LIMITS TO EXOGENOUS GLUCOSE OXIDATION BY SKELETAL MUSCLE DURING PROLONGED, MODERATE-INTENSITY EXERCISE IN MAN

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

by

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" ..... if we do discover a complete theory, it should in time be understandable in broad principles by everyone, not just a few scientists. Then we shall all, philosophers, scientists, and just ordinary people, be able to take part in the discussion of the question of why it is that we and the universe exist. If we find the answer to that, it would be the ultimate triumph of human reason - for then we would know the mind of God."

(Stephen Hawking, A Brief History of Time, 1988)

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To my family and Liz, who have waited so patiently for me to return home. I can never give back the many years I could not share with you. I only hope that, one day, you will understand.

And to Helen. May your dreams come true.

DECLARATION

I, ***John Alan Hawley***, 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 have 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 for a degree in the University or any other university.

This thesis is presented in fulfilment of the requirements for the degree of PhD.

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ABSTRACT

Several factors may determine the rate at which exogenous carbohydrate (CHO) is utilised by the human working muscles during prolonged (> 90 min moderate-intensity (63% of peak sustained power output [PPO]) exercise. These include i) the rate of gastric emptying of an ingested fluid, ii) the rate of digestion, absorption and subsequent transport of glucose into the systemic circulation, and iii) the rate of glucose uptake and oxidation by the working muscles.

To test the hypothesis that the rate of gastric emptying is the primary factor limiting the rate of CHO delivery to the working muscles during exercise, uniformly labelled ^{14}carbon (U-^{14}C) tracer techniques were used in association with conventional gas exchange measurements and post-exercise gastric aspiration to compare the rates of gastric emptying, intestinal CHO delivery and ingested CHO oxidation from 15 g/100 ml solutions of glucose, maltose, a 22 chain-length glucose polymer, and an isocaloric `soluble' starch preparation. Two groups of six highly-trained male cyclists or triathletes each ingested two of the test drinks which were given as a 400 ml loading bolus immediately before and then as eight 100 ml feedings at 10 min intervals during 90 min of continuous cycling at a work rate of 63% of PPO (~70% of maximal oxygen consumption [VO_{2max}]).

The means and standard errors of the means (SEM) of the rates of gastric emptying (glucose 655 ±38 ml/90 min versus maltose 690 ±37 ml/90 min and 22 chain-length glucose polymer 813 ±53 ml/90 min versus `soluble' starch 919 ±47 ml/90 min) and intestinal CHO delivery (glucose 98.3 ±5.7 g/90 min versus maltose 104 ±7.4 g/90 min and glucose polymer 122 ±7.8 g/90 min versus `soluble' starch 138 ±7.1 g/90 min) were not significantly different for the two simple CHO and the two more complex CHO. However, the more complex CHO beverages did empty faster than the simple glucose and maltose drinks ($P < 0.05$).

Despite differences in the rates of gastric emptying, the peak rates of ingested CHO oxidation from glucose (0.9 ±0.04 g/min), maltose (1.0 ±0.04 g/min) and glucose polymer (0.9 ±0.08 g/min) solutions, as well as the total amount of ingested CHO oxidised from these beverages (glucose 49 ±3.7 g; maltose 51 ±3.3 g; glucose polymer 50 ±4.1 g) were all very similar. In absolute terms the ~50 g of ingested CHO oxidised after consumption of these drinks accounted for 20% of total CHO oxidation during the 90 min rides. Unfortunately, retrospective analyses revealed that the calculated rates of ingested CHO oxidation from the `soluble' starch preparation were spurious due to differences in the physical structure and composition of the labelled and unlabelled starches. Hence, rates of oxidation of starch could not be determined with absolute precision.

Since only ~50% of the CHO delivered to the intestine from 15 g/100 ml glucose, maltose and glucose polymer solutions was ultimately oxidised, it is concluded that the rate of gastric emptying does not limit the peak rate of ingested CHO utilisation after 60-75 min of moderate-intensity exercise. Furthermore, the finding that all CHO's were oxidised at the same rate shows that the rate of digestion of at least these relatively simple CHO's, does not appear to limit their rate of oxidation. In practical terms this means that provided the CHO is ingested sufficiently frequently (i.e. every 10-15 min) in appropriate volumes (i.e. 100-150 ml), it is unlikely that there will be any physiologically important differences in the rates of ingested CHO oxidation from 15 g/100 ml solutions of glucose, maltose or glucose polymer drunk repeatedly during exercise. All these CHO's are ultimately oxidised at a peak rate of ~1 g/min after 90 min of moderate-intensity cycling.

