hepatic glycogen reached at the end of exercise, which is determined by the balance between ongoing synthetic and degradative processes as shown by David et al. (95) who demonstrated that both glycogen synthase and phosphorylase are active simultaneously in rat liver.

viii) Effect on FFA concentration

Ingestion of carbohydrate reduced the serum FFA concentrations in rats in which glucose, sucrose or fructose was ingested 30 min prior to exercise, compared to ingestion of water (1). In human subjects, carbohydrate ingested 50 min before (7) or after 90 min (6) of exercise also lowered FFA concentrations and decreased lipolysis. Similarly, the exercise-induced increase in FFA concentration was delayed and reduced by about 60% when subjects ingested 100 g of glucose during exercise at 50% of $VO_{2\,max}$ (220). During exercise at 70% of $VO_{2\,max}$, a single feeding of glucose polymer after 135 min resulted in a plateauing of plasma FFA and blood glycerol concentrations (71).

Plasma FFA concentrations are lower after ingestion of glucose or starch before exercise than after ingestion of fructose, probably as a result of a less marked increase in plasma insulin concentrations after fructose ingestion (135). This is consistent with other studies that have shown that ingestion of fructose during exercise results in higher concentrations of FFA than does the ingestion of other types of carbohydrate (231, 254). However, Massicotte et al. (232) found that the ingestion of fructose, glucose and glucose polymer *all* blunted the plasma FFA and glycerol responses to exercise.

Comparison of the effects of ingestion of glucose, glycerol or placebo showed that glycerol failed to increase exercise time to fatigue, whereas glucose increased time to fatigue by 14% (126). Both glycerol and glucose ingestion attenuated the increase in FFA concentrations during exercise.

Regulatory factors in FFA oxidation

A continual hydrolysis and release of FFA from the triglyceride stores of adipose tissue occurs during prolonged, moderately severe exercise (128). The uptake and oxidation of the plasma FFA by the working skeletal muscles represents a major source of energy during such exercise. During light and moderately intense, prolonged exercise, the release of FFA from the adipose tissue exceeds uptake by peripheral tissue and the net result is an increase in plasma FFA concentrations. Plasma FFA oxidation can contribute 50 - 60% of the energy expenditure during a bout of low intensity exercise of long duration, but more strenuous submaximal exercise requiring 65 - 80% of $VO_{2\ max}$ utilises less fat (10 - 45% of the energy expended) (15). Romijn et al. (296), found that peripheral lipolysis in subjects exercising at 25, 65, and 80% of $VO_{2\ max}$ was highest at the lowest exercise intensity, with decreased fatty acid release into plasma with increasing exercise intensity. Muscle triglyceride lipolysis, however, was stimulated only at higher intensities (296). Plasma FFA oxidation was also influenced by exercise duration, increasing progressively during 2 hr of exercise at 65% $VO_{2\ max}$, whereas muscle glycogen and triglyceride oxidation decreased (296). Inhibition of FFA transport results in decreased FFA oxidation, increased plasma FFA concentrations and an increase in Ra in rats (227). Muscle malonyl Co-A, which inhibits carnitine acetyl transferase I, decreased during exercise in rats (351) and this may be important for allowing the increase in muscle FFA oxidation during exercise.

To determine the effect of FFA concentration on uptake into the cell, intralipid has been infused together with heparin (141). Whereas plasma FFA concentrations increased, FFA uptake did not (141).

After intralipid infusion, glucose uptake has been to be reduced, but muscle glycogenolysis is unaffected, probably due to direct inhibition of glucose transport rather than by the glucose-fatty acid cycle (141, 258). However, other studies have shown lipid oxidation to be unrelated to glucose uptake (217, 285), negatively correlated with glucose oxidation (217, 226) and positively correlated with FFA concentration (168, 217).

The role of plasma glucose concentration in the regulation of lipid metabolism in humans, independent of associated changes in hormone concentrations was examined by Carlson et al. (57). Circulating FFA and glycerol turnover were both reduced by approximately 30% with hyperglycaemia. It was concluded that the equivalent suppression of glycerol and FFA turnover by hyperglycaemia indicated a suppression of lipolysis and not a stimulation of FFA re-esterification (57).

CHAPTER 3

LITERATURE RELATED TO TRACER TECHNIQUES AND DEVELOPMENT OF TRACER METHODOLOGY

To determine fuel substrate kinetics in the different groups of cyclists it was necessary to develop techniques for measuring rate of splanchnic glucose appearance (Ra), exogenous carbohydrate oxidation, and oxidation of plasma glucose (plasma glucose oxidation). For this purpose, the use of stable (^{13}C) glucose as tracer for determination of Ra or oxidation of exogenous carbohydrate was initially considered as this would have enabled the same subject to take part in more than one study, which is preferable to the use of different subjects in each study for statistical purposes. However, the use of stable isotopes for measurement of Ra does not provide the same degree of sensitivity of measurement as radioactive isotopes , necessitating the use of a more than negligible amount of tracer, which renders it unsuitable as a tracer for turnover studies unless very sensitive measurement equipment is available (361).

An added complication is that most foods in South Africa are naturally enriched with ^{13}C. Thus the subjects who had to carbohydrate-load before the experiments would have eaten foods that are high in ^{13}C, and thus their muscle glycogen would have been enriched with ^{13}C which, together with the ^{13}C released from fat during exercise, would have resulted in an overestimation of plasma glucose oxidation or an underestimation of Ra as a result of glycogenolysis during exercise.

It was therefore decided to utilise radiolabelled tracers for determination of Ra and the rate of oxidation of exogenous carbohydrate. Estimation of glucose flux in dogs using stable isotope techniques has been compared with simultaneous measurements made in the same animal with radioactive isotope methods and similar rates of glucose turnover have been found (31).

Measurement of rate of splanchnic glucose appearance

i) Choice of radiolabelled tracer

Depending on the nature and position of the label, different values are obtained in calculation of Ra from isotope dilution measurements (359). Since the use of ^{14}C-glucose tracers would have introduced the problem of re-cycling of the ^{14}C label (12, 103, 282), tritiated (^{3}H) -glucose tracer appeared to be the tracer of choice.

Since the point of interest in the studies in this thesis was whether there was any change in Ra under the different experimental conditions to be studied, a tracer was required that would not measure only glucose appearance from gluconeogenesis, but rather total glucose appearance from any source, including splanchnic glucose output from exogenous sources, hepatic glycogenolysis and gluconeogenesis from

3-carbon products and recycling glucose. According to Wolfe (359), calculation of rate of glucose appearance using 2-^{2}H-glucose tracer includes the rate of total glucose production and the contribution from the glucose cycle. The ^{3}H-label of glucose labelled in the second carbon position is lost in the glucose-6-phosphate - fructose-6-phosphate equilibrium in the glycolytic and gluconeogenic pathways, and thus the incorporation of ^{3}H-glucose into hepatic glycogen is minimised (186), particularly during the pre-exercise infusion period. Thus, with 2-^{3}H-glucose the potential incorporation and subsequent hydrolysis of ^{3}H-glucose labelled glycosyl residues of glycogen, and consequent underestimation of glucose appearance, is limited. However, when glucose is labelled on the third carbon, ^{3}H can be incorporated into hepatic glycogen, which can then be released during glycogenolysis, leading to an underestimation of hepatic glucose appearance (186). An underestimation of glucose turnover will also result if isotope that has been cleared (for example 6-^{3}H-glucose into hepatic glycogen) is recycled into the systemic circulation (53).

Simultaneous infusion into human subjects of 2-^{3}H-glucose, which may undergo futile cycling but does not cycle through glycogen; 6-^{14}C-glucose, which may cycle through glycogen but does not futile cycle; and 3-^{3}H-glucose, which can undergo both futile cycling and cycle through glycogen, showed that glucose turnover determined with 2-^{3}H-glucose was highest, followed by 6-^{14}C-glucose, followed by 3-^{3}H-glucose (23). Altzuler et al. (12), in a study with dogs, have also reported higher rates (150%) for Ra with 2-^{3}H-glucose compared to 6-^{14}C-glucose, after correction for recycling of ^{14}C. Higher values for Ra when 2-^{3}H-glucose is used as tracer have also been found in other studies (153, 280). In a trial by Radziuk et al. (282) in dogs to validate the equations used to calculate glucose appearance, 2-^{3}H-glucose, 6-^{3}H-glucose and 1-^{14}C-glucose were infused at a high, time varying rate to suppress endogenous hepatic glucose production and various models were used to calculate Ra. Mean values of the percentage error in the calculated Ra compared to the rate of infusion of 2-^{3}H-glucose, 6-^{3}H-glucose and 1-^{14}C-glucose were 11.1% for 2-^{3}H-glucose with a mean of 10.5% for all the tracers when a single compartment model with a glucose pool fraction of 0.5 was used; 9.8% and 9.6% respectively for the same model with a pool fraction of 0.65, and 9.4% for both 2-^{3}H-glucose and the other tracers with a pool fraction of 0.75.

Issekutz (164) simultaneously infused 2-^{3}H-glucose to measure hepatic glucose output, 6-^{3}H-glucose to measure hepatic glucose production, and U-^{14}C-glucose to measure carbon recycling, into exercising dogs, to calculate glucose substrate cycling and recycling of radioactive carbon atoms. Substrate cycling was calculated as the difference between Ra measured with 2-^{3}H-glucose and 6-^{3}H-glucose, and recycling carbon was determined from the U-^{14}C-glucose infusion. In resting dogs, substrate cycling represented 13% and recycling carbon 11% of the hepatic glucose production measured with 6-^{3}H-glucose.

Because the use of 2-^{3}H-glucose to measure glucose appearance includes appearance of glucose from substrate cycling as well as glycogenolysis and gluconeogenesis, the validity of its use as a tracer has been questioned. By using a combination of 2-^{3}H-glucose and 6-^{3}H-glucose, the rates of total hepatic glucose output (hepatic glucose production plus glucose cycling), hepatic glucose production, and glucose-

glucose-6-phosphate cycling, have been shown to be increased in conditions in which gluconeogenesis is stimulated (25, 187, 188, 297, 344). This implies that activities of both glucokinase and glucose-6-phosphatase are increased with increased stimulation by glucose counter regulatory hormones and thus, despite the fact that glucose from substrate cycling is not *de novo* glucose production, it is an important and regulated component of hepatic glucose output, which necessitates the use of 2-^{3}H-glucose as a tracer. 2-^{3}H-glucose has previously been used to measure glucose turnover in a number of studies in which *total* glucose appearance from liver was to be determined (35, 186, 192, 218, 222, 266, 318, 344).

ii) Model for computation of glucose kinetics

The validity of the tracer technique depends on several assumptions, one of which is that the selected model of glucose kinetics is valid. Under non-steady state conditions this has recently been challenged (53) for the most commonly used single compartment model of Steele (324).

The model and equation proposed by Steele (324) to compute rates of appearance and disappearance of glucose in non- steady state (a pool fraction model) has been extensively used in experiments in which rate of hepatic glucose appearance has been calculated. The assumption inherent in this equation (and other equations like it) is that the body glucose pool is a single, readily mixable pool. Since the equation was first used in 1959, the fact that this condition is not met has been recognised, and thus included in the equation is a "pool fraction" (p), which represents the fraction of the total glucose pool that behaves as an ideal, readily mixing pool and compensates for non-ideal pool behaviour. This will be discussed subsequently.

Steele's model has been subjected to theoretical analysis by Cobelli et al. (67). They showed that the model introduces errors dependent on the volume of the compartment and configuration of the system, which depend on the time course of changes in specific activity. This analysis suggests that there is no single pool fraction value satisfactory under all non-steady state conditions and that tracer specific activity should be as constant as possible during experiments. In a 1983 paper the conclusions of Cobelli et al. (68) were that the quantitative reliability of predictions provided by the pool fraction model was quite poor. Subsequently, in 1993, a method of estimation of hepatic glucose production which is based on deconvolution and uses a two compartment minimal model to describe a time varying impulse response of the glucose system, was suggested by Caumo and Cobelli (58).

Finegood et al. (113) have suggested a variable one compartment model of glucose kinetics and step increases in rate of tracer infusion in which infusion of tracer was increased two or three fold when endogenous glucose appearance was expected to be changing rapidly, such as the onset of exercise. This was found to provide a slightly more accurate estimate of glucose appearance than the standard fixed volume model and constant tracer infusion.

However, a validation study by Radziuk et al. (282) appears to have been forgotten. In that trial the effects of different models to calculate Ra were evaluated. Data were analysed using a single compartment model

with a number of different volumes of distribution, a two compartment model, and a generalised dispersion (impulse response) model. The calculated rates of infusion (Ra) were 9.5, 8.4, and 7.8% higher than the actual rate for the three different models, respectively. Thus it appears that all models tend to overestimate glucose appearance compared to the actual rate, but that there is little difference in accuracy between the more complex models and the pool fraction (single compartment) models.

In another experiment which compared tracer determined glucose appearance with a known glucose infusion rate, Koivisto et al. (198) compared turnover rates when euglycaemia was maintained by infusing unlabelled glucose mixed with 3-^{3}H or 6-^{3}H-glucose. The isotopically determined glucose disposal rate was virtually identical to the exogenous glucose infusion rate with both tracers (198).

Therefore, the single compartment model described by Steele (324) was used in the current series of experiments.

iii) Pool fraction

Many experiments have attempted to determine the most suitable value for the glucose pool fraction (p), used in the single compartment model equations for calculating glucose appearance. Values range between 0.5 - 0.8 (281), 0.65 - 0.75 (282), and 0.5 - 0.75 (265). Searle (303) states that "Steele now proposes that 0.77 would be a more appropriate value for the calculation of non-steady state events when one employs a continuous infusion technique and assumes a simple mono-compartment system". However, these values were determined at rest and have mostly employed the use of glucose infusions at known rates to determine the accuracy of a simultaneous tracer-determined rate. Most experiments were designed to produce fairly rapid changes in blood glucose concentrations. However, it is known that the slower the change in blood glucose concentration, the higher the value of 'p' becomes, until at an infinitely slow rate of change in glucose concentration, 'p' would be 1.0 (84). In the experiments in this thesis the change in glucose concentrations were very slow, suggesting the use of a fairly high value for 'p'. The data of Cowan and Hetenyi (84) suggest that 'p' becomes larger when rates of glucose appearance increase. During exercise, rates of glucose appearance are much higher than the rates reported in those experiments where 'p' was determined to be 0.65. Cowan and Hetenyi (84) state that the value of 0.65 in their (non-exercising) dogs is probably a minimum value and that 'p' would be higher under more physiological conditions (smaller changes in glucose concentrations). In the current experiments the subjects were of course exercising, suggesting that a higher value would be appropriate.

Most experiments, including data of Radziuk et al. (282), validating the equations for determining rate of glucose appearance and 'p' values, indicate that in real terms a change in the value of 'p' from 0.5 to 0.65 has little effect on the goodness of fit of a tracer-determined rate of glucose appearance and a known rate of infusion. Wolfe et al. (360) report that 40 - 210 ml/kg (which corresponds to a range in 'p' from 0.2 - 1.0) is an acceptable range for the glucose space for calculation of rate of glucose appearance, since the difference in glucose appearance calculated using the extreme values within this range was not more than 10%. This

demonstrates what a small difference (approximately 3%) it would make to calculated glucose appearance if, for example, 0.5 is used for 'p' instead of 0.75. Applying different values for 'p' in the calculation of glucose appearance from the data from the current studies also had little effect on the value calculated for rate of glucose appearance.

It was therefore decided that in the current experiments a value at the high end of the 0.5 - 0.75 range would probably be the most appropriate, and therefore a value of 0.75 was selected. In any event, any error would be the same for all groups.

iv) Constant infusion vs single injection of tracer

The question of whether a constant tracer infusion or a single injection of tracer is the better technique was investigated by Allsop et al. (11). They calculated the rate of appearance of unlabelled glucose from changes in plasma glucose specific activity after a single intravenous injection of labelled glucose and compared the values with the actual constant infusion rate of unlabelled glucose into an anaesthetised dog with all sources of endogenous glucose production surgically removed. The mean steady-state rate of appearance of unlabelled glucose calculated from the area under the specific activity versus time curve was 7% higher than the actual infusion rate (n = 4), but the difference was not statistically significant. The variability in the rate calculated in this manner was, however, greater than the variability reported with rates determined from a primed constant infusion of tracer (11). Thus a constant infusion was used in the experiments in this thesis.

v) Determination of 2-^{3}H-glucose activity for infusion

The amount of 2-^{3}H-glucose to infuse that would result in the minimal radiation exposure, but still be accurately measured from blood (plasma) samples had to be determined. It was also necessary to determine the time taken for the tracer to reach equilibrium in the blood.

The radiation dose accepted as safe in this country is 500 mrem/year or 130 mrem/13 weeks (Information courtesy of Dr E Hering of the Department of Medical Physics, Groote Schuur Hospital, Cape Town). The time for which each cyclist would be infused would be the time taken to reach tracer equilibrium, followed by 3 hr of cycling. Since Jenkins et al. (186) had previously used an infusion rate for ^{3}H-glucose of approximately 900 kBq/hr, a dose somewhat lower than this was decided on for use in a pilot experiment in the hope that plasma levels would still be high enough to measure accurately, so that a second, ^{14}C label could also be used (ingested) while still keeping the total radiation dose low. Thus, after first ascertaining that the glucose tracer was sterile and pyrogen free, a pilot experiment was conducted on the researcher in which 2-^{3}H-glucose (Amersham International, Buckinghamshire, UK) was infused continuously through a 20G cannula (Johnson and Johnson, Halfway House, South Africa) inserted into an antecubital vein of the forearm at a constant rate of 750 kBq/hr from a calibrated auto syringe (Travenol Laboratories Inc., Hooksett, USA). An 18G Jelco cannula was inserted into an antecubital vein of the other arm for blood

sampling (5 ml) every 10 min for later determination of whether the ^{3}H-glucose disintegrations per min (dpm) could be detected, and if so the time taken to reach equilibrium in the blood. For this the glucose was separated from the blood sample, liquid scintillation cocktail (10 ml of Beckman Ready Gel, Fullerton, USA) added and radioactivity (dpm) counted in a liquid scintillation counter (Packard Tri-Carb 4640, Illinois, USA). It was found that ^{3}H dpm could be measured accurately at the chosen rate of infusion, reaching around 1000 dpm/ml of plasma at equilibrium. The technique that was developed for separation of glucose from the other blood components is described subsequently.

vi) Tracer equilibration in blood

Although Radziuk et al. (282) used an unprimed infusion of 80 - 100 min in their experiments, as did Jenkins et al. (185), and although Katz et al. (191) state that "unless the conditions require a very short experimental period, primed infusion appears to us to offer no theoretical advantages over continuous infusion," the pilot experiments showed that tracer equilibration, as evidenced by a constant plasma glucose specific activity, took a minimum of 90 min to be reached, and was somewhat variable. Thus a small priming dose equivalent to 45 min of constant infusion was used in subsequent pilot tests. It was then found that equilibration always occurred between 60 - 75 min after the start of infusion.

vii) Non-glucose contaminants

One potential problem with many commercially available tritiated isotopes is that there have been reports that some may contain non-glucose radioactive contaminants which have a slower clearance rate than glucose under conditions of high glucose turnover. This would result in overestimates of specific activity and underestimates of glucose appearance (53, 271, 302). However, others have not found any non-glucose contaminants in the tritiated glucose that was used in their experiments (198). The glucose tracer used in the experiments in this thesis was tested and also found to be free of non-glucose contaminants.

Calculation of glucose appearance

The rate of total splanchnic (endogenous plus exogenous) glucose appearance (Ra), the rate of glucose appearance from exogenous carbohydrate (Ra_{exog}) and appearance of endogenous glucose from liver (Ra_{end}) were calculated using Steele's equations for non-steady state exercise (324), validated by Radziuk et al. (282) and are shown below:

$$Ra = (I - (pV \times Glu_{tot} \times dSA/dt))/SA$$

$$Ra_{exog} = (I - (pV \times Glu_{lab} \times dSA/dt))/SA$$

$$Ra_{end} = Ra - Ra_{exog}$$

where, Ra, Ra_{exog}, and Ra_{end} are as defined above, in mmol/min; I is the infusion rate of 2-^{3}H-glucose in dpm/min; p is the pool fraction (0.75) in which rapid changes in glucose concentration and specific activity take place (185, 282); V is the glucose distribution volume (19.6% of body mass in l at rest (185); Glu_{tot} is

the mean plasma glucose concentration (^{14}C-labelled and non-labelled) in mmol/l in consecutive samples; Glu_{lab} is the mean plasma glucose concentration of ^{14}C-labelled glucose (^{14}C glucose radioactivity (dpm) of the sample divided by SA of the ^{14}C ingested glucose) in mmol/l in consecutive samples; dSA/dt is the change in plasma 2-^{3}H-glucose specific activities in dpm/mmol over the sample interval in minutes; SA is the mean dpm/mmol 2-^{3}H-glucose specific activity in successive samples and dGlu/dt is the mmol/l/min change in total glucose concentration.

Measurement of exogenous carbohydrate oxidation

One of the aims in this thesis was to compare the oxidation rates of exogenous carbohydrate in carbohydrate-loaded and non-loaded subjects.

i) Exogenous carbohydrate and choice of label

The carbohydrate ingested in these experiments was a short chain glucose polymer (average chain length of 4 glucose monomers). This was used because it has previously been demonstrated that the gastric emptying rate of such carbohydrate solutions may be faster at high concentrations due to the lower osmolality of the glucose polymer (234). In addition, high concentrations of glucose polymer solutions are not as sweet as equivalent concentrations of glucose and are therefore better tolerated, being less likely to cause nausea or gastro-intestinal distress when the rate of ingestion is high for prolonged periods (261).

For reasons described previously in this Chapter, a radiolabelled glucose tracer was used to label the exogenous carbohydrate. The U-^{14}C-glucose tracer was ingested rather than infused so that both exogenous carbohydrate (in subjects ingesting carbohydrate) and total plasma glucose oxidation could be determined from expired $^{14}CO_2$ (described subsequently). Since expired $^{14}CO_2$ and plasma ^{14}C-glucose specific activities were determined simultaneously (described subsequently), the route of ^{14}C-glucose tracer administration did not affect the determination of plasma glucose oxidation.

Experiments in this laboratory (145, 251) conducted under similar conditions to that of the present study, have shown that ^{14}C-glucose tracer can successfully be used to label a glucose polymer of 5x the glucose chain length (Degree of Polymerisation = 22) used in the present study (Degree of Polymerisation = 4). This is shown in Figure 3.1 (n = 6) which shows the relationship between plasma glucose specific activity and that of ingested glucose, maltose and glucose polymer of 22 glucose monomers. The line of ^{14}C-glucose as tracer with the polymer as tracee is superimposed on the lines of the "ideal" tracer-tracee combinations of ^{14}C-glucose-glucose and ^{14}C-maltose-maltose. In addition, the oxidation rates of glucose, glucose polymer, and maltose measured in the study were identical, peak rates being 0.9 ± 0.04, 0.9 ± 0.08 and 1.0 ± 0.04 g/min, respectively. Thus even a polymer with 5 times the molecular mass of the one used in the current study can be successfully traced with a glucose tracer, despite the fact that tracer and tracee are not absolutely identical. A ^{14}C-labelled short-chain polymer was not used as this is not commercially available.

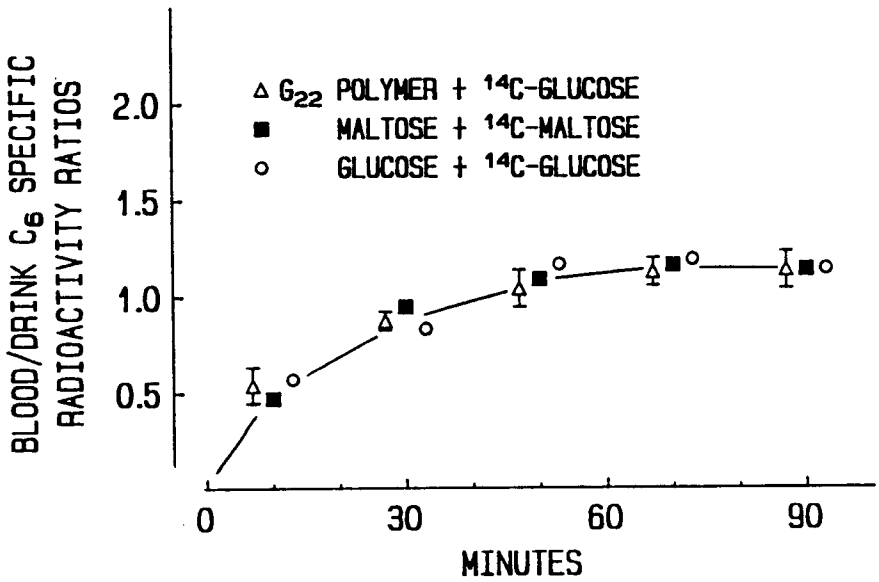

Figure 3.1. Plasma glucose-to-drink specific activity ratios of a glucose polymer of 22 glucose monomers (G22), glucose, and maltose (n = 6). Glucose polymer and glucose were labelled with ^{14}C-glucose and maltose with ^{14}C-maltose. There were no differences in tracking between "ideal" tracer-tracee combinations and ^{14}C-glucose with a chain polymer five times longer than that used in the studies in this thesis.

ii) Activity of U-^{14}C-glucose tracer for ingestion

Since the counting efficiency of ^{3}H-glucose is low compared to ^{14}C-glucose, the radioactivity from the ^{14}C-glucose in blood samples had to be balanced against that of the ^{3}H-glucose to diminish spill-over of ^{14}C counts into the ^{3}H window during dual channel counting of ^{14}C and ^{3}H-glucose in the same plasma sample. Thus pilot tests were conducted in which different amounts of a 7400 kBq/mmol U-^{14}C-glucose (Amersham International, Buckinghamshire, UK) tracer were added to a 10% solution of 4-monomer glucose polymer. Subjects not ingesting carbohydrate during exercise also ingested the ^{14}C tracer added to an artificially sweetened and coloured water placebo. One hundred and seventy mls of either solution was ingested at the start of exercise and a further 170 ml ingested every 20 min while cycling at 70% of $VO_{2\ max}$ (which was the intensity employed in the subsequent studies), so that 500 ml was ingested every hour. Expired CO_2 was trapped every 20 min in a mixture which consisted of 1 ml of hyamine hydroxide in methanol (Packard, Illinois, USA), 1 ml of ethanol and 2 drops of 1% phenolphthalein indicator (309), by bubbling expired air through the mixture until the solution became clear, at which point exactly 1 mmol of CO_2 had been absorbed (309). Liquid scintillation cocktail (10 ml of Beckman Ready Gel, Fullerton, USA) was then added and $^{14}CO_2$ radioactivity was counted in a liquid scintillation counter (Packard Tri-Carb 4640, Illinois, USA).

Results from these pilot tests showed that 200 kBq resulted in measurable $^{14}CO_2$ counts after 20 min of exercise and after 60 min of exercise the $^{14}CO_2$ counts reached around 1000 dpm/mmol.

The total radiation dose (from both tracers) that would be received by each subject was calculated to be approximately 10 mrem, which is well below the 130 mrem/13 weeks or 500 mrem/year safe dose.

Calculation of exogenous carbohydrate oxidation

The rates of exogenous carbohydrate oxidation (R_{exog}) in g/min were calculated from the following equation:

$$R_{exog} = (^{14}CO_2 \times 6)/SA_{exog}) \times VCO_2 \times 1.35$$

where, $^{14}CO_2$ x 6 is the dpm/mmol value multiplied by 6, as there are 6 carbon atoms per molecule of ^{14}C-glucose; SA_{exog} is the specific activity of the carbohydrate ingested in dpm/mmol; VCO_2 is the volume of expired CO_2 in l/min; and 1.35 is the number of grams of glucose oxidised to produce 1 l of CO_2.

Calculation of plasma glucose oxidation

One of the aims in the experiments in the thesis was to determine and compare the rates of plasma glucose oxidation in carbohydrate-loaded or non-loaded subjects, and when ingesting carbohydrate or water during exercise.

The rates of total (endogenous + exogenous) plasma glucose oxidation (R_{ox}) in g/min were calculated from the following equation:

$$R_{ox} = ((^{14}CO_2 \times 6)/SA\ glu) \times VCO_2 \times 1.35$$

where, $^{14}CO_2$ x 6 is the dpm/mmol value multiplied by 6, as there are 6 carbon atoms per molecule of ^{14}C-glucose; SA glu is the plasma ^{14}C-glucose specific activity in dpm/mmol, VCO_2 is the volume of expired CO_2 in l/min; and 1.35 is the number of grams of glucose oxidised to produce 1 l of CO_2.

Calculation of endogenous glucose oxidation

Oxidation of endogenous glucose was calculated by difference between R_{ox} and R_{exog}.

Bicarbonate pool

The formulae to calculate plasma and exogenous glucose oxidation do not take into account the $^{14}CO_2$ retained in the bicarbonate (HCO_3^-) pool.

The body bicarbonate pool can act as a sink for labelled carbon atoms used in the determination of exogenous carbohydrate and plasma glucose oxidation and can account for discrepancies between total and exogenous glucose oxidation in relation to the peak time of occurrence, as well as the absolute quantities (268).

The time required to equilibrate $^{14}CO_2$ with the plasma CO_2/HCO_3^- pool during exercise performed at 60-70% $VO_{2\,max}$ has been stated to vary from 5 min (279), to 15 - 20 min (22, 295), to 30 min (311), to 45 min (96), and to as long as 75 min (293). However, a study by Barstow et al. (22) presents strong evidence that 90% of equilibration has occurred after 16 min of moderate intensity exercise. In the present study, plasma ^{14}C glucose, which would not be affected by the HCO_3^- pool, took ~90 min to reach equilibrium, but $^{14}CO_2$ specific activity closely tracked the increase in plasma ^{14}C glucose specific activity (Figure 3.2). It is therefore unlikely that there was any lag in $^{14}CO_2$ appearance due solely to retention of $^{14}CO_2$ in the HCO_3^- pool. Data on bicarbonate pool kinetics from this laboratory indicate equilibration of the pool within 45 min (A. N. Bosch and S. M. Weltan, unpublished observations).

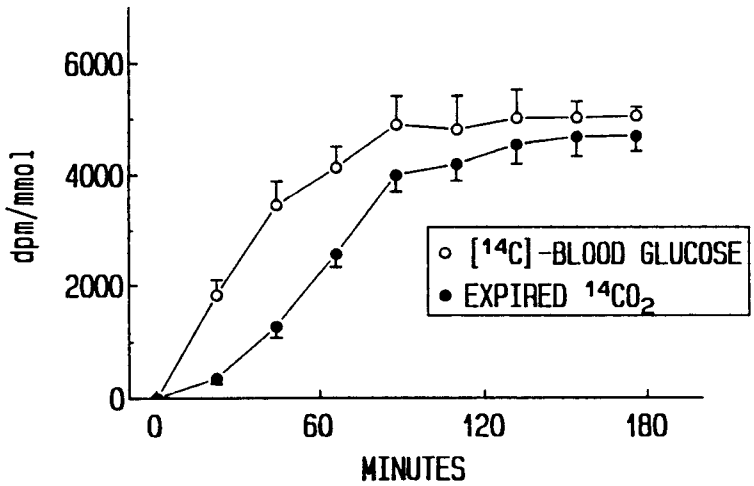

Figure 3.2 Plasma ^{14}C-glucose and expired $^{14}CO_2$ specific activities following ingestion of ^{14}C-glucose. The time course of the increase in $^{14}CO_2$ closely tracked the increase in ^{14}C-glucose, the time course of which would be unaffected by the bicarbonate pool.

A more or less complete equilibration of the bicarbonate/CO_2 pool in 15 to 30 min would also be predicted from the calculated flux of CO_2 through the body bicarbonate stores:- In a 70 kg male, body water content is around 40 l. Of this, 25 l are extracellular fluid which contains 25 mmol/l bicarbonate and 15 l are

intracellular water which contains approximately 10 mmol/l bicarbonate. Hence total body bicarbonate stores are around 775 mmol, which corresponds to 17.5 l of CO_2. Since 85% of the CO_2 produced is carried to the lungs as bicarbonate, a typical exercise VCO_2 of 2.5 l/min would turn over 2.1 l of the CO_2 from bicarbonate per min. Most of the bicarbonate should therefore (theoretically) turn over in 17.5/ 2.1 min i.e. 8 min (44).

Any slight underestimation of the rate of plasma glucose oxidation as a result of HCO_3^- turnover would be similar among groups and therefore would not affect the comparisons.

Determination of plasma glucose and lactate specific activities

The following procedure was developed for the determination of plasma glucose and lactate specific activities.

Since the radioactivity in the blood samples had to be divided by plasma glucose concentration to determine specific activity (disintegrations/ml plasma ÷ mmol glucose/ml of plasma), blood was collected in the same way as for a determination of plasma glucose concentration. As the pilot test had shown adequate dpm for counting in 1 ml of plasma, sufficient blood was drawn at each time to obtain 1 ml of plasma for radioactivity counting as well as measurements of glucose concentration. Thus blood samples (5 ml) were collected into pre-chilled tubes containing potassium oxalate and sodium fluoride and kept on ice until centrifuged at 500 x g for 15 min at 4°C. Since proteins interfere with the efficiency of radioactivity counting, a 1 ml aliquot of the plasma was taken and the concentration of $HClO_4$ needed to deproteinise the sample with the minimum of added volume was determined by experimentation. A low volume was required because the sample would later be run through an ion exchange column and too large a volume would cause saturation of the binding sites from excess anions and would partially flush the glucose from the column. Thus 0.1 ml of 3.5 M $HClO_4$ was added to deproteinise the sample. This also served to drive off ^{14}C-bicarbonate as $^{14}CO_2$. The sample was then centrifuged at 4°C and the protein-free supernatant was removed and kept cold. It was found that the precipitate contained around 10% of the total ^3H-glucose dpm. Thus it was resuspended in 0.4 ml of distilled water (volume kept low for reasons previously described), re-centrifuged and the supernatant added to that previously saved. This resulted in removal of almost all of the radioactivity in the precipitate. 0.6 ml of 4 M K_2CO_3 in 0.5 M PIPES buffer was added slowly to the combined supernatants to return the pH to between 6.8 - 7.2. The buffer was included since it was found that it was difficult to obtain a pH of around 7 after neutralisation because the buffering proteins had been removed, and the ion exchange column requires a pH close to neutral. Since the neutralisation procedure resulted in the formation of another precipitate, this was removed by re-centrifuging at 4°C. The supernatant was then placed on a 5 x 1 cm column of Dowex-1 chloride (50-100 mesh) resin, which would retain charged components such as lactate. Since it was found that the void volume contained some ^3H- and ^{14}C-glucose, this was collected before eluting the remaining glucose from the column with distilled water. Experimentation showed that 5 ml was sufficient to elute all the glucose. Lactate was eluted with 5 ml of

0.2 M $CaCl_2$. A complete separation of glucose and lactate was confirmed by measuring glucose and lactate concentrations in the eluted fractions. A cation exchange resin was not used because it has previously been shown that this is unnecessary when dealing with glucose specific activity (12, 13).

In order to (i) minimise the presence of ^3H_2O (which accounted for ~5% of the glucose counts) from the metabolism of 2-^{3}H-glucose in the glycolytic pathway and (ii) reduce the water/ liquid scintillation cocktail ratio for radioactivity counting, the eluates (~6 ml) were evaporated to near dryness (~0.3 ml) at 70^{0}C over approximately 20 hr. One ml of distilled water was then added to re-dissolve the residue and this solution was then mixed with 15 ml of Ready Gel (Beckman, Fullerton, USA) liquid scintillation cocktail for ^{3}H and ^{14}C radioactivity determinations (dpm) by dual-channel counting (Packard Tri-Carb 4640, Illinois, USA). As small losses of radioactivity during processing are inevitable, whenever samples were processed, a control plasma sample was spiked with a known amount of 2-^{3}H and U-^{14}C-glucose and processed concurrently so that the dpm/min values of experimental samples could be corrected for the percentage recovery. Recovery was around 90%. Since the 1 ml aliquot of plasma used for radiation counting was from the same plasma sample as previously used for the determination of glucose concentration, the specific activity in dpm/ mmol glucose could be calculated.

CHAPTER 4

GENERAL METHODOLOGY

Subjects

Moderately trained (~1 hr cycling daily, 5-6 days per week for a minimum of 6 months) male endurance cyclists took part in the studies which were approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. As radiolabelled tracers were infused and ingested and muscle biopsy and blood samples were taken, the procedures and risks were carefully explained to the subjects and their written informed consents were obtained.

Measurement of maximum oxygen consumption

Each cyclist first came to the laboratory for determination of his $VO_{2\ max}$. This was measured during a progressive exercise test on an electrically braked cycle ergometer (Tunturipyora, Piisparisti, Finland), modified to the configuration of a racing bicycle. The starting work rate was 200 W with 15 W/min increments until exhaustion. This information was used to adjust the work rate in the experimental trial so that each subject exercised at an intensity corresponding to ~70% of $VO_{2\ max}$. Work rate was adjusted during the trial if necessary to maintain exercise at this intensity because of the upward drift that may occur in VO_2 during prolonged exercise.

During the test the subject wore a nose-clip and inspired air via a Hans Rudolph 2700 one-way valve (Vacumed, Ventura, USA) connected to a gas meter. Expired air was passed through a 15 l baffled mixing chamber and a condensation coil to an Ametek S-3A/I O_2 analyser with an N-22M sensor and a CD-3A CO_2 analyser with a P-61B sensor (Thermox Instruments, Pittsburgh, USA). Before each test the gas meter was calibrated with a Hans Rudolph 3 l syringe and the analysers were set with room air and a 4% CO_2 : 16% O_2 : 80% N_2 gas mixture. Instrument outputs were processed by an on-line computer which calculated the average inspired volume, oxygen uptake (VO_2) and carbon dioxide production (VCO_2) over each minute using conventional equations (76).

Measurements of fat free mass

So that measurements of fuel substrate kinetics could be expressed in terms of fat free mass (FFM), the percentage body fat of the subjects was determined from the triceps, biceps, subscapular and supra-iliac skinfold measurements. Percentage body fat was calculated using standard formulae (104).

Subject groups

Following the VO_2 max test, cyclists were randomly assigned to one of the following experimental groups:

Study 1: Subjects started exercise in either a carbohydrate-loaded (CL) or non-loaded (NL) state and ingested non-carbohydrate placebo (P) during exercise (CLP and NLP).

Study 2: Subjects started exercise after carbohydrate-loading (CL) and ingested carbohydrate (C) or non-carbohydrate placebo (P) during exercise (CLC and CLP).

Study 3: Subjects started exercise without prior carbohydrate-loading (NL) and ingested carbohydrate (C) or non-carbohydrate placebo (P) during exercise (NLC and NLP).

Study 4 Subjects started exercise after carbohydrate-loading and ingested non-carbohydrate placebo (CLP) during exercise or did not carbohydrate-load and ingested carbohydrate during exercise (NLC).

When necessary, carbohydrate-loading was achieved by the cyclist resting from training for 3 days and ingesting approximately 600 g of carbohydrate/day, mainly in the form of a commercially available glucose polymer (200 g) in solution, as well as bread, pasta, potato and rice. Subjects who were assigned to experimental groups where carbohydrate-loading was not a requirement also rested from training for 3 days, but continued to eat their normal diet. Total energy intake was similar for all subjects, but the composition was, of course, different.

Subjects participated in only one trial each to ensure that exposure to radiation was kept well below accepted safe limits.

On the day that an experiment was to be conducted, the cyclist returned to the laboratory to start the trial 3 hr after eating a carbohydrate-containing breakfast (70 g carbohydrate) that was similar to that which they would normally ingest before a cycling race. This approximated the conditions under which cyclists would normally race. The breakfast would decrease the likelihood of the subjects becoming hypoglycaemic during the trial and consisted of 1 cup of cereal with 125 ml of milk and 2 slices of toast. A possible negative consequence of this procedure was that if any absorption of the pre-exercise meal was still taking place at the start of the experiment, the specific activity of the exogenous glucose would have been reduced, with a consequent overestimation of the rate of exogenous glucose appearance. However, data of Massicotte et al. (233) show that where differences in plasma insulin concentrations existed between fed and fasted subjects, these disappeared after 20 min of exercise. Only serum FFA concentrations remained lower in fed subjects throughout 120 min of their study, but there were no differences between the groups in total carbohydrate and fat oxidation during either the first or second hours of exercise.

Determination of rates of glucose appearance, exogenous carbohydrate oxidation and plasma glucose oxidation

These are discussed in detail in Chapter 3.

Plasma glucose and blood lactate concentrations

Plasma glucose concentrations were determined using a glucose analyser (Analox LM3, Analox Instruments, London, UK) after collection of blood (5 ml) into chilled tubes containing potassium oxalate and sodium fluoride and centrifugation at 500 x g for 15 min. Blood lactate concentrations were measured by spectrophotometric (Beckman DU-62, Beckman Instruments Inc., Fullerton, Ca, USA) enzymatic assays as previously described (26) after collection of blood into chilled tubes containing 1 ml of 0.6 M $HClO_4$.

Change in leg muscle glycogen

The muscle biopsy technique of Bergstrom (27) as modified by Evans et al.(108) was used to sample muscle from the vastus lateralis muscle before the start of exercise and hourly thereafter. The glycogen concentration of the biopsy samples was measured using conventional methods (269).

Total carbohydrate and fat oxidation

Total carbohydrate and fat oxidation was calculated from the VO_2 and VCO_2 using the formulae of Consolazio et al. (76) and updated by Frayn (119). Non-protein respiratory exchange ratio (RER) was calculated by correcting for urinary urea by having subjects void at the start of the experiment and again on completion of the 3 hr ride. Gram/min values obtained from these formulae were converted to μmol/min/kg FFM units by dividing the values by the molecular weight of glucose or hexose equivalent of glycogen (180), and by the subject's FFM.

Plasma insulin and glucagon concentrations

Plasma insulin and glucagon concentrations were determined by radioimmunoassay (Boehringer Mannheim, Cape Town, South Africa) after collection of blood into tubes containing heparin and centrifugation at 500 x g for 15 min.

FFA concentrations

Serum FFA concentrations were determined by enzymatic techniques (Boehringer Mannheim, Cape Town, South Africa) after collection of blood into siliconised tubes and centrifugation at 500 x g for 15 min

Statistical treatment

All results are presented are means ± SEMs.

As 1 of the 9 NLC subjects stopped after 110 min and 1 after 132 min, means are presented for the 7 subjects who completed the test except where indicated. Of the 8 NLP subjects, 1 stopped after 132 min and 3 after 154 min. Thus, again, statistical analyses for time dependent parameters could only be completed on 7 subjects in each of these groups over 154 min. Although no statistical analyses were performed on the last

data point (n = 4), the mean values for the subjects who completed the trial are presented where appropriate.

Statistical significance ($p < 0.05$) of between-group differences were assessed by a 2-way Analysis of Variance for repeated measures over time and a Sheffe test used for post hoc analysis where necessary. A Fisher's exact test was used to determine significance of the different numbers of CLP and NLP subjects completing the test and a Students' t-test was used for comparison of single data between groups.

CHAPTER 5

STUDY 1: INFLUENCE OF CARBOHYDRATE-LOADING ON FUEL SUBSTRATE TURNOVER AND OXIDATION DURING PROLONGED EXERCISE

Introduction

The carbohydrate-loading diet was first popularised in the late 1960's after it had been found i) that muscle glycogen content could be increased by a high (70%) carbohydrate diet for three or more days before exercise (3, 28, 82, 305) and that ii) these high muscle glycogen stores seemed to enhance performance during subsequent endurance exercise (28, 189). However, the precise mechanism(s) underlying the proposed ergogenic effect of this dietary intervention remains to be established (75).

One possibility is that the higher muscle glycogen stores after carbohydrate-loading may delay the onset of fatigue resulting from muscle glycogen depletion during exercise (28, 89). However, that explanation would depend on the extent to which rates of muscle and hepatic glycogen utilisation during exercise are influenced by a higher pre-exercise muscle glycogen content. A number of studies have shown that rates of muscle glycogen utilisation during exercise are increased after carbohydrate-loading (28, 129, 143, 180, 206, 289, 306), in which case muscle glycogen depletion may not be delayed.

Alternatively, the proposed ergogenic effect of carbohydrate-loading could be due to a slowing of the rate of hepatic glycogen depletion since an increased availability of muscle glycogen would be expected to reduce the muscles' demand for plasma glucose oxidation from endogenous sources. However, a sparing of hepatic glycogen, again, would depend on how the rate of hepatic glycogenolysis is influenced by the pre-exercise hepatic glycogen content, since rates of hepatic glycogenolysis are accelerated by a high hepatic glycogen content after carbohydrate-loading (335).

An important limitation of previous studies is that the interaction between simultaneously increased hepatic and muscle glycogen contents on fuel substrate kinetics has not been studied. Accordingly, the aim of Study 1 was to quantitate the effects of pre-exercise carbohydrate-loading on muscle glycogen utilisation and hepatic glycogen to glucose turnover and oxidation in athletes ingesting only water during prolonged exercise and to determine how these effects on fuel substrate kinetics produced by carbohydrate-loading, might enhance performance during endurance exercise.