In order to determine if by-passing both intestinal absorption and hepatic glucose uptake via an intravenous glucose infusion might increase the rate of muscle glucose oxidation above 1 g/min, an additional ten male endurance-trained subjects were studied during 125 min of cycling at 63% of PPO. During exercise, subjects ingested either a 15 g/100 ml U-^{14}C labelled glucose solution or, in the case of subjects who were infused, water labelled with traces of U-^{14}C glucose. U-^{14}C glucose was administered to determine the rates of ingested CHO oxidation and plasma glucose oxidation (Rox) from plasma ^{14}C glucose

and expired $^{14}CO_2$ specific activities and respiratory gas exchange. Simultaneously, tritiated (2-^{3}H) glucose was infused at a constant rate to estimate glucose turnover (Ra), while in subjects not ingesting glucose, unlabelled glucose (25% dextrose) was infused at increasing rates to maintain plasma glucose concentration at 5 mmol/L.

Despite similar plasma glucose concentrations with glucose ingestion and infusion (5.3 ±0.13 mmol/L versus 5.0 ±0.09 mmol/L), CHO ingestion significantly increased plasma insulin concentrations (12.9 ±1.0 versus 4.8 ±0.5 mU/ml; $P < 0.05$), raised total Rox values (9.5 ±1.2 versus 6.2 ±0.7 mmol/125 min/kg fat free mass [FFM]; $P < 0.05$) and rates of CHO oxidation (37.2 ±2.8 versus 24.1 ±3.9 mmol/125 min/kg FFM; $P < 0.05$). The increased reliance on CHO metabolism with CHO ingestion was associated with a suppression of fat oxidation. Whereas the contribution from fat oxidation to energy production rose to 51 ±10% with glucose infusion, it reached only 18 ±4% with glucose ingestion ($P < 0.05$).

Since the rates of plasma glucose oxidation eventually became similar after 125 min of exercise for both trials (ingestion 93 ±8 umol/min/kg FFM; infusion 85 ±5 umol/min/kg FFM), despite marked differences in plasma insulin concentration and rates of fat oxidation, it is concluded that when sufficient glucose is ingested or infused, its appearance in the systemic blood supply does not limit the rate of exogenous glucose oxidation by the working muscles after the first 90 min of moderate-intensity exercise, at least when plasma glucose concentrations are 5 mmol/L and plasma insulin concentration is not increased above 20 mU/L. Were this not the case, a by-passing of both intestinal absorption and hepatic glucose uptake with an intravenous glucose infusion would have increased the rates of glucose oxidation by muscle above those found with glucose ingestion.

The finding that intravenous glucose infusion, which could provide a more or less unlimited rate of glucose delivery, did not elicit higher rates of muscle glucose oxidation than CHO ingestion suggests that peak rates of glucose oxidation by muscle are primarily

dependent on the prevailing plasma glucose concentration which, in turn, regulates the hepatic appearance of glucose. This hypothesis would explain why the ingestion of a variety of mono-, di-, and oligosaccharides, all of which elicit a similar (i.e. ~5 mmol/L) plasma glucose concentration are limited to ~1 g/min.

Therefore, to establish whether a more rapid rate of glucose delivery into the systemic circulation might increase the rate of plasma glucose oxidation by the working muscles, the effects of euglycaemia (i.e. 5 mmol/L; ET) and hyperglycaemia (i.e. 10 mmol/L; HT) on fuel substrate kinetics were evaluated in another 12 trained subjects (six/group) during 125 min of cycling at 63% of PPO. During exercise, subjects ingested water labelled with traces of U-^{14}C glucose for the determination of Rox, while 2-^{3}H glucose was infused at a constant rate to estimate endogenous Ra, and unlabelled glucose (25% dextrose) was infused at variable rates to maintain plasma glucose concentration at either 5 or 10 mmol/L.