Subjects

Fifteen cyclists took part in the study and were randomly assigned to either carbohydrate-loading (CLP; n=7) or normal diet, non-loading (NLP; n = 8), groups. Both groups rested for 3 days before the trial, with the CLP subjects following a carbohydrate-loading regimen as described in Chapter 4.

At the end of the 3 days of the diet/rest regimen, the cyclists returned to the laboratory to start the trial which followed the procedures as described in Chapters 3 and 4.

Results

Subject details are given in Table 5.1. All had moderately high VO_2 max and peak work rate values, consistent with being well trained, but not elite cyclists. As expected, initial muscle glycogen concentrations were significantly higher in the CLP than NLP subjects and more CLP than NLP subjects completed 180 min of exercise ($p < 0.001$).

Oxygen consumption (Table 5.2) during exercise was relatively constant and not different between groups. However, non-protein respiratory exchange ratio (RER) progressively decreased in NLP subjects and was significantly ($p < 0.05$) lower at 150 and 180 mins than at the start of exercise (Table 5.2).

Although carbohydrate oxidation during exercise was not different between groups (Table 5.2; Figure 5.1, upper panel), the percent contribution to total energy production from carbohydrate oxidation declined with time (Figure 5.1, lower panel) and was significantly lower in the NLP than CLP subjects after 150 min.

Whereas plasma glucose concentrations were maintained above 4.5 mmol/l in CLP subjects (Figure 5.2), they declined significantly in NLP subjects after 150 min. Concentrations were significantly higher in the CLP than NLP subjects after that time.

Despite the decline in plasma glucose concentration in NLP subjects after 150 min, rates of plasma glucose oxidation were not different between groups (Figure 5.3). In both the CLP and NLP subjects, rates of plasma glucose oxidation rose significantly to 80 - 90 μmol/min/kg FFM or around 1 g/min, after 180 min of exercise (Figure 5.3), at which time 53% of all carbohydrate oxidation was derived from plasma glucose in NLP subjects and 37% in the CLP subjects (Figure 5.4).

The contribution of fat to total energy production increased significantly over time to 43 $\pm$ 6% in the NLP subjects, but not in the CLP subjects (27 $\pm$ 8%) (Table 5.2; Figure 5.4). The higher rates of fat oxidation in the NLP subjects at the end of exercise (Table 5.2) were not statistically significant.

Rates of glucose appearance were also not significantly different between groups (Figure 5.5).

Table 5.1. Characteristics of subjects.

	CLP (n=7)	NLP (n=8)
Age (yr)	24 (3.0)	27 (2.5)
$VO_{2\,max}$ (l/min)	3.6 (0.2)	3.7 (0.2)
Peak work rate (W)	315 (25)	325 (20)
Mass (kg)	73.5 (4.0)	70.9 (4.5)
FFM (kg)	63.4 (3.1)	60.7 (3.7)
% Body fat	13 (1.0)	14 (1.0)
Muscle glycogen * (mmol/kg ww)	194 (4)	124 (7)
No completing 180 * min of exercise	7/7	4/8
Exercise time to exhaustion (min) *	180	180 (n=4) 150 (n=4)

Values are Means ± (SEM). CLP indicates carbohydrate-loaded and NLP indicates non carbohydrate-loaded. FFM is fat free mass. * Significantly different ($p < 0.05$).

Table 5.2. Steady state gas exchange data and total carbohydrate oxidation during 180 min of cycling in carbohydrate-loaded (CLP) and non-loaded (NLP) subjects.

		Time (mins)								
		0	22	44	66	88	110	132	150	180
VO_2	CLP	2.63	2.63	2.62	2.54	2.64	2.55	2.44	2.66	2.52
(l/min)		(0.2)	(0.2)	(0.2)	(0.1)	(0.2)	(0.1)	(0.1)	(0.2)	(0.3)
	NLP	2.57	2.57	2.67	2.64	2.64	2.62	2.63	2.69	2.80
		(0.2)	(0.2)	(0.1)	(0.1)	(0.1)	(0.2)	(0.2)	(0.2)	(0.2)
RER	CLP	0.93	0.93	0.92	0.93	0.92	0.92	0.92	0.91	0.90
		(0.03)	(0.03)	(0.04)	(0.03)	(0.03)	(0.03)	(0.03)	(0.02)	(0.02)
	NLP	0.93	0.93	0.92	0.92	0.94	0.92	0.89	0.86*	0.86*
		(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)
CHO_{ox}	CLP	2.76	2.75	2.54	2.57	2.55	2.38	2.30	2.36	2.18
(g/min)		(0.6)	(0.5)	(0.5)	(0.4)	(0.4)	(0.3)	(0.3)	(0.3)	(0.4)
	NLP	2.62	2.62	2.58	2.53	2.72	2.47	2.17	1.83	1.88
		(0.3)	(0.3)	(0.3)	(0.3)	(0.2)	(0.3)	(0.2)	(0.1)	(0.3)
Fat_{ox}	CLP	0.22	0.22	0.29	0.24	0.30	0.32	0.29	0.38	0.38
(g/min)		(0.11)	(0.10)	(0.12)	(0.11)	(0.10)	(0.10)	(0.07)	(0.09)	(0.09)
	NLP	0.27	0.27	0.34	0.34	0.27	0.35	0.47	0.62*	0.65*
		(0.07)	(0.07)	(0.08)	(0.10)	(0.08)	(0.09)	(0.10)	(0.09)	(0.11)

Values are means ± (SEM). VO_2, oxygen consumption; RER, respiratory exchange ratio; CHO_{ox}, carbohydrate oxidation calculated from gas exchange data; Fat_{ox}, fat oxidation from gas exchange data. Differences between groups are not significant. *Significantly different from time 0 ($p < 0.05$).

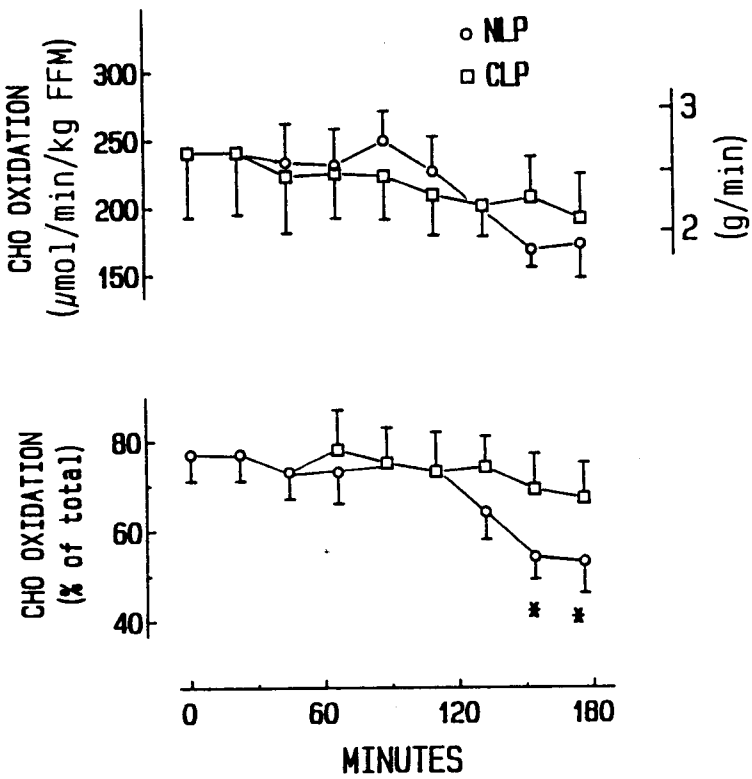

Figure 5.1. Absolute rate of carbohydrate oxidation (upper panel) and percentage contribution to total energy production from carbohydrate oxidation in NLP and CLP subjects (lower panel). * Significantly lower than at the start of exercise in NLP subjects ($p < 0.05$).

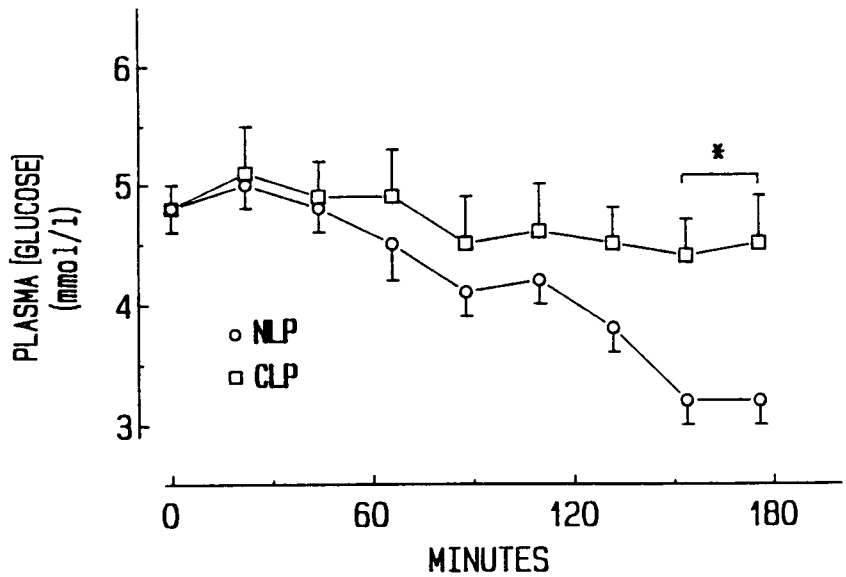

Figure 5.2. Changes in plasma glucose concentration in NLP and CLP subjects. *Significantly lower in NLP than CLP subjects ($p < 0.05$).

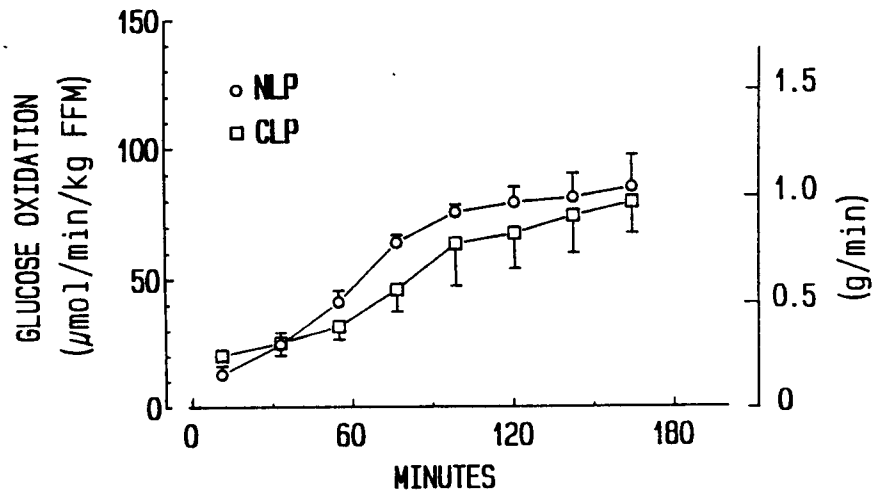

Figure 5.3. Rates of plasma glucose oxidation in NLP and CLP subjects. Increase over time ($p < 0.05$) is significant.

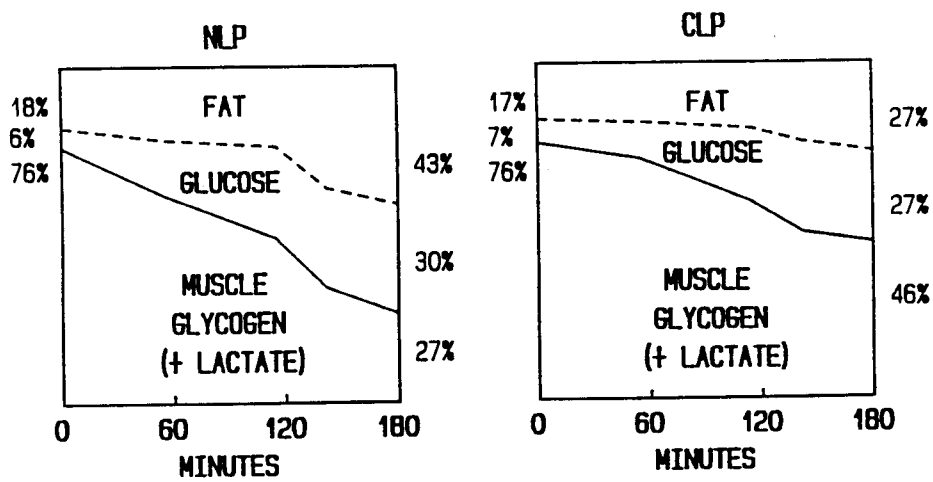

Figure 5.4. Relative contribution of fuel substrates to energy production. Data were obtained by converting g/min values for carbohydrate, plasma glucose and fat oxidation to kilojoules and expressing as a percentage of total energy expenditure.

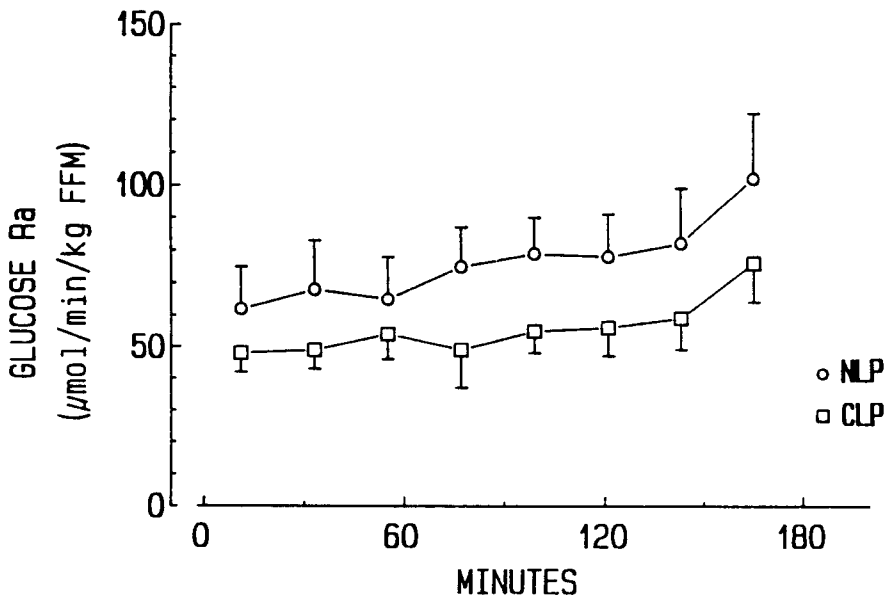

Figure 5.5. Rates of glucose appearance (Ra) in NLP and CLP subjects. Apparent increase in Ra was not significant.

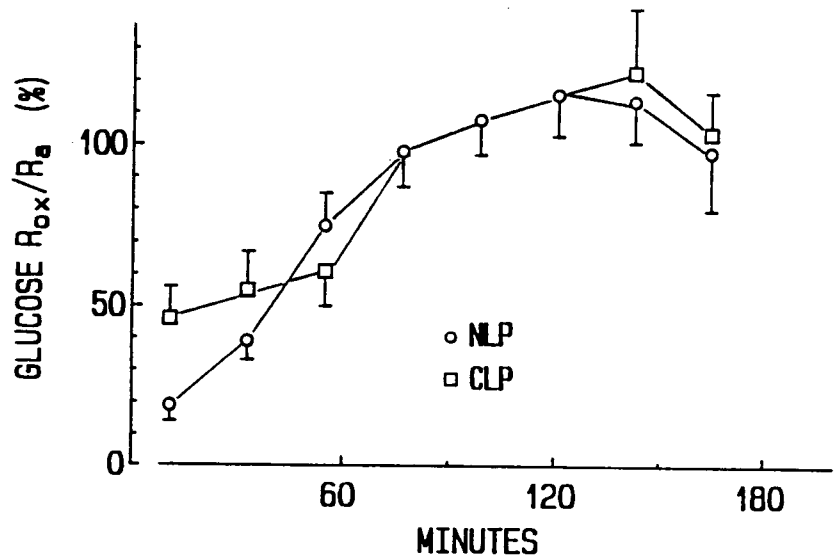

Figure 5.6. Glucose oxidation expressed as a percentage of turnover. Increase over time was significant ($p < 0.05$).

Although the rates of plasma glucose oxidation increased with time ($p < 0.05$; Figure 5.3), rates of total splanchnic (endogenous plus exogenous) glucose appearance (Ra) were already high at the onset of exercise and tended to increase over time (Figure 5.5), but again this rise was not significant. As a result, the rate of glucose turnover exceeded the rate of glucose oxidation during the first 75 min of exercise (Figure 5.6).

Changes in muscle glycogen concentrations with time are shown in Figure 5.7. Muscle glycogen concentrations were significantly higher in CLP than in NLP subjects throughout the 180 min of exercise. In the 4 NLP subjects who failed to complete the 180 min of exercise, the mean muscle glycogen concentration at exhaustion appeared to be lower (22 $\pm$ 4 mmol/kg ww) than that calculated to be present at the same time (39 $\pm$ 12 mmol/kg ww) in the 4 NLP subjects who completed 180 min of exercise (Figure 5.7), but with such small subject numbers this was not statistically significant.

Rates of muscle glycogen disappearance in the first hour of exercise were similar in both groups (48 vs 54 mmol/kg ww/hr in CLP and NLP respectively) (Figure 5.7). However, during the second hour of exercise, rates of glycogen disappearance were significantly higher in CLP than in NLP subjects (57 vs 26 mmol/kg ww/hr). During this period, rates of muscle glycogenolysis similar to those in the first hour were maintained in CLP subjects, in which a decline only occurred in the third hour. In contrast, rates of muscle glycogenolysis fell significantly to ~26 mmol/kg ww/hr after the first hour in the NLP subjects, at which time muscle glycogen concentration had declined to ~70 mmol/kg ww. During the third hour rates were once again not significantly different between groups.

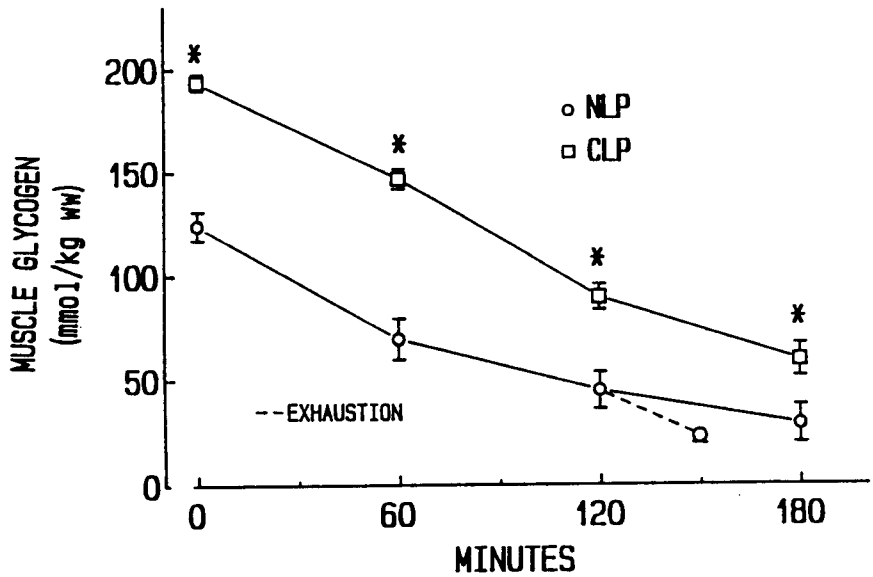

Figure 5.7. Changes in muscle glycogen concentration with time. Glycogen concentration was higher in CLP subjects throughout exercise.

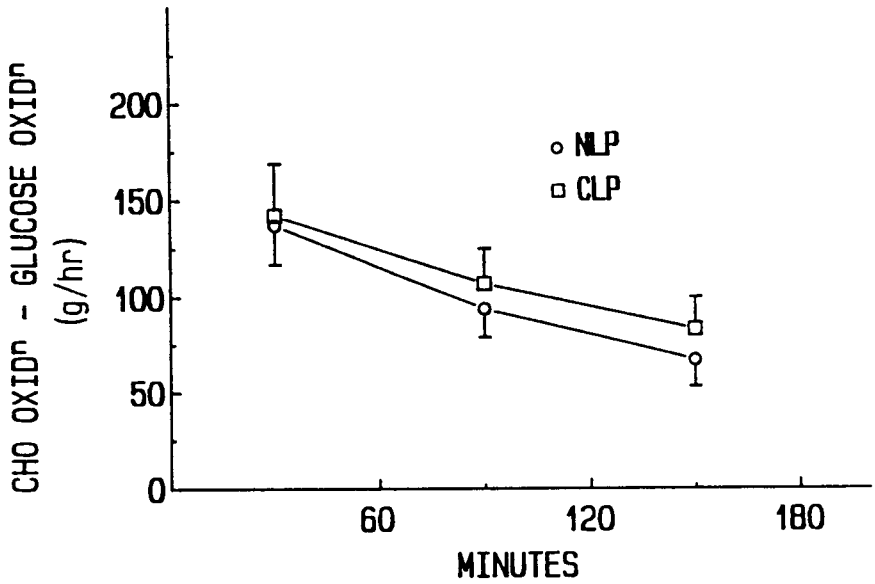

Figure 5.8. Rates of muscle glycogen plus lactate oxidation. Data were calculated by difference between total carbohydrate oxidation and plasma glucose oxidation.

Unlike muscle glycogen utilisation in the working muscles, which was significantly different after the first hour of exercise, total muscle glycogen plus lactate oxidation, calculated from the difference between total carbohydrate oxidation and plasma glucose oxidation, was not significantly different between the groups at any time and fell gradually during the trial (Figure 5.8).

Discussion

One of the important findings of this study was that 50% of subjects who did not carbohydrate-load before exercise were unable to complete the 180 min trial. In contrast, all those who carbohydrate-loaded finished the 180 min exercise bout (Table 5.1). An increased time to exhaustion is compatible with the findings from field and laboratory studies showing an ergogenic effect of carbohydrate-loading (28, 129, 189, 236, 273).

The clearest metabolic effects of carbohydrate-loading were the maintenance of a high percentage contribution to energy production from carbohydrate oxidation (Figure 5.1) and the maintenance of plasma glucose concentrations during the last 30 minutes of exercise (Figure 5.2). In addition, muscle glycogen concentration in the CLP subjects was higher throughout exercise (Figure 5.7).

One possible explanation for the ergogenic effect of carbohydrate-loading in the present study might be that this dietary manipulation maintained plasma glucose concentrations, since this was significantly higher in

CLP than NLP subjects (Figure 5.2). However, plasma glucose concentrations were not different in NLP subjects who either terminated the trial prematurely due to exhaustion at 150 min or who completed 180 min of exercise (Table 5.3).