In the ET, endogenous Ra progressively declined from 22.4 ±4.9 to 6.5 ±1.4 umol/min/kg FFM ($P < 0.05$) while in the HT, Ra values were not significantly different from zero, suggesting that endogenous Ra was, most likely, largely suppressed. In contrast Rox increased to 151.8 ±20.8 at the end of the HT and 92.3 ±8.6 umol/min/kg FFM at the end of the ET ($P < 0.05$). These values correspond to rates of glucose oxidation of 1.81 ±0.28 and 1.16 ±0.13 g/min, respectively. Hyperglycaemia (i.e. 10 mmol/L) and the resulting hyperinsulinaemia (24.5 ±0.9 uU/ml) also increased total CHO oxidation from 203.4 ±6.9 umol/min/kg FFM (ET) to 310.1 ±3.1 umol/min/kg FFM ($P < 0.0001$) and suppressed fat oxidation from 51.3 ±2.5 umol/min/kg to 17.5 ±1.7 umol/min/kg FFM ($P < 0.0001$).

These results indicate that by doubling plasma glucose concentration from 5 mmol/L (i.e. euglycaemia) to 10 mmol/L (i.e. hyperglycaemia) did the rate of glucose oxidation by working muscle increase above 1 g/min at the end of 125 min of exercise. Hence, when plasma glucose concentrations are maintained at 5 mmol/L (by either CHO ingestion or an

intravenous infusion of glucose), plasma insulin concentrations are not greatly increased and the rate of muscle glucose oxidation appears to be limited to ~1 g/min.

In conclusion, the results of this thesis suggest that the rate at which ingested CHO is utilised by the working muscles during prolonged, moderate-intensity exercise, is neither limited by the rate of gastric emptying of an ingested solution, nor by the rate of digestion and subsequent transport of glucose into the systemic circulation, at least for solutions of simple CHO's (glucose, maltose and glucose polymers) ingested repetitively throughout exercise. Rather it would appear that the oxidation of glucose by muscle is regulated by the prevailing plasma glucose concentration during exercise, which, in turn, regulates the hepatic appearance of ingested (or infused) glucose. Only when hyperglycaemic (i.e. 10 mmol/L) are trained subjects capable of oxidising exogenous glucose at rates of > 1 g/min. The practical implication of this postulate is that it is the physiological concentration of glucose normally present during prolonged moderate-intensity exercise which may ultimately limit the rate of glucose oxidation by working skeletal muscle.

PUBLICATIONS

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

i) Noakes TD, Hawley JA, Dennis SC. Fluid and Energy Replacement During Prolonged Exercise. In: Shephard RJ, Torg JS (Editors), *Current Therapy in Sports Medicine*, Mosby-Year Book Inc., (in press), 1994

ii) Hawley JA, Dennis SC, Noakes TD. Carbohydrate, Fluid and Electrolyte Replacement During Prolonged Exercise. In: Kies C (Editor), *Sports Nutrition: Minerals and Electrolytes*, American Chemical Society, CRC Press Inc., Florida, (in press), 1994

iii) Hawley JA, Bosch AN, Weltan SM, Dennis SC, Noakes TD. Effects of glucose ingestion and glucose infusion on fuel substrate kinetics during prolonged exercise. *European Journal of Applied Physiology and Occupational Physiology*, (in press), 1994

iv) Hawley JA, Bosch AN, Weltan SM, Dennis SC, Noakes TD. Glucose kinetics during prolonged exercise when hyperglycaemic. *European Journal of Physiology, Pflügers Archives*, (in press), 1994

v) Hawley JA, Dennis SC, Noakes TD. Carbohydrate metabolism during prolonged exercise: An historical perspective. *New Zealand Journal of Sports Medicine*, (in press), 1993

vi) Hawley JA, Dennis SC, Bosch AN, Noakes TD. Exogenous starch oxidation using ^{14}C labeling. Letter to the Editor (Reply). *Journal of Applied Physiology* 73: 2719-2722, 1992

vii) Hawley JA, Dennis SC, Noakes TD. Oxidation of exogenous carbohydrate ingested during prolonged endurance exercise. *Sports Medicine* 14: 27-42, 1992

viii) Hawley JA, Dennis SC, Nowitz A, Brouns F, Noakes TD. Exogenous carbohydrate oxidation from maltose and glucose ingested during prolonged exercise. *European Journal of Applied Physiology and Occupational Physiology* 64: 523-527, 1992

ix) Hawley JA, Dennis SC, Laidler BJ, Bosch AN, Noakes TD, Brouns F. High rates of exogenous carbohydrate oxidation from starch ingested during prolonged exercise. *Journal of Applied Physiology* 71: 1801-1806, 1991

Abstracts and/or professional presentations:

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ii) Weltan SM, Bosch AN, Hawley JA, Noakes TD. Influence of hyperglycaemia on carbohydrate kinetics and blood glucose oxidation (Abstract). *Medicine and Science in Sports and Exercise* (Supplement) 25: S71, 1993

iii) Hawley JA, Dennis SC, Noakes TD. Oxidation of carbohydrate ingested during prolonged exercise (Abstract). *New Zealand Journal of Sports Medicine* 21: 13, 1993

iv) Hawley JA, Derman KD, Dennis SC, Noakes TD, Brouns F. Faster rates of exogenous carbohydrate oxidation from amylopectin than from amylose ingested during exercise (Abstract). *Medicine and Science in Sports and Exercise* 24 (Supplement): S122, 1992

v) Hawley JA, Bosch AN, Noakes TD. Effect of glucose polymer chain-length on gastric emptying and exogenous U-^{14}C carbohydrate oxidation during exercise (Abstract). *Medicine and Science in Sports and Exercise* 23 (Supplement): S70, 1991

vi) Hawley JA, Bosch AN, Noakes TD. Effect of glucose polymer chain-length on gastric emptying and exogenous U-^{14}C carbohydrate oxidation during exercise (Abstract). *South African Journal of Food, Science and Nutrition* 3: 88, 1991

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CHAPTER ONE

INTRODUCTION, BACKGROUND AND AIMS OF THE THESIS

1.0 Introduction and Background

Investigations of the effects of carbohydrate (CHO) ingestion by athletes during prolonged (> 90 min) continuous exercise of moderate to high intensity (60-75% of maximal oxygen uptake [VO_{2max}]) had their origins in the first quarter of the century (Levine et al. 1924; Gordon et al. 1925; Carpenter and Fox 1931; Dill et al 1932). Indeed, nearly seventy years ago it was suggested that fatigue could be postponed and endurance (running) performance improved by the ingestion of CHO during prolonged moderate intensity exercise (Gordon et al. 1925).

A number of studies conducted between 1925 and 1939 (Gordon et al. 1925; Carpenter and Fox 1931; Dill et al 1932; Boje 1936; Christensen and Hansen 1939a, 1939b, 1939c) established the importance to performance of CHO when ingested by endurance-trained subjects during prolonged, exhaustive exercise. Due to the technical constraints of the time, however, these early studies could provide no information of the source of the endogenous substrate oxidised by the exercising muscles and how this might be influenced by the nature of the ingested CHO. Yet, despite such limitations, it was still possible in 1932 for Dill and co-workers to suggest that the factor limiting the performance of prolonged exercise "seems to be merely the quantity of easily available fuel ..... in the form of blood-borne glucose" (Dill et al. 1932).

These findings were, however, largely ignored by the athletic community. Athletes were also unaware of the early industrial and military investigations showing the importance of adequate fluid replacement for exercise (Adolph 1947; Pitts et al. 1944).

For the next two decades there was a scarcity of research in exercise physiology, in part, due to the disruptive effects of World War II (Noakes 1991).

The first official reference to fluid replacement during long-distance running can be found in the 1953 Handbook of the International Amateur Athletic Federation (IAAF). The 1953 IAAF rules controlling marathon races stated that "*refreshments shall (only) be*

provided by the organisers of a race after 15 km or 10 miles, and thereafter every 5 km or 3 miles. No refreshment may be carried or taken by a competitor other than that provided by the organisers." At that time water was the only drink available to runners at official refreshment stations.

Studies of CHO utilisation during exercise became popular again in the 1960's, with the reintroduction of the percutaneous needle biopsy technique by Scandinavian physiologists (Ahlborg et al. 1967a, 1967b; Bergstrom and Hultman 1966, 1967; Bergstrom et al. 1967, 1969; Hermansen et al. 1967). These studies demonstrated the crucial role of starting muscle glycogen stores for performance during prolonged exercise and focused attention on pre-exercise nutritional strategies aimed at maximising working muscle glycogen content prior to exercise.

By this time the IAAF had *slightly* modified their rules governing the provision of refreshments during marathon races. In 1967 the IAAF Handbook stated that "*refreshments could be provided after (only) 11 km or 7 miles.*" Presumably the term 'refreshments' referred to water.