Table 5.3. Comparison of metabolic variables of subjects in the NLP group who either completed 180 mins of cycling or who terminated exercise prematurely

Variable	NLP non-finishers at 150 min	NLP finishers at 150 min	NLP finishers at 180 min
Glucose (mmol/l)	3.8 (0.4)	3.1 (0.2)	3.2 (0.2)
Ra (mmol/min)	4.03 (0.62)	5.24 (1.07)	5.74 (1.05)
$Glucose_{ox}$ (g/min)	0.90 (0.15)	0.90 (0.11)	0.93 (0.13)
CHO_{ox} (g/min)	2.19 (0.31)	1.76 (0.19)	1.88 (0.28)
RER	0.90 (0.03)	0.86 (0.02)	0.86 (0.02)
Glycogen (mmol/kg ww)	22 (4)	39* (12)	34 (15)

Values are means ± SEM. n = 4 subjects in each group. Ra, rate of appearance of glucose from liver; $Glucose_{ox}$, plasma glucose oxidation; CHO_{ox}, carbohydrate oxidation. Differences between groups are not significant. * Calculated from individual rates of utilisation.

There were also no significant differences in any other aspects of carbohydrate kinetics between NLP subjects who either completed or failed to complete the trial and between NLP and CLP groups. Thus total carbohydrate oxidation, plasma glucose oxidation and Ra were not significantly different ($p > 0.05$) between the NLP finishers and non-finishers (Table 5.3) or between NLP and CLP groups (Figures 5.1, 5.3, 5.4 and 5.5). Despite the lower plasma glucose concentrations in the NLP than CLP subjects after 150 min, rate of plasma glucose oxidation was maintained in both NLP finishers and non-finishers and it is therefore

impossible that factors related to plasma glucose oxidation were the cause of 50% of the NLP subjects being unable to complete the trial. However, the low plasma glucose concentration *per se* in the NLP subjects may have contributed to their inability to complete the trial. Coyle et al. (89) found that hypoglycaemia was the cause of subjects in their experiment becoming fatigued, despite muscle glycogen concentrations of around 40 mmol/kg ww, a value sufficiently high so as not to be the cause of exhaustion (189). Hypoglycaemia has previously been shown to influence RPE (and therefore possibly exhaustion) only when glycogen becomes depleted (327).

Glycogenolysis in non-working muscles during exercise has been shown previously to provide a source of lactate for gluconeogenesis (49, 241). This may be the mechanism whereby plasma glucose concentration was maintained in CLP subjects. However, it is surprising that Ra was not found to be higher in this group of subjects or even between NLP finishers and non-finishers.

Although the muscle glycogen concentration of the 4 NLP subjects who became exhausted after only 150 min of exercise were not significantly lower than those predicted from the final two biopsies to be present at the corresponding time in the NLP subjects who completed the experiment, the means were well separated (22 $\pm$ 4 mmol/kg ww vs 39 $\pm$ 12 mmol/kg ww) and the lack of significance can probably be attributed to the small subject numbers. Since there were no differences in the plasma glucose concentrations of the NLP finishers and non-finishers, it is likely that the exhaustion of the NLP non-finishers can be ascribed to low muscle glycogen concentrations. It is interesting that the low (22 $\pm$ 4 mmol/kg ww) muscle glycogen values in the NLP subjects who failed to complete the trial are consistent with the reported muscle glycogen values of 17-28 mmol/kg ww below which optimal race pace cannot be maintained (189). Since there were only 4 subjects in each of the NLP sub-groups, more data are needed to determine whether the non-finishers were limited by low muscle glycogen concentrations. In the present study there are only sufficient subject numbers to address differences between CLP and NLP groups as a whole. These data are discussed subsequently.

The values of 22 $\pm$ 4 mmol/kg ww for muscle glycogen in the cyclists who became prematurely exhausted are substantially lower than the values of around 40 mmol/kg ww reported in the study of Coyle et al. (89) in which subjects were able to continue exercise for longer when ingesting carbohydrate. As mentioned previously, this was ascribed to the maintenance of high rates of plasma glucose oxidation by the muscle and it was concluded that exhaustion was due primarily to an inadequate supply of carbohydrate for oxidation.

Although there were no statistically significant differences in glycogen concentration between the NLP subjects who either completed or failed to complete the trial, NLP subjects as a group had significantly lower glycogen values than CLP subjects at the end of exercise. Thus, the data from this study show that carbohydrate-loading may enhance endurance performance by delaying the time at which muscle glycogen

concentration becomes very low (Figure 5.7); a state which may be consistent with exhaustion (3, 19, 20, 28, 29, 37, 46, 47, 81, 132, 136, 157, 159, 162, 174, 211, 212, 263, 264, 270, 298, 299, 317, 364, 365).

In the CLP subjects, high rates of muscle glycogen utilisation (48 mmol/kg ww/ hr) were maintained for the first 2 hrs of exercise (Figure 5.7). However in the NLP subjects such high rates of glycogen utilisation were only observed during the first hour of exercise. Once the muscle glycogen concentration had declined to around 70 mmol/kg ww the rate of glycogen utilisation decreased for the second and third hours (Figure 5.7), suggesting that when muscle glycogen decreases to an apparent critical concentration of around 70 mmol/kg ww the rate of muscle glycogenolysis decreases. It has previously been reported that there is a decline in the rate of utilisation when muscle glycogen concentration reaches 80 mmol/kg ww, during short, intense contraction (286).

Although overall carbohydrate metabolism was not influenced by carbohydrate-loading, total muscle glycogen disappearance was greater in the carbohydrate-loaded subjects because high rates of muscle glycogen utilisation were maintained during the second hour when glycogen concentration was still high in the CLP subjects (Figure 5.7). Others (28, 129, 143, 152, 206, 289, 306) have also shown that the rate of glycogen disappearance during exercise is increased from muscles with high starting glycogen content. However, in those studies, muscle biopsy samples were taken only at the start and end of exercise, making it impossible to determine a time-course of utilisation. Furthermore, in only one of these studies (28) was exercise prolonged (3 hr). In the present study, muscle biopsies were performed each hour, which made it possible to identify the time period during which higher rates of utilisation occurred.

The finding that total muscle glycogen disappearance was greater in CLP subjects would predict that either overall carbohydrate oxidation (as measured by gas exchange) should be increased in CLP subjects, or that plasma glucose oxidation should be reduced in proportion to the increased muscle glycogen contribution to total carbohydrate oxidation. However, this was not the case. Despite apparent differences in rates and extent of muscle glycogen utilisation, plasma glucose oxidation (Figure 5.3), total carbohydrate oxidation (Upper panel of Figure 5.1) and overall muscle glycogen plus lactate oxidation (calculated from the differences between the rates of total carbohydrate and plasma glucose oxidation; Figure 5.8) were the same in both groups. Thus, for the energy equation to balance, more lactate must have been oxidised by the NLP than CLP subjects during the second hour of exercise. A breakdown of glycogen in non-working muscles to provide lactate as an oxidisable substrate for glycogen depleted working muscles has previously been argued to be an important mechanism of redistributing carbohydrate during exercise (49, 345).

Thus, from this study, it can be concluded that: i) carbohydrate-loading may have extended endurance by increasing the time taken for muscle glycogen and plasma glucose concentrations to become critically low

in subjects not ingesting carbohydrate, ii) carbohydrate-loading did not reduce hepatic glycogenolysis or gluconeogenesis, or both since hepatic glucose appearance was unaffected by carbohydrate-loading, and iii) carbohydrate-loading did not influence the rate of plasma glucose oxidation.

CHAPTER 6

STUDY 2: INFLUENCE OF CARBOHYDRATE INGESTION ON FUEL SUBSTRATE TURNOVER AND OXIDATION DURING PROLONGED EXERCISE

Introduction

It has been well established that carbohydrate ingestion during exercise can enhance endurance performance by supplying carbohydrate for oxidation (28, 69, 71, 89, 138, 172, 189, 350).

In Study 1 (Chapter 5) it was found that muscle glycogen concentration at the start of exercise had little effect on energy substrate kinetics when water was ingested during prolonged (180 min) cycling at 70% of maximal oxygen uptake ($VO_{2\ max}$). Particularly, the rate of hepatic glucose turnover and plasma glucose oxidation was not different in subjects who started exercise with either very high (194 mmol/kg ww) or normal (124 mmol/kg ww) muscle glycogen concentrations. However, as found in Study 1 (Chapter 5), muscle glycogen utilisation was greater in carbohydrate-loaded than non-loaded subjects during the second and third hours of exercise. Despite higher rates of glycogenolysis later in exercise, muscle glycogen concentration was still higher in carbohydrate-loaded subjects at the end of 180 min of cycling (Study 1, Chapter 5). Further, muscle glycogen concentration appeared to be critically important for the continuation of exercise, as 50% of cyclists who did not carbohydrate-load before the trial failed to complete the 180 min of cycling. Hence carbohydrate-loading had an ergogenic effect as previously shown (28, 129, 189) that could not be attributed to a measurable effect on plasma glucose turnover and, instead, appeared to be due to a local effect associated with adequate muscle glycogen within the active muscles.

Besides the ergogenic effect of carbohydrate-loading described above, there is also an ergogenic effect of carbohydrate ingestion during prolonged exercise that was first demonstrated by Christensen and Hansen in 1939 (64), and has subsequently been repeatedly confirmed (34, 43, 69, 71, 89, 91, 126, 138, 170, 172, 235, 248, 252, 256, 304, 350, 362). For this effect to be manifest, it appears that more than 4.2 g (260), to 24 g (247) of carbohydrate per hour has to be ingested and the duration of exercise has to be greater than 1 hr (260).

The oxidation rates of different types of carbohydrate (98, 135, 145, 231, 232, 251) and their effects on performance (254) have been found to be very similar. The effect of ingesting varying amounts of carbohydrate during the exercise period on blood glucose oxidation rate has also been studied (2, 248, 251, 253, 267, 308). Despite all these data, the precise effects of carbohydrate ingestion on total (splanchnic) and endogenous hepatic glucose appearance, blood glucose oxidation, and muscle glycogenolysis have not been studied simultaneously. Hence the aim of this investigation was to determine the effect of carbohydrate ingestion during exercise on the kinetics of hepatic glucose turnover, exogenous and endogenous glucose oxidation and muscle glycogen utilisation in carbohydrate-loaded cyclists who ingested either a

carbohydrate drink or a placebo during prolonged exercise and whether carbohydrate ingestion altered either muscle glycogen utilisation or hepatic glucose turnover in a way that might explain the established ergogenic effect of ingesting carbohydrate during exercise.

Subjects

Fourteen cyclists took part in the study and were randomly assigned to either carbohydrate ingestion (CLC; n = 7) or placebo ingestion (CLP; n = 7) groups. Both groups rested for 3 days before the trial, with both groups following a carbohydrate-loading regimen, as described in Chapter 4.

At the end of the 3 days of the diet/rest regimen, the cyclists returned to the laboratory to start the trial which followed the procedures as described in Chapters 3 and 4.

Results

Subject characteristics are given in Table 6.1. All had moderately high $VO_{2\ max}$ and peak work rate values, consistent with being well trained but not elite cyclists. All subjects completed the 180 min of exercise.

Oxygen consumption (Table 6.2) during exercise was relatively constant as the exercise intensity of the subjects was maintained at close to 70% of $VO_{2\ max}$ and was not significantly ($P < 0.05$) different between groups. Respiratory exchange ratio was also not significantly different between the groups, but fell significantly during the trial (Table 6.2). There were no significant differences between groups in total carbohydrate or fat oxidation during exercise (Table 6.2), both changing significantly over time.

Plasma glucose concentrations (Figure 6.1) were not significantly different between groups and remained between 4.1 ± 0.3 and 5.1 ± 0.4 mmol/l throughout exercise. However, Ra was significantly ($p < 0.05$) higher in CLC than CLP subjects (Figure 6.2), but endogenous Ra (Ra_{end}) was less in the CLC than CLP group (Figure 6.2, lower solid line) and remained relatively constant. Exogenous Ra (Ra_{exog})in the CLC group increased significantly ($p < 0.05$) until a plateau was reached after ~75 min (Figure 6.3), at which time 60 - 67% of Ra was accounted for from exogenous carbohydrate.

Plasma glucose oxidation (Figure 6.4) also rose progressively ($p < 0.05$) over the first 100 min of exercise from 22 ± 4 to 100 ± 10 μmol/min/kg FFM (0.25 ± 0.05 to 1.14 ± 0.18 g/min) in CLC subjects, after which there was little further increase with the final value at the end of the trial being 107 ± 8 μmol/min/kg FFM (1.24 ± 0.10 g/min)). In CLP subjects oxidation increased from 19 ± 4 to 62 ± 17 μmol/min/kg FFM (0.22 ± 0.05 to 0.70 ± 0.19 g/min) in the first 100 min and thereafter increased more slowly to 76 ± 13 μmol/min/kg FFM (0.88 ± 0.15 g/min) (Figure 6.4, solid lines).

Table 6.1. Characteristics of subjects.

	CLP (n=7)	CLC (n=7)
Age (yr)	25 (3)	26 (2)
$VO_{2\ max}$ (l/min)	3.64 (0.19)	3.93 (0.18)
Peak work rate (W)	332 (23)	330 (20)
Mass (kg)	74.5 (3.5)	73.4 (3.0)
FFM (kg)	64.6 (2.5)	62.7 (2.6)
% Body fat	13 (1.0)	15 (1.0)
Muscle glycogen (mmol/kg ww)	195 (5)	210 (10)
Completed 180 min of exercise	7 of 7	7 of 7

Values are Means ± (SEM). CLC, carbohydrate ingestion; CLP, water ingestion; FFM, fat free mass. No significant differences existed between groups.

Table 6.2. Steady state gas exchange data and total carbohydrate oxidation during 180 min of cycling in subjects ingesting carbohydrate (CLC) or water placebo (CLP).

		Time (mins)								
		5	22	44	66	88	110	132	150	18
VO_2	CLC	2.79	2.79	2.85	2.73	2.69	2.61	2.61	2.72	2.7
(l/min)		(0.15)	(0.15)	(0.18)	(0.13)	(0.12)	(0.14)	(0.13)	(0.14)	(0.1
	CLP	2.51	2.51	2.52	2.46	2.54	2.53	2.42	2.59	2.5
		(0.21)	(0.21)	(0.15)	(0.11)	(0.14)	(0.13)	(0.11)	(0.13)	(0.1
RER	CLC	0.94	0.94	0.92	0.93	0.90	0.89	0.89	0.88	0.8
		(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.02)	(0.01)	(0.02)	(0.0
	CLP	0.91	0.91	0.91	0.91	0.89	0.89	0.90	0.88	0.8
		(0.04)	(0.04)	(0.03)	(0.03)	(0.03)	(0.03)	(0.02)	(0.03)	(0.0
CHO_{ox}	CLC	2.75	2.75	2.65	2.59	2.28	2.10	2.03	2.07	2.0
(g/min)*		(0.23)	(0.23)	(0.21)	(0.30)	(0.24)	(0.20)	(0.18)	(0.24)	(0.2
	CLP	2.37	2.37	2.29	2.32	2.13	2.08	2.05	2.00	1.9
		(0.58)	(0.57)	(0.47)	(0.42)	(0.41)	(0.35)	(0.27)	(0.37)	(0.3
Fat_{ox}	CLC	0.28	0.28	0.35	0.31	0.41	0.44	0.46	0.50	0.5
(g/min)*		(0.07)	(0.07)	(0.08)	(0.10)	(0.08)	(0.08)	(0.06)	(0.08)	(0.0
	CLP	0.30	0.30	0.34	0.33	0.41	0.41	0.38	0.48	0.4
		(0.11)	(0.11)	(0.10)	(0.11)	(0.11)	(0.11)	(0.08)	(0.11)	(0.1

Values are means ± (SEM). VO_2, oxygen consumption; RER, respiratory exchange ratio; CHO_{ox}, carbohydrate oxidati calculated from gas exchange data; Fat_{ox}, fat oxidation from gas exchange data. * Significant change over ti ($p < 0.05$). There were no significant differences between groups, however carbohydrate oxidation tended to be hig and fat oxidation lower for the first 90 min of exercise in the CLC subjects.

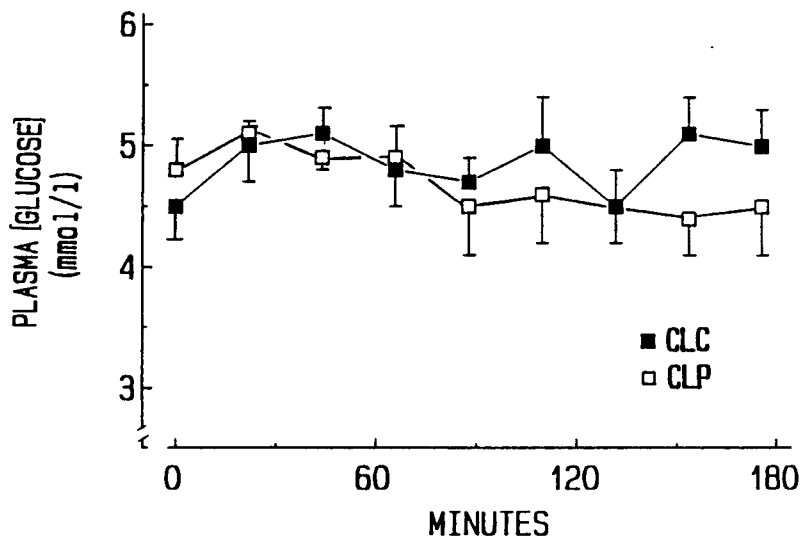

Figure 6.1. Plasma glucose concentrations were not significantly different between groups ingesting either carbohydrate or water placebo, remaining relatively constant throughout the trial.

Rates of plasma glucose oxidation were significantly ($p < 0.05$) higher in the CLC than CLP subjects (Figure 6.4, solid lines) with a large contribution from exogenous carbohydrate (Figure 6.5). Exogenous (ingested) glucose oxidation reached 63 ± 8 μmol/min/kg FFM (0.71 ± 0.08 g/min) after 90 min, with little further increase (Figure 6.5). At the end of exercise 65 ± 8% of all carbohydrate oxidation was derived from plasma glucose in the CLC subjects and 53 ± 11% ($p < 0.05$) in the CLP subjects.

Although Ra exceeded the rate of glucose oxidation in both groups during the first 75 min of exercise, thereafter Ra equalled the rate of plasma glucose oxidation (Figure 6.6).

Plasma insulin concentrations decreased progressively in both groups from 5.6 ± 0.3 to 4.2 ± 0.45 and from 2.7 ± 0.7 to 1.2 ± 0.45 μU/ml in CLC and CLP groups, respectively, between 60 and 180 min of exercise. The differences between groups were significant ($P < 0.05$) at all time points.

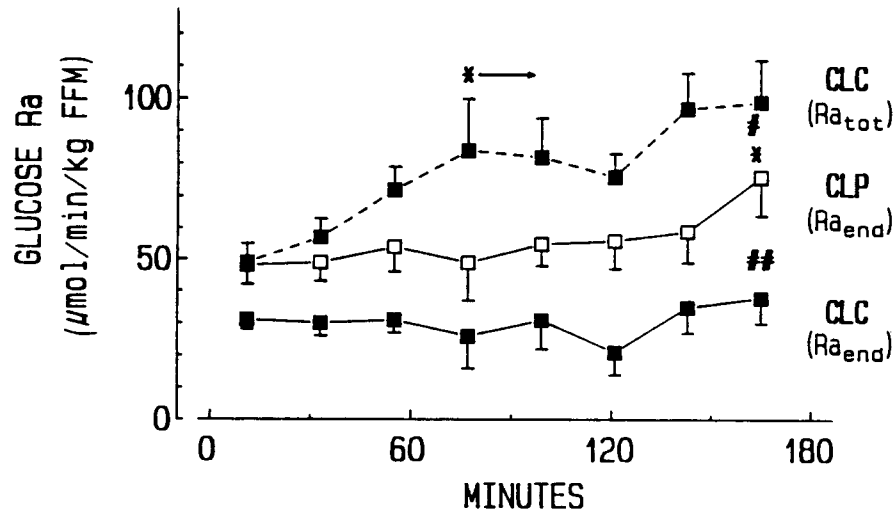

Figure 6.2. Rate of total glucose appearance (Ra) increased significantly in both groups during the trial. Rate of endogenous glucose appearance (Ra_{end}) in subjects ingesting carbohydrate (CLC) (solid line) was significantly less than in subjects ingesting placebo (CLP), although Ra in the CLC subjects (dotted line) was significantly greater ($p < 0.05$). * Significantly higher than at the start ($p < 0.05$). # Ra in the CLC subjects significantly higher than the CLP subjects after 60 min of exercise ($p < 0.05$). ## CLC (Ra_{end}) significantly less than CLP (Ra_{end}) throughout exercise ($p < 0.05$).

Serum FFA concentrations increased significantly in both groups during the trial from 0.51 ± 0.07 to 0.92 ± 0.14 and from 0.46 ± 0.05 to 1.02 ± 0.01 mmol/l in the CLC and CLP subjects, respectively. There were no significant differences between groups.

A more or less linear decline in muscle glycogen concentration during the trial, which was similar in both the CLC and CLP subjects throughout the 180 min of exercise, is shown in Figure 6.7. Mean values at the end of exercise were 74 ± 16 and 55 ± 10 mmol/kg ww (CLC and CLP, respectively).

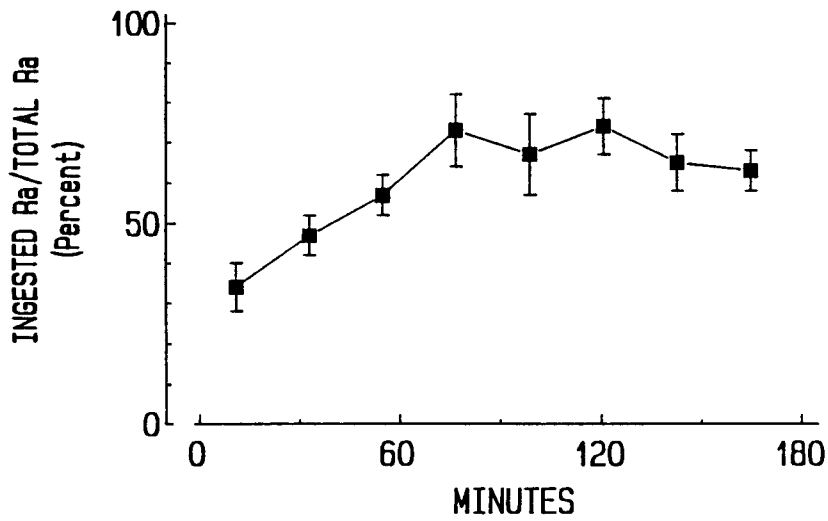

Figure 6.3. Percentage contribution of ingested (exogenous) carbohydrate to Ra in subjects ingesting carbohydrate. A plateau was reached after 75 min.