For the next decade, the idea that water rather than CHO replacement was more important during exercise was accepted. This was largely the result of a single study, which showed that runners who became dehydrated during a 20 mile (32 km) road-race had elevated post-race rectal temperatures (Wyndham and Strydom 1969). Further, those runners who were the most dehydrated had the highest post-race temperatures, a finding that led Wyndham and Strydom (1969) to speculate that the athletes' level of dehydration and their elevated post-race temperatures were causally related, and that dehydration alone was the most important factor determining rectal temperature during prolonged exercise (Wyndham and Strydom 1969; Wyndham 1977). Unfortunately, due to a failure to note that rectal temperatures are more a function of metabolic rate than of dehydration (Noakes et al. 1991b), the concept that fluid replacement alone was of primary importance for optimising performance during prolonged exercise was promoted. The CHO studies of

the 1920's and 1930's were largely ignored, and water, in large volumes, was considered the optimum fluid replacement for ingestion during prolonged exercise. Accordingly, the IAAF modified their rules so that competitors during a marathon could now have *"refreshments provided by the organisers of the race after approximately 5 km or 3 miles."*

Thus, during the 1970's it was universally accepted that CHO ingestion during prolonged exercise was of little benefit. Several studies at this time, however, did *suggest* that CHO feedings during exercise could potentially delay fatigue (Brooke and Green 1974; Brooke et al. 1975; Green and Bagley 1972, Ivy et al. 1979). These investigations showed that when trained cyclists or canoeists were fed various CHO preparations during prolonged, strenuous exercise, blood glucose concentrations were elevated above pre-exercise levels, high rates of CHO oxidation were sustained during exercise, and endurance capacity was improved.

Despite this long-standing interest into the efficacy of CHO feedings for enhancing performance during endurance exercise, it was not until the 1980's that a series of well controlled experiments, largely undertaken in one laboratory in the United States, conclusively demonstrated that CHO ingestion during prolonged exercise could delay fatigue, at least under the specific conditions tested in those trials (Coyle et al. 1983, 1986; Coggan and Coyle 1987, 1988, 1989). These studies, utilising highly-trained cyclists who had fasted 12 hr before exercise, showed that the primary effect of CHO feedings during continuous cycling is to maintain plasma glucose concentrations at euglycaemic levels (i.e. 4-5 mmol/L) and to sustain high rates of CHO oxidation late in exercise. These studies emphasised the importance of plasma glucose as a critical substrate for exercising muscle at a time when endogenous CHO stores (muscle and liver glycogen) were nearly depleted (Coggan and Coyle 1991).

A limitation of *all* of the studies discussed so far is that assessment of the contribution of plasma glucose to oxidative metabolism has relied upon indirect calorimetry or plasma

glucose concentrations, or both, in order to *estimate* CHO metabolism during exercise. As such, these studies cannot differentiate between endogenous and exogenous sources of glucose in subjects who were fed (or infused) with CHO during exercise. For example, although it has been frequently inferred that, providing adequate glucose availability is maintained, plasma glucose can *potentially* provide all of the CHO energy needed to support exercise at 70-75% of VO_{2max} during the latter stages of prolonged exercise (Coggan and Coyle 1991; Coyle et al. 1986), this postulate has never been verified experimentally. For instance, it is not known whether CHO ingestion enhances plasma glucose oxidation *throughout* or only during the latter stages of prolonged exercise.

The recent use of isotopic tracers has allowed the quantification of the extent to which ingested substrates are oxidised during exercise (Massicotte et al. 1986, 1989, 1990, 1992; Moodley et al. 1992; Pallikarakis et al. 1986; Pirnay et al. 1977a, 1977b, 1982; Rehrer et al. 1992; Saris et al. 1993). The results of these studies suggest that ingested CHO can make a substantial contribution to oxidative energy supply during prolonged exercise. A shortcoming of *some* of these investigations, however, is that they have employed untrained subjects during treadmill walking exercise at intensities ranging from 40-50% of VO_{2max}. It is unlikely that the conclusions from such studies can be applied to well-trained athletes who are able to maintain higher absolute and relative work rates for sustained (i.e. 3-4 hr) periods. Further, at the low work rates that have been utilised in some of these investigations, *fat* oxidation and not CHO metabolism would be expected to account for most of the energy utilisation during exercise.

The rate at which energy (i.e. CHO) contained in a solution ingested during exercise reaches the working muscles where it can be utilised depends on the rate at which it is emptied from the stomach and on the rate of absorption in the small intestine. Of these two processes, it has been assumed that the rate of gastric emptying is *the* factor controlling the rate at which the fuel supplied exogenously in that solution will be oxidised by the muscles (Costill and Saltin 1974). As such, there has been considerable interest on

the influence of solute characteristics (osmolality, caloric density) on the rate of gastric emptying and intestinal CHO delivery (Costill 1990).

However, it is important to note that despite the wide acceptance of this belief, most of the measurements of gastric emptying and intestinal transport have been obtained under resting conditions using a single bolus feeding and a single aspiration of the stomach contents usually at a fixed time interval (i.e. 60 min) after ingestion. The results obtained using this procedure led to the belief that gastric emptying occurs at a constant rate independent of the residual gastric volume. However, the development of a repeated sampling technique has shown that gastric emptying follows a curvilinear exponential time course (Leiper and Maughan 1988; Rehrer et al. 1990). Thus, it is quite possible that conclusions drawn from studies performed *at rest* using a single bolus feeding may not be applicable to conditions present *during* exercise.

Indeed, recent investigations utilising uniformly labelled radioactive carbon fourteen (U-^{14}C) isotopes and naturally labelled stable carbon thirteen (^{13}C) tracer techniques in association with conventional gastric aspiration, suggest that the factors limiting muscle glucose oxidation lie distal to the stomach. Thus, it has been found that the rate at which the exercising muscles oxidise exogenous CHO is far less than the rate at which the ingested CHO leaves the stomach (Moodley et al. 1992; Rehrer et al. 1992; Saris et al. 1993).

Finally, it is perhaps surprising that despite renewed and vigorous scientific interest in the field of CHO metabolism, undoubtedly fostered by extensive commercial patronage, the optimal *type* and *amount* of CHO to be ingested by athletes during prolonged exercise has not been formulated. Most studies have investigated the effects of ingesting glucose or glucose polymer solutions upon exercise metabolism (Massicotte et al. 1989; Pallikarakis et al. 1986; Rehrer et al. 1992), thereby ignoring the possibility that other CHO sources may be as good, if not better sources of energy for the exercising muscles. In formulating the optimal CHO solution for ingestion by endurance athletes, *all* of the factors that can

potentially influence the rate at which the active muscles take up and oxidise the ingested CHO during exercise should be systematically investigated.

1.1 Aims and scope of this thesis

The experiments described in this thesis comprise a series of related, but independent studies into one (or more) of the factors which may ultimately determine the rate at which ingested (or infused) CHO (or glucose) is utilised by the working muscles during exercise. These factors are i) the rate of gastric emptying of an ingested fluid, ii) the rate of digestion and subsequent transport of glucose into the systemic blood supply, and iii) the rate of muscle glucose oxidation.

In order to identify the optimal CHO *type* for ingestion during prolonged exercise, the first study, described in Chapter Four, compares the rates of gastric emptying and oxidation of 15 g/100 ml solutions of U-^{14}C labelled maltose and U-^{14}C labelled glucose ingested by trained subjects during 90 min of moderate-intensity (i.e. ~70% of VO_{2max}) cycling. If the rate of gastric emptying, CHO digestion, and subsequent transport of glucose into the systemic blood supply limits the rate of ingested glucose oxidation, then the rate of ingested CHO oxidation for maltose should be less than that for glucose. This is the first study to examine the metabolic fate of maltose ingested by human subjects during exercise.

Chapter Five pursues the search for the ideal CHO solution for ingestion during exercise by comparing the rates of gastric emptying and ingested CHO oxidation of a 22-chain length glucose polymer solution and `soluble' starch, the longest glucose polymer that can be ingested in suspension form. It may well be that isocaloric CHO sources with different solubility are oxidised at different rates. Specifically, this study examined whether increasing the glucose polymer chain length influences the rates of gastric emptying and ingested CHO oxidation during exercise. To date, the ingestion of starch by athletes during exercise has received little attention.