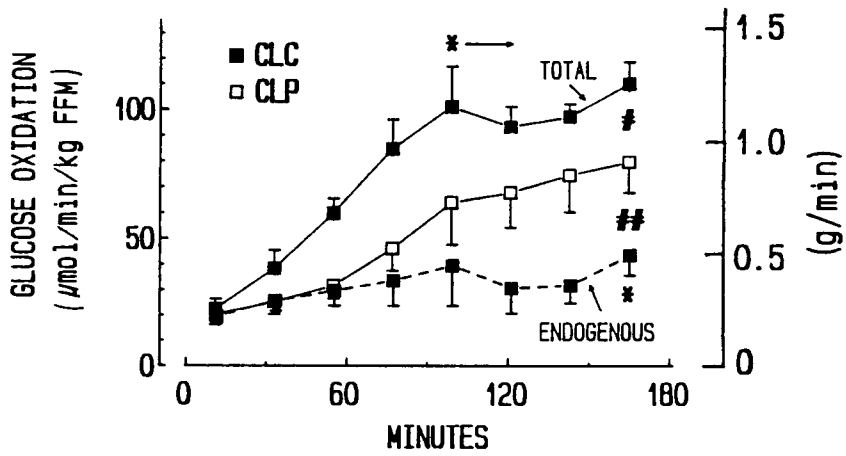

Figure 6.4. Plasma glucose oxidation increased significantly in both groups ($p < 0.05$) during the trial. Endogenous glucose oxidation in subjects ingesting carbohydrate (CLC) (dotted line) was significantly ($p < 0.05$) less than in subjects ingesting placebo (CLP), although total glucose oxidation in the CLC subjects (solid line) was significantly ($p < 0.05$) greater. * Plateau reached after 100 min. # CLC (total) significantly higher than CLP ($p < 0.05$). ## CLP significantly higher than endogenous glucose oxidation in CLC ($p < 0.05$).

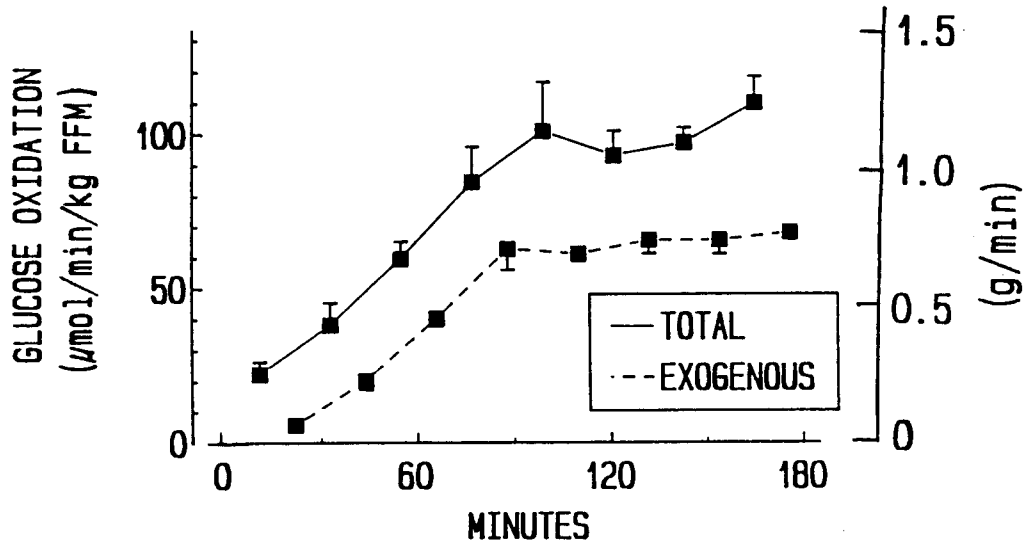

Figure 6.5. Effect of carbohydrate ingestion on plasma glucose oxidation. Exogenous carbohydrate contributed significantly to total plasma glucose oxidation.

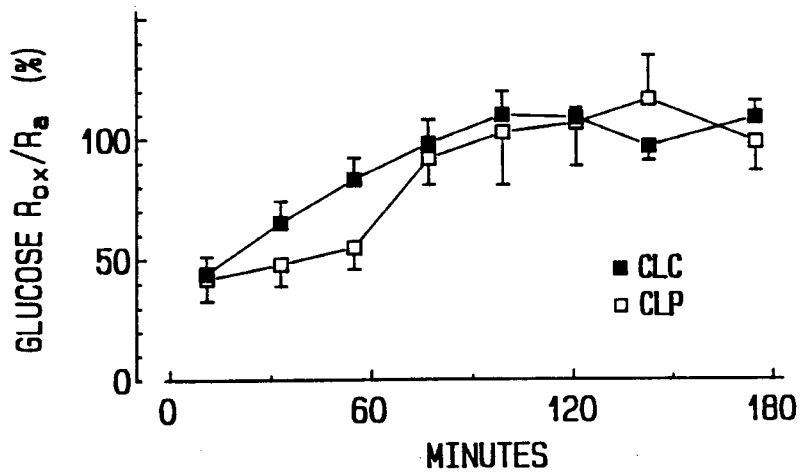

Figure 6.6. Plasma glucose oxidation shown as a percentage of rate of total glucose appearance (Ra). Ra exceeded the rate of oxidation for the first 75 min of exercise, but thereafter Ra and oxidation rate were equal.

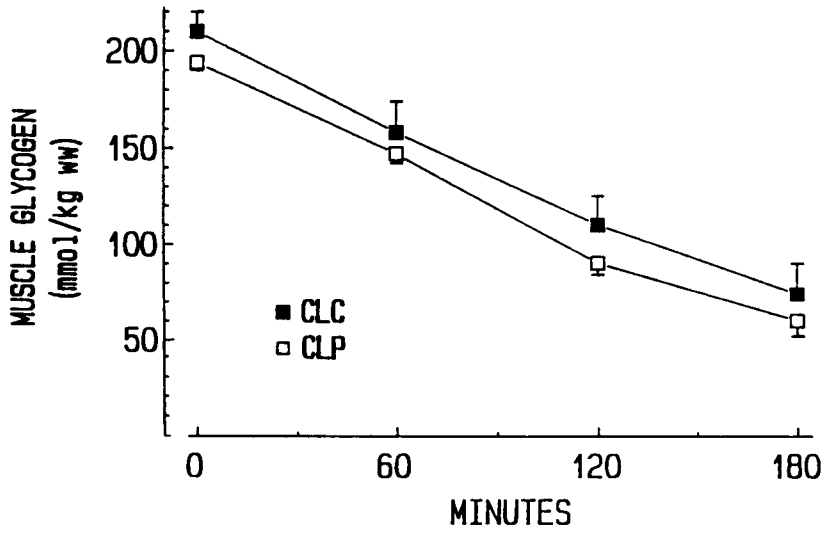

Figure 6.7. Rate of muscle glycogen disappearance was significant over time ($p < 0.05$), but not between groups.

Discussion

Studies such as those of Massicotte et al. (231, 232) and Pirnay et al. (277) have shown by calculation of the difference between total carbohydrate and exogenous carbohydrate oxidation, that less endogenous carbohydrate is oxidised when carbohydrate is ingested during exercise, but the nature of this sparing remains to be elucidated. In particular, the relationship between carbohydrate ingestion, Ra, and the rates of oxidation of blood glucose originating from exogenous carbohydrate and endogenous sources has not been determined. Radziuk et al. (280) partly answered the question of the effects of exogenous carbohydrate on Ra_{end} by using tracer techniques, but these studies were in non-exercising subjects and gave no information about blood glucose oxidation rates and the influence of the ingested carbohydrate on muscle glycogen utilisation.

Hence the first important finding of this study was that ingestion of carbohydrate during exercise had a significant hepatic glycogen sparing effect or caused a reduction in the rate of gluconeogenesis, or both, since Ra_{end} was significantly reduced in CLC subjects (Figure 6.2).

The reduced Ra_{end} remained relatively constant throughout exercise despite an increase in Ra (Figure 6.2). The increase in Ra was similar to that reported by Wahren et al. (343) and occurred as a result of a significant contribution of exogenous carbohydrate to Ra which increased during exercise, reaching a plateau of around 67% of Ra at approximately 75 min (Figure 6.3). Carbohydrate ingestion resulted in the

Ra of the CLC subjects being higher than that of the CLP subjects (Figure 6.2), but over the duration of the trial their mean Ra_{end} was only 65% of that of the CLP subjects (35 ± 8 vs 53 ± 5 μmol/ min) or a total of 72 vs 111 g. Thus an estimated total of 46 g of glucose was "spared". The total Ra_{end} in the CLC subjects of 72 g over the 180 min of exercise gives an average Ra_{end} of 0.4 g/min. Thus the glucose spared (46 g) would be sufficient for an additional 115 min of exercise before the same total amount of glucose (111 g) had been produced as in the CLP subjects.

As might be expected from the Ra data (Figure 6.2), oxidation of exogenous carbohydrate showed a progressive increase until 90 min (Figure 6.5) with the rate of increase being less after that point. This reflects the increase and plateau that occurred in Ra_{exog} (Figure 6.3). Previous studies in which ^{13}C glucose was ingested during exercise (114, 201, 233, 267, 275, 293) also showed a progressive increase in the rate of oxidation of ingested carbohydrate. The oxidation rate of the ingested carbohydrate at 90 min was 63 ± 8 μmol/min/kg FFM (0.71 ± 0.08 g/min). This value is similar to that found in studies that have measured rates of oxidation of exogenous carbohydrate using ^{13}C techniques, where rates have varied from 0.4 to 0.9 g/min, depending on exercise intensity and amount of carbohydrate ingested (98, 114, 135, 179, 201, 231, 232, 267).

Total plasma glucose oxidation (Figure 6.4) also mirrored Ra (Figure 6.2) in the CLC subjects, progressively increasing until reaching a plateau between 90 and 110 min. In CLP subjects the pattern of increase was similar. Utilising the arterio-venous difference technique, others have also shown a progressive increase in the rate of blood glucose oxidation with increasing exercise duration (6, 343). This has also been shown indirectly in glucose clamp experiments (186) and is probably the result of a decrease in muscle glycogen levels as suggested by Gollnick et al. (129).

Plasma glucose oxidation reached 65 ± 8 % and 53 ± 11 % of total carbohydrate oxidation by the end of exercise in CLC and CLP subjects, respectively. These values are similar to those reported by Wahren (343) and agree with the conclusions of Coyle et al. (89) that after 2 - 3 hrs of exercise at 70 - 75% of $VO_{2\ max}$, blood glucose represents a major source of carbohydrate for oxidation.

As with Ra, the increase in plasma glucose oxidation was significantly greater in CLC subjects and occurred mainly as a result of an increase in oxidation of plasma glucose originating from the ingested carbohydrate (Figure 6.5). Interestingly, Ra_{end} (Figure 6.2) and endogenous plasma glucose oxidation (Figure 6.4) remained relatively constant and low in the CLC subjects, but not completely suppressed, throughout the trial. Had the rate of ingestion of carbohydrate been higher, a complete suppression of Ra_{end} may have occurred. In CLC subjects, it appears that the higher Ra as a result of carbohydrate ingestion resulted in more plasma glucose being available for oxidation, but since a plateau was reached in both Ra_{exog} (Figure 6.3) and exogenous plasma glucose oxidation (Figure 6.5), there appeared in this study to be an upper limit to these parameters amounting to an oxidation rate of ingested carbohydrate that varied

between 0.71 ± 0.08 g/min after ~90 min of exercise and 0.77 ± 0.03 g/min after 180 min of exercise. Since the carbohydrate concentration (10%) of the drink ingested amounted to an average rate of carbohydrate intake of 0.83 g/min, it appears that the rate of oxidation closely matched the rate of supply. This suggests that the ingestion of more carbohydrate may have resulted in an increase in Ra, with a subsequent increase in availability of plasma glucose for oxidation. If this were the case, it may explain the substrate kinetics of different studies in which higher rates of oxidation of exogenous carbohydrate are measured when the rates of carbohydrate ingestion are higher (98, 114, 135, 179, 201, 231, 248, 251, 253, 267, 308). In the CLP subjects, plasma glucose oxidation showed a progressive increase, but still did not reach the oxidation rates measured in the CLC subjects (Figure 6.4). The decline in muscle glycogen during exercise may be a cause of the increasing plasma glucose oxidation with time during the exercise period, since Gollnick et al. (129) have found that glucose uptake by exercising muscle increases in proportion to the number of glycogen depleted fibres and may compensate for the reduced glycogen availability by providing carbohydrate for oxidation by the muscle.

The Ra and oxidation data from the current study may explain the numerous studies which have shown an increase in exercise time to exhaustion when carbohydrate is ingested during exercise (34, 64, 69, 71, 89, 91, 126, 138, 170, 172, 235, 248, 252, 256, 350, 362). The higher Ra after carbohydrate ingestion provides more glucose for oxidation while, in addition, sparing hepatic glycogen and thus delaying the time at which these stores would otherwise become depleted. This latter benefit of carbohydrate ingestion is important because if Ra becomes inadequate, blood glucose available for oxidation will decrease and when this occurs, as shown by Coggan and Coyle (69) and Coyle et al. (91), total carbohydrate oxidation becomes insufficient for exercise to be continued at fairly high intensity (70 % of $VO_{2\ max}$). In the present study, the contribution of the exogenous carbohydrate to total carbohydrate oxidation peaked at 40 %.

A second consequence of inadequate Ra is that blood glucose concentration could become sufficiently low to cause hypoglycaemia (343), which would result in a reduction in exercise intensity or termination of exercise before muscle fatigue or muscle glycogen depletion occurs. Hypoglycaemia would be compounded by an increasing rate of plasma glucose oxidation at the same time that Ra is decreasing. Thus the interaction between Ra and rate of plasma glucose oxidation is important and is shown in Figure 6.6. This Figure shows that at times the rate of plasma glucose oxidation exceeded Ra in the CLP subjects but this was not significantly different from 100%. If Ra did not match the rate of plasma glucose oxidation, this would lead to the development of hypoglycaemia, as previously suggested (343).

The second important finding in this study was that all the subjects were able to complete the 180 min trial, in contrast to Study 1 in which 50% of the NLP subjects were unable to complete 180 mins of exercise. In that study mean muscle glycogen of those subjects who fatigued prematurely, reached very low concentrations (22 ± 4 mmol/kg ww) which have previously been reported to be consistent with fatigue (189). In the current study, carbohydrate ingestion had no effect on muscle glycogen utilisation

(Figure 6.7). A lack of muscle glycogen sparing during exercise in subjects ingesting carbohydrate has previously been shown (78, 89, 91, 98, 116, 140, 214, 256, 262, 316), while sparing has been shown in the studies of Bergstrom and Hultman (29), Hargreaves et al. (138), and others (34, 204, 214, 364). At the end of exercise mean glycogen concentration was still relatively high (55 ± 10 and 74 ± 16 mmol/kg ww in the CLC and CLP groups, respectively; Figure 6.7). Thus, even though CLP subjects ingested only water during exercise, all could complete the trial, further evidence that when carbohydrate-loaded, as all these subjects were, there may be an ergogenic effect as a result of the higher muscle and hepatic glycogen levels at the start of exercise. An increased time to exhaustion is compatible with the findings from other studies showing an ergogenic effect of carbohydrate-loading (28, 43, 129, 189, 273).

Total carbohydrate oxidation (Table 6.2) in the two groups was not significantly different and declined during the trial by 17 and 25 % in the CLP and CLC subjects, respectively. Other studies have demonstrated similar reductions in total carbohydrate oxidation and RER when carbohydrate was ingested during exercise at around 70% of $VO_{2\ max}$ (74, 91). This may be due to the progressive increase in plasma epinephrine concentrations shown by Kjaer (194) during prolonged exercise at 70% of $VO_{2\ max}$ The effect of increasing epinephrine concentrations would be an increase in lipolysis resulting in the increase in serum FFA concentration that occurred in both groups during the trial (0.51 ± 0.07 to 0.92 ± 0.14 and 0.46 ± 0.05 to 1.02 ± 0.01 mmol/l, in the CLC and CLP subjects, respectively). Increased serum FFA concentrations have been shown by Rennie and Holloszy (287) and Hargreaves et al. (141) to inhibit glucose uptake and glycogenolysis (200, 287, 288) in muscle.

Since total carbohydrate oxidation (Table 6.2) and muscle glycogen disappearance (Figure 6.7) did not differ between groups, and since plasma glucose oxidation was higher in CLC than CLP subjects after 45 min of exercise (Figure 6.4), to balance the energy equation oxidation of other carbohydrate substrates such as lactate must have been lower in the CLC subjects after that time. Thus in the absence of carbohydrate ingestion, there appears to have been a greater rate of lactate utilisation as a fuel substrate after 45 min of exercise. A breakdown of glycogen in non-working muscles to provide lactate for gluconeogenesis has previously been demonstrated (8).

Similar to previous results reported by Coyle et al. (91), carbohydrate ingestion did not produce hyperglycaemia (Figure 6.1) and consequently plasma insulin levels remained low in both groups throughout the trial. Although the values were low, the difference between groups was significant and since sensitivity to insulin is increased during exercise (21, 52), it is possible that the higher insulin levels in the CLC subjects contributed to increased plasma glucose oxidation by increasing glucose uptake by muscle (99, 106, 131, 228, 243, 292, 360).

In summary: i) carbohydrate ingestion during prolonged exercise had a marked hepatic glycogen sparing effect or caused a reduction in gluconeogenesis, or both, that should ultimately extend exercise time to

exhaustion by delaying the onset of hypoglycaemia, ii) carbohydrate ingestion increased plasma glucose oxidation, iii) the rates of ingestion and oxidation of the ingested carbohydrate were closely matched, and iv) carbohydrate ingestion did not have a muscle glycogen sparing effect.

CHAPTER 7

STUDY 3: INFLUENCE OF CARBOHYDRATE INGESTION ON FUEL SUBSTRATE TURNOVER AND OXIDATION IN NON CARBOHYDRATE-LOADED CYCLISTS

Introduction

Carbohydrate-loading before, and carbohydrate ingestion during prolonged exercise have both been found to enhance endurance by maintaining an adequate supply of carbohydrate for oxidation throughout exercise (28, 69, 71, 89, 91, 138, 172, 189, 350).

In Study 2 (Chapter 6) in which the effect of carbohydrate ingestion on fuel substrate kinetics in subjects who had carbohydrate-loaded before prolonged cycling was investigated, ingestion of carbohydrate reduced the rate of appearance of glucose from endogenous stores but did not reduce the rate of glycogenolysis in the exercising muscles. Conversely, in Study 1 (Chapter 5), it was found that muscle glycogen concentration at the start of exercise had no effect on Ra when water was ingested during prolonged cycling (43), but muscle glycogen utilisation was greater in carbohydrate-loaded than non - loaded subjects during the second and third hours of exercise. This suggests that exogenous and endogenous sources of carbohydrate are oxidised in preference to fat as a fuel substrate late in exercise if adequate carbohydrate is available, as previously suggested (89). However, the effect of carbohydrate ingestion on fuel substrate kinetics when the muscles are not glycogen-loaded may be different from the kinetics found after carbohydrate-loading.

Therefore, the aim of Study 3 was to determine the effect of carbohydrate ingestion during prolonged exercise on the kinetics of hepatic glucose turnover, exogenous and endogenous glucose oxidation and muscle glycogen utilisation in cyclists who had not previously carbohydrate-loaded.

Subjects

Seventeen cyclists took part in the study and were randomly assigned to either carbohydrate ingestion (NLC; n = 9) or placebo ingestion (NLP; n = 8) groups. Neither group carbohydrate-loaded before the experimental trials, all subjects following their normal diets.

After 3 days of only light training, the cyclists returned to the laboratory to start the trial which followed the procedures as described in Chapters 3 and 4.

Results

Subject characteristics are given in Table 7.1. $VO_{2\ max}$ and peak work rate values were consistent with the subjects being moderately trained, but not elite cyclists. Two NLC subjects and four NLP subjects were unable to complete the 180 min of exercise ($p<0.05$).

Table 7.1. Characteristics of subjects.

	NLP (n=8)	NLC (n=9)
Age (yr)	27 (3)	27 (3)
$VO_{2\ max}$ (l/min)	3.8 (0.2)	3.7 (0.2)
Peak work rate (W)	330 (20)	322 (22)
Mass (kg)	70.9 (4.5)	78.2 (4.0)
FFM (kg)	60.7 (3.7)	64.6 (2.5)
% Body fat	14 (1)	17 (2)
Completed 180 min of exercise *	4 of 8	7 of 9

Values are Means ± (SEM). NLC, carbohydrate ingestion; NLP, water ingestion; FFM, fat free mass. * significantly different ($p < 0.05$).

Oxygen consumption (Table 7.2) during exercise was relatively constant (subjects were maintained at close to 70% of $VO_{2\ max}$) and not significantly ($P < 0.05$) different between groups. Respiratory exchange ratio was also not significantly different between the groups, but fell significantly during the trial (Table 7.2). There were no significant differences between groups in total carbohydrate or fat oxidation during exercise (Table 7.2), but both changed significantly over time.

Table 7.2. Steady state gas exchange data and total carbohydrate oxidation during 180 min of cycling in subjects ingesting carbohydrate (NLC) or water placebo (NLP). (n=7)

		Time (mins)								
		5	22	44	66	88	110	132	150	18
VO_2	NLC	2.40	2.40	2.40	2.46	2.52	2.55	2.54	2.61	2.5
(l/min)		(0.22)	(0.22)	(0.20)	(0.17)	(0.18)	(0.17)	(0.16)	(0.17)	(0.1
	NLP	2.56	2.56	2.69	2.62	2.66	2.66	2.67	2.69	2.8
		(0.20)	(0.20)	(0.15)	(0.11)	(0.11)	(0.17)	(0.17)	(0.15)	(0.2
RER*	NLC	0.90	0.90	0.89	0.89	0.89	0.88	0.88	0.85	0.8
		(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.02)	(0.02)	(0.0
	NLP	0.93	0.93	0.91	0.91	0.93	0.91	0.88	0.86	0.8
		(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.01)	(0.0
CHO_{ox}	NLC	2.02	2.03	1.90	1.91	1.97	1.82	1.88	1.55	1.5
(g/min)*		(0.25)	(0.25)	(0.23)	(0.14)	(0.21)	(0.15)	(0.16)	(0.15)	(0.1
	NLP	2.56	2.56	2.49	2.39	2.66	2.40	2.05	1.83	1.8
		(0.38)	(0.38)	(0.35)	(0.30)	(0.27)	(0.31)	(0.22)	(0.14)	(0.2
Fat_{ox}	NLC	0.37	0.37	0.42	0.44	0.45	0.52	0.49	0.65	0.6
(g/min)*		(0.05)	(0.05)	(0.04)	(0.04)	(0.08)	(0.10)	(0.09)	(0.11)	(0.1
	NLP	0.28	0.28	0.37	0.38	0.30	0.40	0.53	0.62	0.6
		(0.08)	(0.08)	(0.08)	(0.10)	(0.09)	(0.09)	(0.09)	(0.09)	(0.1

Values are means ± (SEM). VO_2, oxygen consumption; RER, respiratory exchange ratio; CHO_{ox}, carbohydrate oxidat calculated from gas exchange data; Fat_{ox}, fat oxidation from gas exchange data. * Significant change over time ($p < 0.0$ There were no significant differences between groups.