In Chapter Six, studies using both U-^{14}C and tritiated (2-^{3}H) glucose isotopic tracer techniques to measure simultaneously rates of ingested CHO oxidation, whole body glucose appearance (Ra) and disappearance (Rd), and plasma glucose oxidation (Rox) are described. These experiments were designed to compare the effects of maintaining euglycaemia (i.e. a plasma glucose concentration of 5 mmol/L) by either a continuous variable-rate intravenous glucose infusion or by multiple feedings of moderately concentrated (15 g/100 ml) glucose, on fuel substrate kinetics during 125 min of cycling. The primary aim of this study was to determine whether the peak rate of exogenous CHO oxidation is limited by the regulation of a constant glucose output into the systemic circulation by the liver, or if by-passing both intestinal absorption and hepatic glucose uptake via intravenous glucose infusion might permit skeletal muscle to oxidise glucose at a rates greater than those seen with CHO ingestion.

If the exercise-induced Ra is precisely matched to the rate of systemic glucose supply (Jenkins et al. 1985), then it might be possible to inhibit hepatic glycogenolysis completely as a consequence of either intravenous glucose infusion, or by the repetitive CHO ingestion, the latter of which would be expected to cause a progressively rising glucose concentration in the portal circulation. As depletion of liver glycogen stores probably precedes depletion of muscle glycogen stores at exercise intensities of 70-75% of VO_{2max} (Noakes 1992), and as glucose ingested or infused into the bloodstream does not reduce the rate of muscle glycogen utilisation during prolonged, moderate-intensity exercise (Bosch et al. 1993a; Coyle et al. 1986, 1991; Fielding et al. 1985; Flynn et al. 1987; Hargreaves and Briggs 1988; Noakes et al. 1988), such a suppression of endogenous liver glycogen stores could potentially enhance athletic performance. A further purpose of this investigation, therefore, was to determine whether CHO ingestion increases the rate of plasma glucose oxidation during only the latter stages of prolonged exercise, as has been previously proposed (Coggan and Coyle 1991), or whether CHO ingestion increases plasma glucose oxidation *throughout* exercise.

Finally, in Chapter Seven the effects of euglycaemia (i.e. a plasma glucose concentration of 5 mmol/L) and hyperglycaemia (i.e. a plasma glucose concentration of 10 mmol/L) on fuel substrate kinetics in highly-trained subjects during prolonged exercise are described. Plasma glucose concentration was maintained via variable-rate intravenous glucose infusion, while a constant infusion of 2-^{3}H glucose, in association with ^{14}C tracer methodology allowed the determination of the rates of liver glucose turnover and plasma glucose oxidation. This study sought to establish whether the rate of glucose oxidation by muscle could be increased when plasma glucose concentration was elevated above normal physiological concentrations (i.e. from 5 to 10 mmol/L). If this were the case, it would indicate that the rate at which exogenous glucose is utilised by the working muscles under physiological conditions is limited by the rate of working muscle glucose uptake, which, in turn, is regulated by liver glucose uptake and release.

CHAPTER TWO

REVIEW OF LITERATURE

CARBOHYDRATE SUPPLEMENTATION DURING PROLONGED MODERATE-INTENSITY EXERCISE

2.1 Historical development.

In 1911, Zuntz established from respiratory exchange ratio (RER) measurements in exercising human subjects that both dietary fat and CHO can serve as substrates for energy metabolism (Zuntz 1911). Subsequently, Krogh and Lindhard (1920) confirmed these findings. They also showed that the proportional contribution of these two substrates to energy production was influenced by the pre-exercise diet; if individuals were fed a high fat diet prior to exercise, low intensity work could be performed with little contribution from CHO to oxidative metabolism (Krogh and Lindhard 1920).

These early investigations were primarily concerned with identifying the fuels utilised to provide the energy for muscular activity. As such, they relied exclusively upon RER values or plasma glucose concentrations or both to estimate indirectly the rates of CHO metabolism during short periods of exercise.

It was in the mid 1920's that the pioneering studies of CHO metabolism during prolonged exercise began. Levine and colleagues (1924), of the Peter Bent Brigham Hospital in Boston, studied 12 runners in the 1923 Boston Marathon and observed a marked decline in post-race plasma glucose concentrations (< 2.8 mmol/L) in three of the runners. They proposed that this hypoglycaemia, which was manifested as "asthenia, nervous irritability, extreme pallor and prostration," could explain the fatigue and poor physical condition of these runners at the finish of the race. In the same marathon the following year they tested this possibility by feeding CHO, in the form of candy, to a number of athletes 24 km into the race. This practice, in combination with a high CHO diet 24 hr before the race, enhanced running performance and prevented hypoglycaemia (Gordon et al. 1925). This was probably the first study to *suggest* that CHO ingestion could postpone fatigue and improve endurance performance during prolonged exercise of moderate to high intensity (60-75% of VO_{2max}).