Plasma glucose concentrations (Figure 7.1) remained fairly constant throughout exercise in the NLC subjects, starting at 4.3 ± 0.2 and ending at 4.4 ± 0.3 mmol/l. However, in NLP subjects glucose concentrations declined to 3.2 ± 0.2 mmol/l after 154 min, which was significantly less than in the NLC subjects at that time. The mean final value of the subjects who completed the trial (n = 4) was 3.2 ± 0.2 mmol/l.

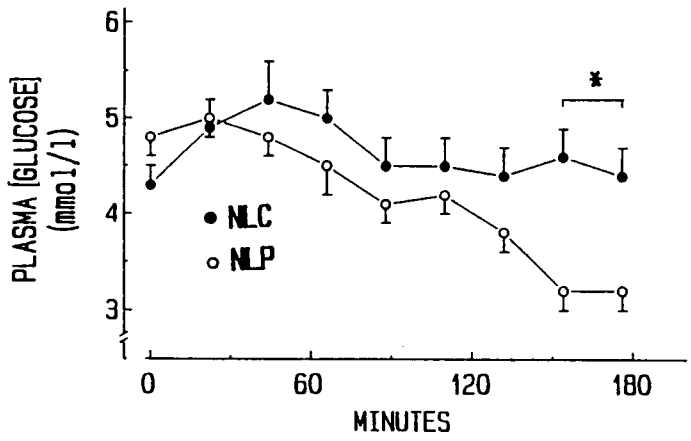

Figure 7.1. Plasma glucose concentrations were significantly lower at the end of the trial in NLP subjects than NLC subjects and were significantly lower than at the start of the trial in NLP subjects. The last data point for NLP subjects is n=4.

Ra was not significantly different between groups and remained relatively constant during the experiment (Figure 7.2). Ra_{end}, however, was significantly ($p < 0.05$) lower in the NLC than NLP group (Figure 7.2, dashed line).

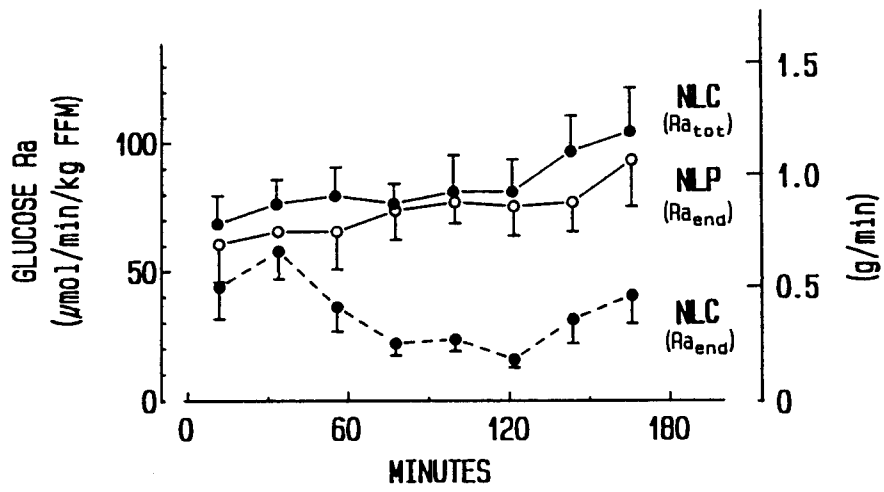

Figure 7.2. Rate of total glucose appearance (Ra) did not increase significantly in either group during the trial. The increase at the last data point in NLP subjects may be due to subjects dropping out of the trial at the previous time point. Rate of endogenous glucose appearance in subjects ingesting carbohydrate (NLC) (dashed line) was significantly less than in subjects ingesting placebo (NLP), although Ra in the NLC subjects (solid line) was not significantly different from NLP subjects.

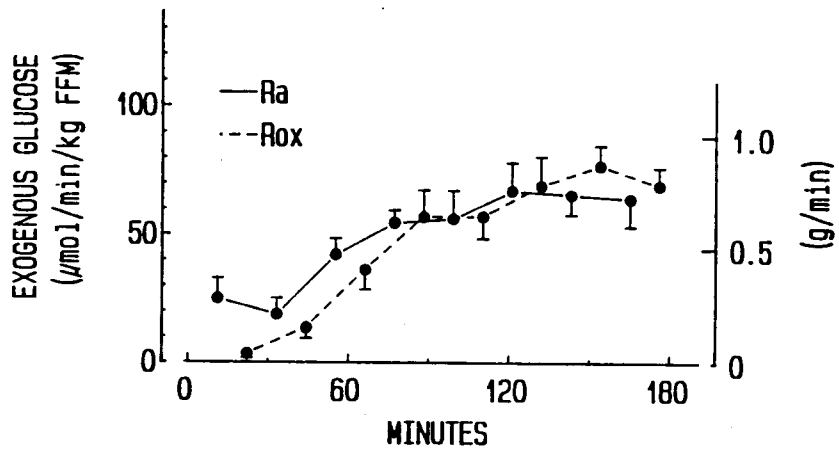

Figure 7.3 Rate of appearance (Ra) and oxidation (R_{ox}) of glucose derived from exogenous carbohydrate in NLC subjects.

Ra_{exog} in the NLC group increased significantly ($p < 0.05$) until a plateau was reached after ~120 min (Figure 7.3), at which time 80% of Ra was accounted for from exogenous carbohydrate.

Plasma glucose oxidation (Figure 7.4) rose progressively ($p < 0.05$) throughout exercise until 154 min (last data point of n=7) in the NLC subjects from 13 ± 3 to 98 ± 13 μmol/min/kg FFM (0.15 ± 0.04 to 1.12 ± 0.15 g/min). In NLP subjects oxidation increased fairly rapidly during the first 90 min of exercise from 11 ± 6 to 72 ± 5 μmol/min/kg FFM (0.12 ± 0.06 to 0.82 ± 0.03 g/min), but thereafter a more gradual increase occurred. The final value for n=7 data at the end of 154 min was 81 ± 7 μmol/min/kg FFM (0.89 ± 0.10 g/min). The values after 180 min in NLC (n=7) and NLP (n = 4) were 117 ± 16 and 90 ± 7 μmol/min/kg FFM (1.34 ± 0.19 vs 0.93 ± 0.13 g/min), respectively. Rates of plasma glucose oxidation

were not significantly higher in the NLC than NLP subjects (Figure 7.4, solid lines) at the last time point at which n=7 data were available for both groups (154 min). However, at the end of 180 min plasma glucose oxidation was higher than at 154 min in NLC subjects and was significantly higher than in NLP subjects at 180 min.

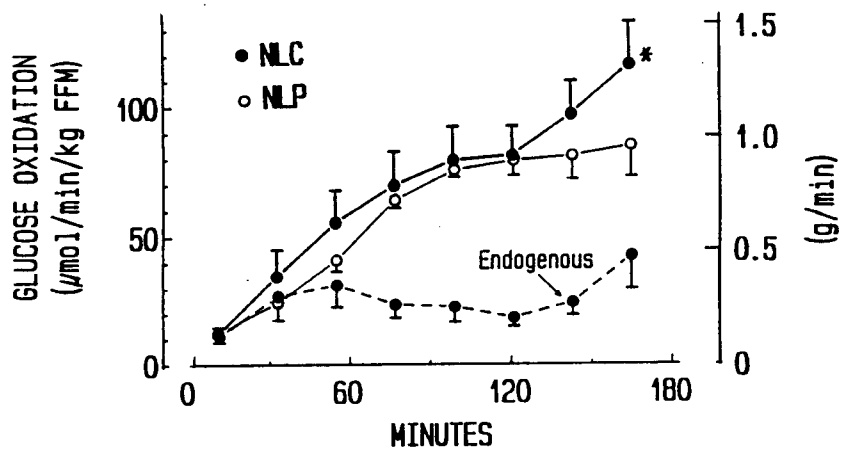

Figure 7.4. Plasma glucose oxidation increased significantly in both groups ($p < 0.05$) during the trial but was not significantly different between groups except at the end of the trial (*). Endogenous plasma glucose oxidation was significantly less in NLC subjects.

Oxidation of exogenous carbohydrate increased during exercise (Figure 7.3) and contributed significantly to the total plasma glucose oxidation (80% after 154 min). After 90 min, plasma glucose oxidation contributed more to total carbohydrate oxidation in NLC subjects than in NLP subjects ($p < 0.05$; Figure 7.5). At the end of exercise, plasma glucose oxidation constituted 69 ± 11 and 48 ± 6% of all carbohydrate oxidation in the NLC and NLP subjects, respectively, after 154 min and 93 ± 13 vs 52 ± 6% after 180 min (n=4 in NLP subjects).

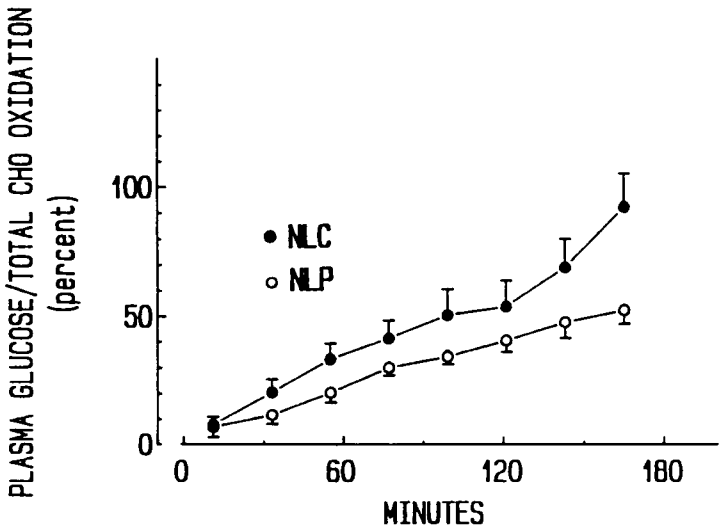

Figure 7.5. Percentage contribution of plasma glucose to total carbohydrate oxidation increased progressively throughout exercise and was significantly greater in NLC than NLP subjects throughout exercise.

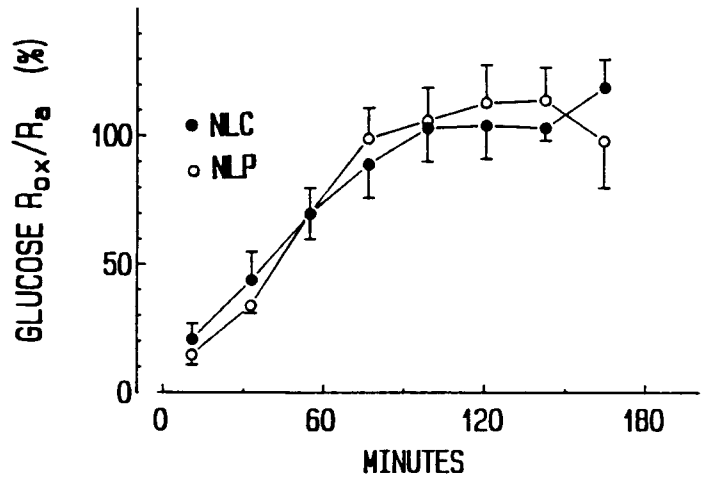

Figure 7.6. Plasma glucose oxidation expressed as a percentage of total plasma glucose appearance (Ra) i.e. the percentage of Ra that is oxidised. Ra initially exceeded oxidation, but thereafter Ra and oxidation rate were equal (100%).

Ra exceeded the rate of glucose oxidation in both groups during the first 90 min of exercise, thereafter Ra equalled the rate of plasma glucose oxidation (Figure 7.6).

Insulin concentrations at the end of exercise were 3.0 ± 0.5 and 0.8 ± 0.4 μU/ml in the NLC and NLP subjects, respectively ($P < 0.05$), and corresponding FFA concentrations were 1.09 ± 0.05 and 0.91 ± 0.08 mmol/l ($p < 0.05$).

Muscle glycogen concentration was similar at the start of exercise (Figure 7.7) and declined similarly in both groups during the first 2 hr of exercise. During the 3rd hr, glycogen utilisation continued to decline at a similar rate as in the previous hour in NLP subjects, but in NLC subjects no nett utilisation of glycogen occurred during the final hour (values were 56 ± 12 and 60 ± 10 mmol/kg ww after 2 and 3 hr, respectively). Less muscle glycogen was utilised in the NLC than NLP subjects (69 ± 9 vs 95 ± 12 mmol/kg ww).

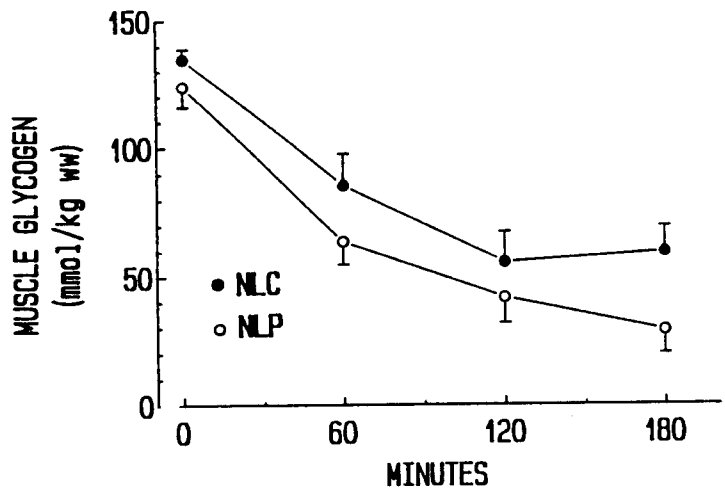

Figure 7.7. Rate of muscle glycogen disappearance was linear in NLC subjects during the first 120 min of exercise, but no nett glycogen utilisation occurred during the final hour of exercise. In NLP subjects, muscle glycogen declined more slowly after 60 min and continued to decline until the end of exercise.

Discussion

Less endogenous carbohydrate is oxidised when carbohydrate is ingested during exercise. This was initially suggested by indirect calculation (231) and confirmed by tracer techniques in Study 1 and Study 2 (43, 45)

which determined the relationship between carbohydrate ingestion, Ra_{exog} and Ra_{end} , and the rates of oxidation of plasma glucose originating from both exogenous and endogenous carbohydrate sources. Since the latter study was performed on subjects who had carbohydrate-loaded before the trial, it did not answer the question whether the effect of ingesting carbohydrate during exercise is different when not preceded by carbohydrate-loading, since the lower glycogen stores would potentially make exogenous carbohydrate a more important fuel substrate.

Although most of the differences in carbohydrate kinetics between the NLC and NLP subjects were similar to those previously found in the carbohydrate-loaded subjects in Study 2 (Chapter 6), there were some significant differences between the two groups in the current study which were not found in the previous study and which can therefore be attributed to the fact that the subjects in the present study were not carbohydrate-loaded.

As found previously in carbohydrate-loaded subjects in Study 2 (45), there was a hepatic glycogen sparing effect or a reduction in the rate of gluconeogenesis, or both, in NLC subjects, since Ra_{end} was significantly reduced in NLC compared to NLP subjects (Figure 7.2).

Ra in both NLC and NLP subjects (Figure 7.2) remained constant until 132 min of exercise and was sufficient to maintain euglycaemia and the increasing rate of plasma glucose oxidation. Subsequent to 132 min, Ra increased in NLC subjects, maintaining euglycaemia (Figure 7.1). However, Ra did not increase in NLP subjects (Figure 7.2) and this may explain the decline in plasma glucose concentrations in those subjects towards the end of exercise.

The contribution of exogenous carbohydrate to Ra increased during exercise, reaching a peak of 82% of Ra after approximately 120 min of exercise. Since exogenous carbohydrate contributed up to 82% of Ra in the NLC subjects, it provided most of the plasma glucose for oxidation, whereas the most likely sources of plasma glucose in the NLP subjects was hepatic glycogenolysis and, in the later stages of exercise when hepatic glycogen stores were likely to have been low, gluconeogenesis. Ra_{exog} (Figure 7.3) was similar to that previously found in carbohydrate-loaded subjects in Study 2 (45).

Despite the large contribution of exogenous carbohydrate to Ra, there was no significant difference in Ra between NLC and NLP subjects. However, mean Ra_{end} in NLC subjects was only 47% of that of the NLP subjects (34 ± 8 vs 72 ± 16 μmol/ min/kg FFM) or a total of 60 vs 122 g. Thus an estimated total of 62 g of glucose was "spared" by exogenous carbohydrate ingestion in these non-loaded subjects. The total Ra_{end} in the NLC subjects of 60 g during the 154 min when there were equal subject numbers in both groups, gives an average Ra_{end} of 0.4 g/min. Thus the carbohydrate spared (62 g) by carbohydrate ingestion in the NLC subjects would be sufficient for an additional 155 min of exercise before they had oxidised the same total

amount of glucose (122 g) as in the NLP subjects. This is similar to that previously reported for carbohydrate-loaded subjects in Study 2 (45).

As might be expected, Ra_{exog} and oxidation of exogenous carbohydrate (Figure 7.3) increased at a similar rate, reaching a peak oxidation rate of 0.9 g/min. Previous studies in which ^{13}C glucose was ingested during exercise also showed a progressive increase in the rate of oxidation of exogenous carbohydrate (109, 120, 201, 231, 232, 267, 293).

Total plasma glucose oxidation (Figure 7.4) increased significantly ($P<0.05$) in both groups. A progressive increase in the rate of plasma glucose oxidation with increasing exercise duration has previously been shown utilising the arterio-venous difference technique (6, 343) and indirectly in glucose clamp experiments (186). This probably results from the progressive decrease in muscle glycogen content as suggested by Gollnick et al. (129).

Plasma glucose oxidation reached 69 ± 11 % and 48 ± 6 % of total carbohydrate oxidation by the end of 154 min of exercise in NLC and NLP subjects, respectively, which was similar to that found in carbohydrate-loaded subjects (Study 2). However, in NLC subjects, the percentage contribution of plasma glucose to total carbohydrate oxidation reached 93% by the end of 180 min. Data from this time, however, may be unreliable as 2 subjects failed to complete the trial and thus the final data point reflects the values for the remaining 7 subjects only. Despite this, there appears to be a higher percentage contribution from plasma glucose to total carbohydrate oxidation at the end of exercise in subjects who are not carbohydrate-loaded, but who have an exogenous source of carbohydrate available for oxidation. The values for percentage plasma glucose contribution to total carbohydrate oxidation after 154 min of 69 ± 11 % and 48 ± 6 % are similar to the data of Wahren (343). Together with the data from carbohydrate-loaded subjects in Study 2 (45), these findings support the conclusions of Coyle et al. (89) that after 2 - 3 hrs of exercise at 70 - 75 % of $VO_{2\ max}$, plasma glucose represents a major source of carbohydrate for oxidation. This may be particularly true in subjects who have not carbohydrate-loaded but who ingest carbohydrate during exercise.

The increase in plasma glucose oxidation resulted from an increased oxidation of exogenous carbohydrate in NLC subjects (Figure 7.3). Interestingly, Ra_{end} (Figure 7.2) and endogenous plasma glucose oxidation (Figure 7.4) remained relatively constant. Since a plateau was reached in both Ra_{exog} (55 μmol/ min/kg FFM; Figure 7.3) and exogenous plasma glucose oxidation (58 μmol/ min/kg FFM; 0.8 g/min; Figure 7.3), this study appears to show an upper limit in these values amounting to a mean rate of exogenous carbohydrate oxidation of 0.83 ± 0.1 g/min from 110 min to the end of exercise. Since the carbohydrate concentration (10%) of the drink ingested amounted to an average rate of carbohydrate intake of 0.83 g/min, the rate of oxidation matched the rate of supply, which is similar to the findings in carbohydrate-loaded subjects (Study 2). The matching of these rates suggests that the ingestion of more

carbohydrate might have increased Ra with a subsequent increase in availability of exogenous glucose for oxidation.

In both groups, plasma glucose oxidation increased during exercise (Figure 7.4) until the rate of oxidation equalled Ra after ~ 90 min (Figure 7.6). Inadequate Ra, compounded by increasing blood glucose uptake by muscle (190), would cause hypoglycaemia (343). This would result in a reduction in exercise intensity or termination of exercise before muscle fatigue or muscle glycogen depletion occurs. Thus, the interaction between Ra and rate of plasma glucose oxidation is important and is shown in Figure 7.6. This Figure shows that early in exercise, the rate of Ra exceeded the rate of oxidation of plasma glucose. However, the progressive increase in the rate of plasma glucose oxidation (Figure 7.4) with a fairly constant Ra (Figure 7.2), resulted in all glucose produced being oxidised subsequent to 66 min of exercise (Figure 7.6).

The Ra and oxidation data from this study may explain why exercise time to exhaustion is increased when carbohydrate is ingested during exercise (28, 69, 71, 89, 91, 138, 172, 189, 350) since the utilisation of less endogenous carbohydrate would delay the time at which these stores would otherwise become depleted. This is important because an inadequate Ra would decrease the amount of plasma glucose available for oxidation as shown by Coggan and Coyle (69) and Coyle et al. (91). When this occurs, total carbohydrate oxidation is not adequate to sustain exercise of a moderately high intensity (70% of $VO_{2\ max}$). In the present study, the contribution of the exogenous carbohydrate to total carbohydrate oxidation peaked at 62%, compared to 40% in carbohydrate-loaded subjects in Study 2, probably as a consequence of the higher percentage contribution of plasma glucose to total carbohydrate oxidation in non-loaded subjects compared to carbohydrate-loaded subjects (69 ± 11 vs 47 ± 4%, respectively) after 132 min of exercise. In NLP subjects plasma glucose concentration (3.2 ± 0.2 mmol/l) was lower than in NLC subjects (4.5 ± 0.3 mmol/l) at that time (Figure 7.1) and lower than in carbohydrate-loaded subjects (4.1 ± 0.3 mmol/l). Carbohydrate ingestion during exercise therefore appears to be particularly important for subjects who are not carbohydrate-loaded at the start of exercise to maintain plasma glucose concentrations and provide a source of carbohydrate for oxidation.

Another important finding in this study was that in neither group were all the subjects able to complete the 180 min trial. In Study 2 in which subjects carbohydrate-loaded before exercise, all subjects were able to complete 180 min of exercise regardless of whether water or carbohydrate was ingested during exercise. In the current study, 50% of the NLP subjects, who ingested only water during the trial and did not carbohydrate- load beforehand, were unable to complete 180 mins of exercise. The NLC subjects fared somewhat better, with 7 of the 9 subjects (78%) able to complete 180 min. The mean muscle glycogen concentration of the NLP subjects who fatigued prematurely was 22 ± 4 mmol/kg ww, a value which has previously been reported to be consistent with fatigue (189). In the case of the NLC subjects, the 2 subjects who were unable to complete the trial due to exhaustion had the lowest muscle glycogen concentrations at the start of exercise (85 mmol/kg ww), compared to the group mean of 124 mmol/kg ww. At exhaustion,

their muscle glycogen concentration had declined to 6 mmol/kg ww. However, their plasma glucose concentrations were euglycaemic. This strongly suggests that muscle glycogen depletion was the cause of exhaustion.

In Study 2, in which subjects were carbohydrate-loaded, and as shown by others (89, 190, 214), carbohydrate ingestion had no effect on muscle glycogen utilisation. However, in the current study, significantly less muscle glycogen was utilised in NLC subjects than NLP subjects (69 ± 9 vs 95 ± 12 mmol/kg ww, NLC and NLP respectively), particularly during the last hour of exercise (Figure 7.7). During the last hour of exercise no significant nett glycogen utilisation occurred in NLC subjects, whereas glycogen utilisation was 14 ± 12 mmol/kg ww in NLP subjects.

Exogenous carbohydrate failed to produce a muscle glycogen sparing effect in carbohydrate-loaded subjects in Study 2 (45), and glycogen values as low as those in the NLC subjects in the present study were reached only at the end of 3 hours, which was the end of the trial. However, in Study 1, in which carbohydrate-loaded subjects ingested only water during exercise, the rate of muscle glycogen utilisation declined when glycogen concentration decreased to below ~70 mmol/kg ww. In the current study, when exogenous carbohydrate was available, no nett glycogen utilisation occurred after ~70 mmol/kg ww was reached (NLC), whereas in the NLP subjects glycogen utilisation continued, although at a decreased rate. Thus it appears that muscle glycogen sparing occurs when glycogen concentration reaches ~70 mmol/kg ww if exogenous carbohydrate is available. Hence no sparing effect could have been expected in carbohydrate-loaded subjects (Study 2) if this occurs only when muscle glycogen concentrations are extremely low. Sparing of muscle glycogen has previously been shown by Bergstrom and Hultman (29), Hargreaves et al. (138) and others (34, 204, 214, 364).

Total carbohydrate oxidation (Table 7.2) in the two groups was not significantly different and declined during the trial by 27 and 25% in the NLP and NLC subjects, respectively. Other studies have demonstrated similar reductions in total carbohydrate oxidation when carbohydrate was ingested during exercise at around 70% of $VO_{2\ max}$ (74, 91). The reduction of carbohydrate oxidation and concomitant increase in fat oxidation can be attributed to the declining insulin concentration and rising FFA concentration in both groups (360). The progressive increase in plasma epinephrine concentrations shown by Kjaer (194) during prolonged exercise at 70% of $VO_{2\ max}$ may also be a contributory factor, since increasing epinephrine concentrations would result in an increase in lipolysis, which in turn would result in an increase in serum FFA concentration. FFA concentrations increased during the trial in the 3 subjects from each group in which they were measured. Increased serum FFA concentrations have been shown to inhibit glucose uptake (141, 287) and glycogenolysis (41, 200, 270, 273, 287, 288, 321) in muscle. No effect on glycogenolysis has been reported (141).

Although insulin concentrations remained low in both groups, at the end of exercise insulin concentrations were significantly higher in NLC subjects (3.0 ± 0.5 vs 0.8 ± 0.4 μU/ml) and may have contributed to increased plasma glucose oxidation by increasing glucose uptake by muscle (99, 106, 131, 228, 243, 292, 360).

In summary, it can be concluded that: i) carbohydrate ingestion during prolonged exercise without prior carbohydrate-loading had a marked hepatic glycogen sparing effect or caused a reduction in gluconeogenesis, or both, a response identical to that which occurred in carbohydrate-loaded subjects, ii) unlike the finding in carbohydrate-loaded subjects, in non carbohydrate-loaded subjects, carbohydrate ingestion did not increase plasma glucose oxidation, but plasma glucose oxidation contributed a large percentage of total carbohydrate oxidation in both groups, as did exogenous carbohydrate oxidation in NLC subjects, iii) carbohydrate ingestion maintained plasma glucose concentration, iv) carbohydrate ingestion had a muscle glycogen sparing effect late in exercise when muscle glycogen concentrations reached ~ 70 mmol/kg ww, and v) carbohydrate ingestion did not prevent fatigue when muscle glycogen concentrations became very low.

CHAPTER 8

STUDY 4: EFFECTS OF CARBOHYDRATE-LOADING VS CARBOHYDRATE INGESTION ON FUEL SUBSTRATE KINETICS DURING PROLONGED EXERCISE

Introduction

Both carbohydrate ingestion during exercise (69, 71, 89, 138, 170, 172, 350) and carbohydrate-loading before exercise (28, 129, 189) can enhance endurance performance.

In Study 2, in carbohydrate-loaded subjects and Study 3, in non-loaded subjects, it was shown that when carbohydrate was ingested during exercise there was a significant hepatic glycogen sparing effect or a reduction in the rate of gluconeogenesis, or both. It has previously been shown that ingestion of carbohydrate does not have a muscle glycogen sparing effect (37, 78, 89, 91, 98, 116, 137, 139, 140, 256, 262, 316). Likewise, the ingestion of carbohydrate did not have a muscle glycogen sparing effect in the carbohydrate-loaded subjects in Study 2, but a sparing effect was shown in non-loaded subjects (Study 3). A sparing effect has previously been shown in some other studies (29, 34, 138, 204, 214, 364). In contrast, there was no reduction in hepatic glycogenolysis or gluconeogenesis, or both in Study 1 (43) which investigated the effect of carbohydrate-loading on Ra and associated carbohydrate kinetics in subjects who ingested only water during exercise. All subjects in Study 2, who were carbohydrate-loaded at the start of the trial, were able to complete 180 min of cycling, but 50% of non-loaded subjects in Study 1 (43) were unable to complete the 180 min trial. Non-loaded subjects who ingested carbohydrate during the trial (Study 3) did somewhat better, with 7 of the 9 subjects completing the trial.

The individual effects on fuel substrate kinetics of carbohydrate-loading when only water is ingested during exercise (Study 1), and the effect on kinetics of carbohydrate versus water ingestion in subjects who have carbohydrate-loaded (Study 2) or not carbohydrate-loaded (Study 3) before exercise have been determined. The options available to athletes to provide adequate carbohydrate during competition, however, are to either carbohydrate-load before the event and ingest water during the event (Studies 1 and 2), to carbohydrate-load before and ingest carbohydrate during the event (the most likely regimen to provide adequate carbohydrate substrate) (Study 2), or to dispense with carbohydrate-loading and merely ingest carbohydrate during the event. Thus the aim of this study was to determine what differences may exist in the fuel substrate kinetics of glucose turnover, exogenous and endogenous glucose oxidation, and muscle glycogen utilisation when carbohydrate-loading is done before exercise and water ingested during exercise, as compared to carbohydrate ingestion during prolonged exercise without prior carbohydrate-loading.

Subjects

Sixteen cyclists took part in the study and were randomly assigned to a group which would either carbohydrate-load before the trial and ingest water (placebo) during the trial (CLP; n = 7) or not carbohydrate-load and ingest carbohydrate (NLC; n = 9). Carbohydrate-loading was achieved as detailed in Chapter 4. NLC subjects also rested from training for 3 days, but continued to eat their normal diet. Methods were as described in Chapters 3 and 4.

Results

Subject characteristics are given in Table 8.1. All had modestly high $VO_{2\ max}$ and peak work rate values, consistent with the subjects being moderately trained cyclists. All subjects who had carbohydrate-loaded and ingested placebo (CLP) completed the prescribed 180 min of exercise, whereas 2 of the 9 subjects who did not carbohydrate-load but ingested carbohydrate during the trial were unable to complete 180 min.

Oxygen consumption (Table 8.2) during exercise was relatively constant as subjects were maintained at close to 70% of $VO_{2\ max}$ and was not significantly different between groups. Respiratory exchange ratio was also not significantly different between the groups, but fell significantly during the trial (Table 8.2). There were no significant differences between groups in total carbohydrate and fat oxidation during exercise (Table 8.2), both changing significantly over time.

Plasma glucose concentrations (Figure 8.1) were not significantly different between groups and remained between 4.3 ± 0.2 and 5.4 ± 0.5 mmol/l throughout exercise. However, Ra was significantly ($p < 0.05$) higher in NLC than CLP subjects (Figure 8.2) and increased significantly over time. Ra_{end} was significantly lower in the NLC than CLP group (Figure 8.2, dotted line) but did not change significantly over time.

However, Ra_{exog} in the NLC group increased significantly ($p < 0.05$) until a plateau was reached after ~120 min (Figure 8.3), at which time 80% of Ra (Figure 8.4) was accounted for from exogenous carbohydrate.

Total plasma glucose oxidation (Figure 8.5) also rose progressively ($p < 0.05$) from 13 ± 3 to 115 ± 16 μmol/min/kg FFM (0.15 ± 0.04 to 1.34 ± 0.19 g/min) in NLC subjects and from 19 ± 4 to 74 ± 11 μmol/min/kg FFM (0.22 ± 0.05 to 0.85 ± 0.12 g/min) in CLP subjects (Figure 8.5, solid lines). Rates of total plasma glucose oxidation were significantly higher in NLC subjects towards the end of exercise, however oxidation of plasma glucose derived from endogenous sources was significantly lower in NLC than CLP subjects (Figure 8.5, dotted line and open circles).

Table 8.1. Characteristics of subjects.

	CLP (n=7)	NLC (n=9)
Age (yr)	25 (3)	27 (3)
$VO_{2\ max}$ (l/min)	3.64 (0.19)	3.53 (0.26)
Peak work rate (W)	332 (23)	322 (22)
Mass (kg)	74.5 (3.5)	75.8 (4.0)
FFM (kg)	63.4 (3.1)	63.6 (3.0)
% Body fat	13 (1.0)	16 (2.0)
Completed 180 min of exercise	7 of 7	7 of 9

Values are Means ± (SEM). NLC, non carbohydrate-loaded with carbohydrate ingestion; CLP, carbohydrate-loaded with water ingestion during exercise; FFM, fat free mass.

Table 8.2. Steady state gas exchange data and total carbohydrate oxidation during 180 min of cycling in subjects who were not carbohydrate-loaded and ingesting carbohydrate (NLC) or carbohydrate-loaded and ingesting water placebo during exercise (CLP).

		Time(mins)								
		5	22	44	66	88	110	132	150	180
VO_2	NLC	2.40	2.40	2.40	2.46	2.52	2.55	2.54	2.61	2.57
(l/min)		(0.22)	(0.22)	(0.20)	(0.17)	(0.18)	(0.17)	(0.16)	(0.17)	(0.17)
	CLP	2.56	2.56	2.52	2.48	2.52	2.51	2.54	2.58	2.58
		(0.20)	(0.20)	(0.15)	(0.11)	(0.15)	(0.12)	(0.13)	(0.14)	(0.15)
RER*	NLC	0.90	0.90	0.89	0.89	0.89	0.88	0.88	0.85	0.85
		(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
	CLP	0.94	0.93	0.92	0.90	0.90	0.90	0.87	0.88	0.88
		(0.03)	(0.03)	(0.03)	(0.03)	(0.02)	(0.03)	(0.02)	(0.03)	(0.03)
CHO_{ox}*	NLC	2.02	2.03	1.90	1.91	1.97	1.82	1.88	1.55	1.50
(g/min)		(0.25)	(0.25)	(0.23)	(0.14)	(0.21)	(0.15)	(0.16)	(0.15)	(0.17)
	CLP	2.62	2.60	2.39	2.16	2.22	2.15	1.82	1.97	1.94
		(0.49)	(0.49)	(0.43)	(0.42)	(0.36)	(0.36)	(0.30)	(0.38)	(0.37)
Fat_{ox}*	NLC	0.37	0.37	0.42	0.44	0.45	0.52	0.49	0.65	0.65
(g/min)		(0.05)	(0.05)	(0.04)	(0.04)	(0.08)	(0.10)	(0.09)	(0.11)	(0.12)
	CLP	0.23	0.24	0.30	0.36	0.36	0.38	0.52	0.48	0.49
		(0.11)	(0.11)	(0.10)	(0.11)	(0.09)	(0.11)	(0.09)	(0.11)	(0.10)

Values are means ± (SEM). VO_2, oxygen consumption; RER, respiratory exchange ratio; CHO_{ox}, carbohydrate oxidation calculated from gas exchange data; Fat_{ox}, fat oxidation from gas exchange data. * Significant change over time ($p < 0.05$). There were no significant differences between groups.

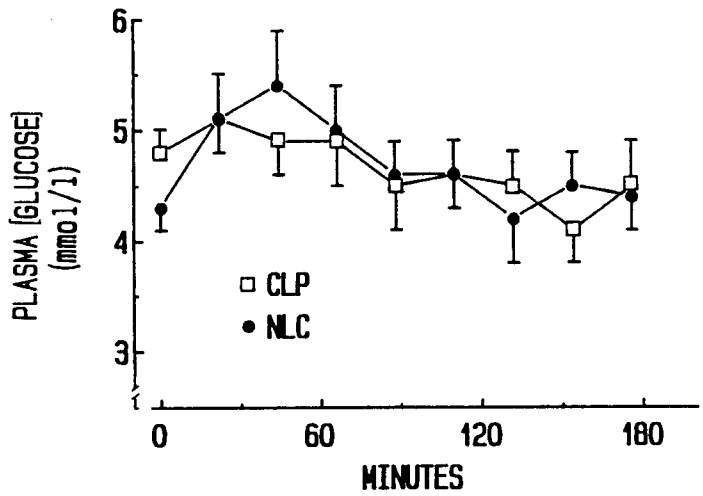

Figure 8.1. Plasma glucose concentrations were not significantly different between non carbohydrate-loaded subjects ingesting carbohydrate (NLC) and carbohydrate-loaded subjects ingesting water placebo (CLP). There was no significant change during the trial.

Thus, in NLC subjects there was a large contribution from exogenous carbohydrate (Figure 8.6) which reached 69 ± 7 μmol/min/kg FFM (0.80 ± 0.08 g/min) after 180 min. At the end of exercise 93 ± 13% of all carbohydrate oxidation was derived from plasma glucose in the NLC subjects and in the CLP subject this was 53 ± 11% ($p < 0.05$) (Figure 8.7).

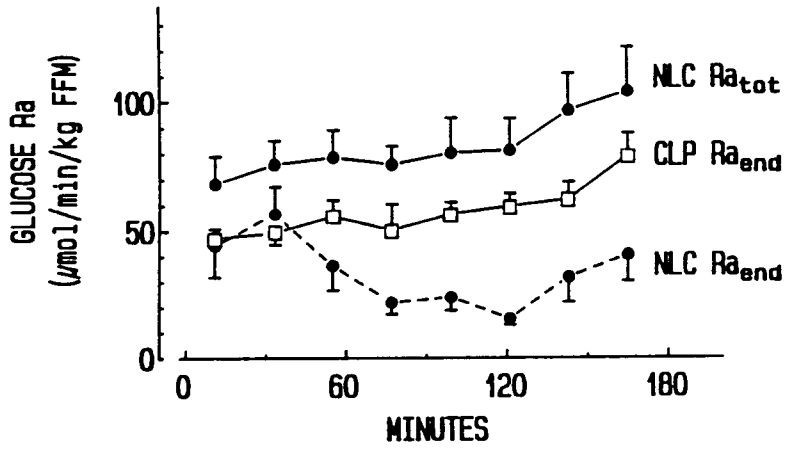

Figure 8.2. Rate of glucose appearance (Ra) increased significantly in both groups during the trial. Rate of endogenous glucose appearance (Ra_{end}) in non carbohydrate-loaded subjects ingesting carbohydrate (NLC) (dashed line) was significantly less than in carbohydrate-loaded subjects ingesting water placebo (CLP), although Ra from endogenous and exogenous sources together was significantly greater ($p < 0.05$) in the NLC subjects (solid line) than Ra in CLP subjects.

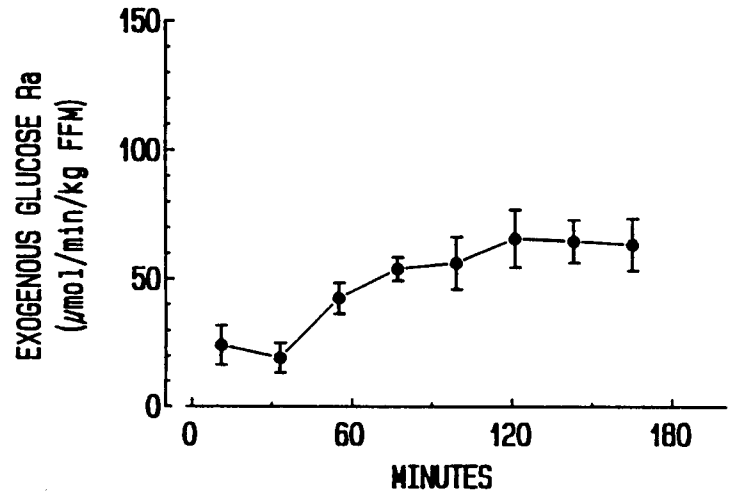

Figure 8.3. Rate of appearance of glucose derived from ingested (exogenous) (Ra_{exog}) carbohydrate in non carbohydrate-loaded subjects (NLC).

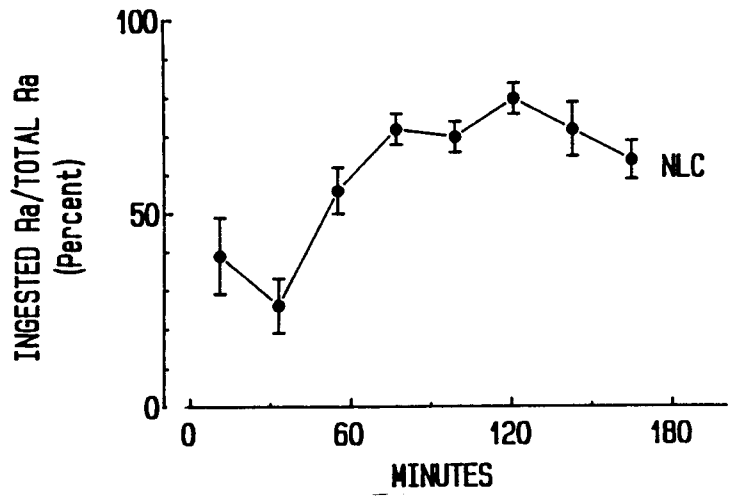

Figure 8.4. Percentage contribution of exogenous (ingested) carbohydrate to total plasma glucose appearance (Ra) in non carbohydrate-loaded subjects ingesting carbohydrate (NLC). A plateau was reached after 75 min.

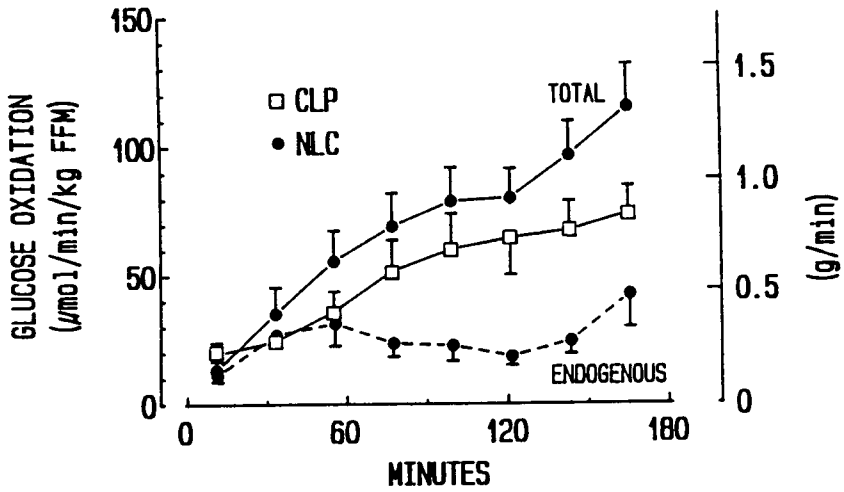

Figure 8.5. Plasma glucose oxidation increased significantly in both groups ($p < 0.05$) during the trial. Endogenous glucose oxidation in non carbohydrate-loaded subjects ingesting carbohydrate (NLC) (dotted line) was significantly ($p < 0.05$) less than in carbohydrate-loaded subjects ingesting water placebo (CLP), although total glucose oxidation in the NLC subjects (solid line) was significantly ($p < 0.05$) greater.

Although Ra initially exceeded the rate of glucose oxidation in both groups, oxidation of plasma glucose increased until it was not significantly different from Ra (Figure 8.8).

Insulin concentrations were 4.4 ± 0.4 and 2.7 ± 0.7 μU/ml at the start of exercise in NLC and CLP groups, respectively, and decreased to 3.0 ± 0.5 and 1.2 ± 0.5 μU/ml respectively at the end of exercise. The difference between groups at the end of exercise was significant ($P < 0.05$). The low initial values indicate that the breakfast ingested by the subjects was probably absorbed by the time exercise started.

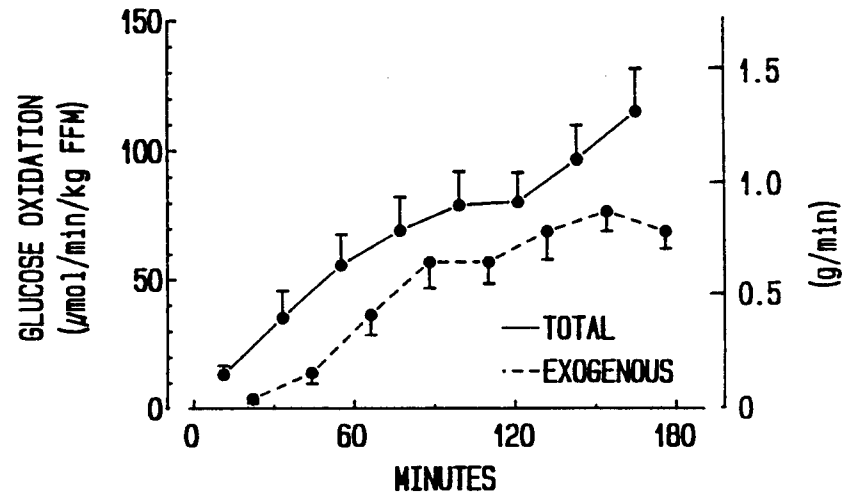

Figure 8.6. Contribution of exogenous (ingested) carbohydrate to plasma glucose oxidation. Exogenous carbohydrate contribution reached a plateau and contributed significantly to total plasma glucose oxidation, which continued to increase throughout exercise.

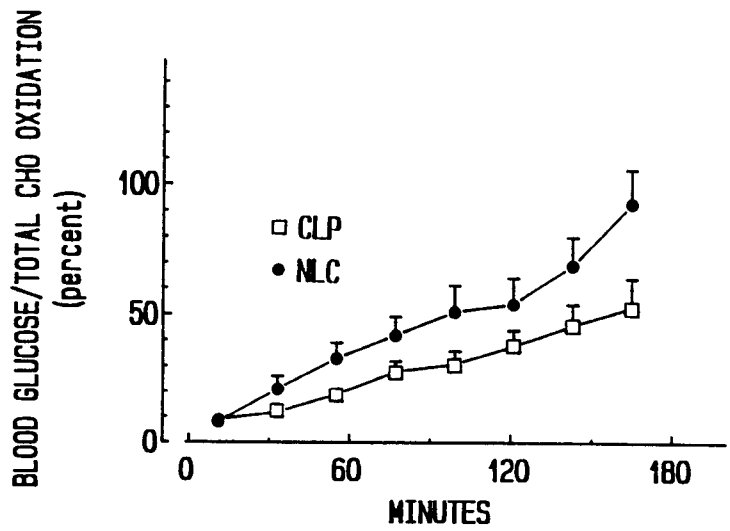

Figure 8.7. Percentage contribution of plasma glucose to total carbohydrate oxidation increased progressively throughout exercise and was significantly greater in non carbohydrate-loaded subjects ingesting carbohydrate (NLC) than in carbohydrate-loaded subjects ingesting water placebo (CLP) at the end of exercise.

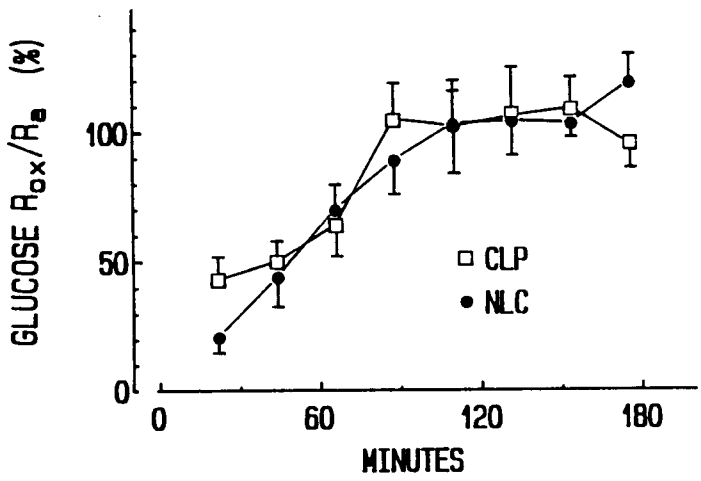

Figure 8.8. Plasma glucose oxidation expressed as a percentage of total plasma glucose appearance (Ra) i.e. the percentage of Ra that is oxidised. Ra exceeded the rate of oxidation for the first 75 min of exercise, but thereafter Ra and oxidation rate were equal (100%).

Muscle glycogen concentration declined linearly during the trial in CLP subjects, whereas in NLC subjects the rate of utilisation decreased after the first hour (Figure 8.9). Thus at the end of the trial mean values were identical (60 ± 10 and 60 ± 8 mmol/kg ww in NLC and CLP, respectively).

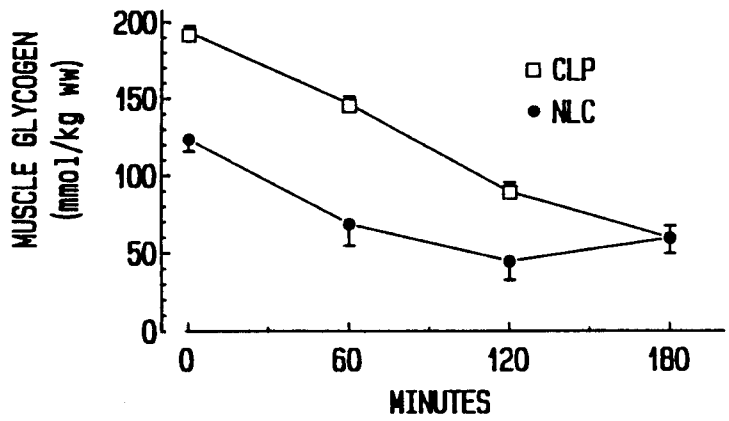

Figure 8.9. Rate of muscle glycogen disappearance was linear in carbohydrate-loaded subjects ingesting water placebo (CLP) throughout the trial. In non carbohydrate-loaded subjects ingesting carbohydrate (NLC), the rate was the same as in the CLP subjects over the first hour of exercise, but thereafter the rate of utilisation slowed.

Discussion

Although the separate effects of carbohydrate-loading and carbohydrate ingestion on fuel substrate kinetics have been determined in Studies 1-3, and although other studies have investigated carbohydrate metabolism during exercise (89, 114, 189), the relative effects of carbohydrate-loading compared to carbohydrate ingestion on fuel substrate kinetics and the possible importance for athletes participating in endurance events, has not been assessed.

Carbohydrate ingestion resulted in the Ra of the NLC subjects being higher than that of the CLP subjects, despite which Ra_{end} was reduced (Figure 8.2) throughout exercise. Over the duration of the trial, their mean Ra_{end} was only 60% of that of the CLP subjects (2.17 ± 0.53 vs 3.63 ± 0.39 mmol/ min), or a total of 70 vs 118 g. Thus 48 g of glucose was "spared". The total Ra_{end} in the NLC subjects of 70 g over the 180 min of exercise gives an average of 0.4 g/min. Thus the glucose spared (48 g) would be sufficient for an additional 120 min of exercise before the same total amount of glucose (118 g) had been produced as in the CLP subjects. These findings are virtually identical to those in Study 2, in which subjects in both groups had carbohydrate-loaded and one ingested carbohydrate and the second water, during exercise.

The higher Ra was similar to that reported by Wahren (343) and occurred as a result of a significant contribution of exogenous carbohydrate to Ra (Ra_{exog}) which increased during exercise (Figure 8.3), reaching a peak of around 80% of Ra after 120 min (Figure 8.4). The higher Ra in NLC subjects (Figure 8.2) can be attributed to carbohydrate ingestion rather than lower muscle glycogen content, since it was found in Study 1 that carbohydrate-loading does not influence Ra, whereas Study 2 showed that carbohydrate ingestion does. Ra, the percentage contribution of exogenous carbohydrate to Ra and Ra_{end} in NLC subjects were the same in the present study as in subjects in Study 2 who were both carbohydrate-loaded and ingested carbohydrate during exercise.

Oxidation of exogenous carbohydrate increased rapidly (mean rate of increase 0.63 μmol/min/kg FFM) until 90 min (Figure 8.6), with the rate of increase being less thereafter (0.31 μmol/min/kg FFM), thus reflecting the increase and plateau that occurred in Ra_{exog} (Figure 8.3). Previous studies in which ^{13}C-glucose was ingested during exercise (114, 201, 233, 267, 275, 293) also showed a progressive increase in the rate of oxidation of exogenous carbohydrate. The oxidation rate of exogenous carbohydrate at 90 min was 57 ± 10 μmol/min/kg FFM (0.66 ± 0.12 g/min). This value is similar to that found in Study 2, suggesting that oxidation of exogenous carbohydrate is independent of muscle glycogen status. It is also similar to that found in studies that have measured rates of oxidation of exogenous carbohydrate using ^{13}C techniques, where rates have varied from 0.4 to 0.9 g/min, depending on exercise intensity and the amount of carbohydrate ingested (98, 114, 135, 179, 201, 231, 232, 267).

As occurred with Ra (Figure 8.2), total plasma glucose oxidation (Figure 8.5) also increased progressively. Others, utilising the arterial - venous difference technique (343), have also shown a progressive increase in the rate of blood glucose oxidation with increasing exercise duration. This has also been shown indirectly in glucose clamp experiments (186) and is probably the result of decreasing muscle glycogen content as suggested by Gollnick et al. (129). The higher rate of plasma glucose oxidation in the NLC than CLP subjects towards the end of exercise is probably attributable to the higher insulin levels in the NLC subjects, in accordance with findings of Wolfe et al. (360).

The influence of muscle glycogen content on the rate of plasma glucose oxidation is further shown by the finding that the percentage of plasma glucose contribution to total carbohydrate oxidation (Figure 8.7) was significantly higher in NLC than in CLP subjects at the end of exercise (93 ± 13 % and 53 ± 11 % of total carbohydrate oxidation, respectively), whereas the percentage contribution in subjects who were carbohydrate-loaded and ingesting carbohydrate during exercise (Study 2) was 57 ± 6%, which was not significantly different from the CLP subjects. Thus carbohydrate-loading had a greater effect on the percentage contribution of plasma glucose to total carbohydrate oxidation than did carbohydrate ingestion. Further evidence for this is found when the rate of muscle glycogen utilisation is compared with the percentage contribution of plasma glucose to total carbohydrate oxidation. Figure 8.9 shows a linear decline in muscle glycogen concentrations, indicating a constant rate of utilisation in CLP subjects, and Figure 8.7

shows a corresponding linear increase in the percentage contribution of plasma glucose to total carbohydrate oxidation. However, in the case of the NLC subjects, muscle glycogen utilisation decreased to zero, since muscle glycogen concentration remained constant between 120 and 180 min after the glycogen concentration had reached approximately 55 mmol/kg ww (Figure 8.9). This was accompanied by a rapid increase ($p < 0.05$) in the percentage contribution of plasma glucose to total carbohydrate oxidation (Figure 8.7). These observations agree with the conclusions of Coyle et al. (89) that after 2 - 3 hrs of exercise at 70 - 75 % of $VO_{2\ max}$, at which time muscle glycogen content would be very low, blood glucose represents a major source of carbohydrate for oxidation. Likewise, Gollnick et al. (129) have found that glucose uptake by exercising muscle increases in proportion to the number of glycogen depleted fibres and somewhat compensates for the reduced glycogen availability.

The increase in plasma glucose oxidation in NLC subjects occurred mainly as a result of an increase in oxidation of plasma glucose originating from exogenous carbohydrate (Figure 8.6). Interestingly, Ra_{end} (Figure 8.2) and endogenous plasma glucose oxidation (Figure 8.5) remained low in the NLC subjects throughout the trial, but was not completely suppressed. Had the rate of ingestion of carbohydrate been higher, a complete suppression of Ra_{end} may have occurred. In NLC subjects it appears that the higher Ra resulting from carbohydrate ingestion resulted in an increased amount of plasma glucose available for oxidation. But since plateau's were reached in both Ra_{exog} (Figure 8.3) and exogenous plasma glucose oxidation (Figure 8.6), there appeared to be an upper limit for the rate of oxidation of exogenous carbohydrate of $\sim 0.80 \pm 0.08$ g/min. Since the carbohydrate concentration (10%) of the drink ingested amounted to an average rate of carbohydrate intake of 0.83 g/min, the rate of oxidation closely matched the rate of supply. This suggests that the ingestion of more carbohydrate may have resulted in an increase in Ra, with a subsequent increase in availability of plasma glucose for oxidation. If this were the case it may explain the substrate kinetics of different studies in which higher rates of oxidation of exogenous carbohydrate are measured when the rates of carbohydrate ingestion are higher (98, 114, 135, 179, 201, 231, 248, 251, 253, 267, 308). The kinetics of exogenous carbohydrate availability for oxidation is similar to the findings in subjects who were carbohydrate-loaded and ingested carbohydrate during exercise (Study 2), suggesting that these kinetics are independent of muscle glycogen status.

A consequence of inadequate Ra is hypoglycaemia (190, 343), which would result in a reduction in exercise intensity, or termination of exercise before muscle fatigue or muscle glycogen depletion occurs. Hypoglycaemia would be compounded by an increasing rate of plasma glucose oxidation at the same time that Ra is decreasing. Thus the interaction between Ra and rate of plasma glucose oxidation in these groups is important and is shown in Figure 8.8. Oxidation was approximately the same as Ra in both groups, but in NLC subjects a large percentage of the Ra was from exogenous carbohydrate (Figure 8.3), whereas in CLP subjects the entire Ra was from endogenous sources (Figure 8.2). It appears from mean values in Figure 8.8 that at times oxidation exceeded Ra, particularly at 154 min. It can be speculated, after examination of plasma glucose concentration (Figure 8.1), Ra (Figure 8.2) and plasma glucose oxidation (Figure 8.5)

shows that at 154 min, plasma glucose concentration declined significantly in CLP subjects to 4.1 ± 0.3 mmol/l. Oxidation of plasma glucose at this time exceeded Ra, causing the plasma glucose concentration to fall. This was followed during the period 154 - 180 min by a significant increase in Ra, which provided sufficient glucose to both maintain plasma glucose oxidation and simultaneously restore plasma glucose concentration to euglycaemic levels. The decline in plasma glucose to around 4 mmol/l may have activated hormonal and non-hormonal counter-regulatory mechanisms (33, 165, 185, 195, 224, 225, 257, 272, 290, 311, 314, 333, 337, 343, 360) causing a subsequent increase in Ra. A similar interaction was observed at around 90 min when mean rate of oxidation was 105% of Ra. Plasma glucose oxidation was increasing (Figure 8.5), with little increase in Ra (Figure 8.2), with a resultant decrease in plasma glucose concentration (Figure 8.1). However, the subsequent increase in Ra was less than at 154 min, probably because plasma glucose concentration, despite decreasing, remained euglycaemic at around 4.5 mmol/l and thus did not provoke a dramatic increase in Ra. Similar findings with respect to the relationship between Ra and glucose uptake were also reported by Wolfe et al. (360). There is close agreement between the measurements of Ra and rate of oxidation, despite the fact that independent tracer techniques were used in the determination of each.

The ability of the glucoregulatory mechanisms to maintain euglycaemia and sufficient plasma glucose for oxidation is dependent on the availability of sufficient endogenous substrate. In Study 1, 50% of subjects who had not carbohydrate-loaded and who ingested only water, failed to complete 180 min of exercise. The fact that CLP subjects in the present study not only completed the 180 min of exercise but maintained plasma glucose oxidation rates equal to those of the NLC subjects, indicates that carbohydrate-loading provides sufficient endogenous carbohydrate for exercise of this duration and intensity.

Two of the NLC subjects were unable to complete the 180 min trial. Although this was not statistically significant, in Study 1 (43), 50% of the subjects who ingested only water during the trial, and who were not carbohydrate-loaded at the start of exercise, were unable to complete 180 mins of exercise. In that study, mean muscle glycogen concentrations of those subjects who fatigued prematurely reached very low levels (22 ± 4 mmol/kg ww). These low values have previously been reported to be consistent with fatigue (189). The two NLC subjects who did not complete the trial in the present study also had very low (< 22 mmol/kg ww) muscle glycogen levels when they stopped exercise. Even though CLP subjects ingested only water during exercise, all could complete the trial, further evidence that when carbohydrate-loaded there may be an ergogenic effect as a result of the higher muscle and hepatic glycogen levels at the start of exercise. An increased time to exhaustion is compatible with the findings from other studies showing an ergogenic effect of carbohydrate-loading (28, 129, 189).

The rate of muscle glycogen disappearance decreased after 60 min in the NLC subjects compared with CLP subjects. This is most likely an effect of the lower muscle glycogen levels at the start of exercise in NLC subjects, and not a result of carbohydrate ingestion, since others (89), and the results of Study 2, have

shown that when subjects are carbohydrate-loaded the rate of muscle glycogen disappearance is the same irrespective of carbohydrate or water ingestion. The rate of muscle glycogen disappearance was less, however, in subjects who had not carbohydrate-loaded and who ingested carbohydrate during exercise, than it was in non-loaded subjects who ingested water (Study 3).

Total carbohydrate oxidation (Table 8.2) in the two groups was not significantly different and declined during the trial by 26 and 25% in the CLP and NLC subjects, respectively. Other studies have demonstrated similar reductions in total carbohydrate oxidation and RER despite carbohydrate ingestion, during exercise at around 70% of $VO_{2\ max}$ (45, 91).

Since total carbohydrate and plasma glucose oxidation did not differ between groups, and since muscle glycogen utilisation was higher in CLP subjects, oxidation of other substrates such as lactate (from non-working muscle glycogen) must have been higher in the NLC subjects, if the energy equation is to balance.

In summary: i) carbohydrate ingestion had a hepatic glycogen sparing effect or caused a reduction in gluconeogenesis or both, that should delay the onset of hypoglycaemia, whereas carbohydrate-loading before exercise reduced the relative contribution of plasma glucose oxidation to total carbohydrate oxidation and may prolong time to exhaustion due to higher muscle glycogen concentrations. Since these effects of carbohydrate ingestion and carbohydrate-loading on fuel substrate kinetics occur independently, both procedures may be necessary for the maintenance of optimal fuel substrate kinetics in endurance events of several hours. The benefits of these interventions on cycling performance have been demonstrated in a recent study by Widrick et al. (349); ii) a progressive increase in plasma glucose oxidation occurred that was related to muscle glycogen status and occurred irrespective of whether carbohydrate was ingested or not, and iii) carbohydrate ingestion did not prolong exercise time to exhaustion at a constant workload if glycogen levels became very low.

CHAPTER 9

SUMMARY AND CONCLUSIONS

The aim of this thesis was to establish some of the mechanism(s) underlying the ergogenic effects of carbohydrate-loading and carbohydrate ingestion.

Higher muscle glycogen stores after carbohydrate-loading may delay the onset of fatigue resulting from either hepatic or muscle glycogen depletion during exercise, while carbohydrate ingestion may spare hepatic or muscle glycogen.

The options available to athletes to provide adequate carbohydrate during competition are to either carbohydrate-load before and ingest carbohydrate during the event (the most likely regimen to provide adequate carbohydrate substrate), carbohydrate-load before the event and ingest water during the event, or only ingest carbohydrate during the event.

Thus the first study assessed the effects of carbohydrate-loading and showed that total carbohydrate oxidation during exercise was similar in the CLP and NLP subjects, as were plasma glucose oxidation and Ra, but plasma glucose concentrations were lower towards the end of exercise in NLP subjects. However, total muscle glycogen utilisation was greater in CLP than NLP subjects, and CLP subjects had higher muscle glycogen concentrations at the start and throughout the trial. Whereas high rates of muscle glycogen breakdown were maintained throughout the trial in CLP subjects, rates of muscle glycogenolysis decreased after 60 min of exercise in NLP subjects, when their muscle glycogen concentrations had declined to approximately 70 mmol/kg ww. Comparable rates of plasma glucose and overall carbohydrate oxidation in CLP and NLP subjects, despite a slowing of muscle glycogenolysis in the NLP group, could be explained by an accelerated breakdown of glycogen in the non-working muscles to redistribute carbohydrate to the working muscles for oxidation. Low muscle glycogen concentrations appeared to be consistent with fatigue in the NLP subjects, since a significantly greater number of NLP than CLP subjects became exhausted before completion of the trial. However, although plasma glucose concentrations were low at the end of exercise in all the NLP subjects, those who failed to complete the trial had plasma glucose concentrations that were the same as NLP subjects who completed the trial. Since rates of plasma glucose oxidation and Ra in both groups were not significantly different, these data suggest that high initial muscle glycogen levels increase endurance by postponing muscle glycogen depletion rather than by sparing hepatic glycogen.

In both carbohydrate-loaded and non-loaded cyclists, carbohydrate ingestion reduced Ra_{end}. This may have been due to a reduction in hepatic glycogenolysis or gluconeogenesis, or both. Another effect of carbohydrate ingestion was to increase Ra, which was accompanied by an increase in plasma glucose oxidation. The rate of oxidation of the ingested carbohydrate closely matched the rate of ingestion. The

contribution of blood glucose to total carbohydrate oxidation was highest in non-loaded subjects who ingested carbohydrate, and a sparing of muscle glycogen occurred. However, carbohydrate ingestion did not have a muscle glycogen sparing effect in carbohydrate-loaded subjects. If glycogen levels become very low, carbohydrate ingestion may not prolong exercise time to exhaustion, as 22% of the NLC subjects failed to complete the trial.

Comparison of the effects of carbohydrate ingestion in the absence of carbohydrate-loading, and carbohydrate-loading without carbohydrate ingestion, showed that carbohydrate ingestion, as before, reduced Ra_{end}. The rate of muscle glycogenolysis was lower in non-loaded subjects who ingested carbohydrate, but from the results of the first 3 studies, this cannot be ascribed to carbohydrate ingestion alone, as the glycogen sparing effect of carbohydrate ingestion was only evident when muscle glycogen content was not high at the start of exercise.

In conclusion, the results of these studies suggest that: i) The kinetics of carbohydrate-loading and carbohydrate ingestion operate independently, ii) glycogen utilisation is higher in carbohydrate-loaded subjects because of a continued high rate of utilisation of muscle glycogen after the first hour of exercise. The rate of utilisation appears to decrease once glycogen concentrations reach approximately 70 mmol/kg ww, iii) carbohydrate-loading prolongs exercise time to exhaustion, primarily by delaying muscle glycogen depletion, which coincided with exhaustion, iv) in these studies, exhaustion did not appear to be related to hypoglycaemia and carbohydrate ingestion may not prolong exercise time to exhaustion if glycogen concentration becomes very low, v) carbohydrate ingestion reduces hepatic glycogenolysis or gluconeogenesis, or both regardless of muscle glycogen concentration at the start of exercise, vi) carbohydrate ingestion does not spare muscle glycogen when glycogen concentrations at the start of exercise are high, but muscle glycogen sparing occurs in non-loaded subjects when carbohydrate is ingested, vii) plasma glucose concentrations can be maintained if carbohydrate is ingested or if muscle glycogen concentrations are high at the start of exercise, viii) carbohydrate ingestion results in an increased rate of blood glucose oxidation and this is not influenced by carbohydrate-loading, ix) the percentage contribution of blood glucose to total carbohydrate oxidation is highest in non-loaded subjects who ingested carbohydrate, x) the rate of oxidation of ingested carbohydrate closely matches the rate of ingestion and xi) cyclists participating in competition may benefit most from both carbohydrate-loading before the event and ingesting a carbohydrate-containing drink during the event. If only one of these procedures is possible, then carbohydrate loading is preferable.

CHAPTER 10

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