The next study to highlight the importance of CHO during exercise was that of Bock et al. in 1928. These workers reported that the contribution of CHO oxidation to energy metabolism increased with increasing exercise intensity (Bock et al. 1928).

The possibility that hypoglycaemia might be a factor explaining exhaustion at the end of long-distance races was raised again by Best and Partridge (1930), who studied ten runners who completed the 1928 Amsterdam Olympic Marathon. Three of the ten runners studied were hypoglycaemic at the end of the race. However, as plasma glucose concentrations were measured up to 20 min after the athletes had crossed the finish line, it is possible that the incidence of hypoglycaemia could have been higher. Delays in blood sampling would have allowed plasma glucose concentrations to increase from the values present at the end of the race. Based on notes taken on the condition of the subjects at the finish, and the food they had consumed before and during the race, Best and Partridge (1930) observed that "*the carbohydrate reserves of the body at the start of the race*" and "*the ingestion of food during the race*" were important factors in the prevention of hypoglycaemia. In the same year, low plasma glucose concentrations after a marathon race were also reported by Schenk and Craemer (1930).

In 1931 Carpenter and Fox (1931) confirmed the earlier finding of Bock et al. (1928), that CHO oxidation is elevated with increasing exercise intensity. On two separate occasions these workers had a subject cycle at different work rates immediately after a 50 gram glucose feeding. They observed that "*the more intense the work, the greater was the amount of carbohydrate burned*". Their RER measurements also indicated that "*when glucose is available, work is performed more at the expense of carbohydrate than at the expense of fat*". Carpenter and Fox (1931) concluded that "*when glucose is ingested (before exercise), part of the ingested glucose is used for the performance of muscular work*".

The significance of CHO for improving work capacity was further demonstrated by Dill and his colleagues during studies conducted at the Harvard Fatigue Laboratory in Boston

in 1932 (Dill et al. 1932). They showed that when their dogs, Joe and Sally, ran without being fed CHO they became hypoglycaemic and fatigued after 4-6 hr of running. However, when provided with CHO at regular intervals during exercise (20 g/hr), the same dogs could run for 17-23 hr, covering up to 150 km before becoming exhausted. Dill et al. (1932) concluded that the limiting factor in the performance of prolonged moderate intensity exercise "*seems to be merely the quantity of easily available fuel.....in the form of blood-borne glucose*" for oxidation by the working muscles.

In 1936 Boje (1936) showed that feeding CHO solutions to individuals at exhaustion could restore exercise capacity. These early studies prompted Grace Eggleton (1936) to surmise that "*when long distance runners have run to exhaustion, the levels of blood sugar are found to be abnormally low. Presumably the glycogen stores of the liver are entirely depleted; if the eating of sugar candy during a race was encouraged by Athletic Organisations, it seems possible that new records might be achieved in very long-distance running*".

In the classic studies of Christensen and Hansen (1939a, 1939b), the essential role of CHO for the performance of prolonged exercise was confirmed. Using different dietary manipulations, these workers found that endurance time was decreased after a high fat diet, but increased markedly after a high CHO diet. The premature fatigue experienced by subjects after the high fat-low CHO diet was accompanied by ketosis, a decreased rate of CHO oxidation, and hypoglycaemia severe enough to result in symptoms of neuroglucopenia.

In an additional experiment aimed to distinguish between these possible causes of fatigue, Christensen and Hansen (1939c) had two subjects ingest a large quantity of glucose (200 g) at the point of "*almost complete exhaustion*". This single feeding resulted in a rapid increase in plasma glucose concentration and relief of the neuroglucopenic symptoms, and enabled the subjects to perform an additional hour of exercise. As had been found by Boje (1936), the subject's RER values did not alter significantly either before or after glucose

ingestion indicating that the reversal of fatigue after CHO ingestion was not associated with changes in the rate of CHO oxidation. Instead, these observations suggest that hypoglycaemia caused fatigue during prolonged exercise by affecting the functioning of the central nervous system.

These investigations, performed over 50 years ago, strongly suggested the importance of ingesting CHO during prolonged, moderate-intensity exercise. That information, together with the importance of fluid replacement during prolonged exercise (Adolph 1947; Pitts et al. 1944) was, however, largely ignored by the athletic community. The first mention of any type of fluid replacement during long-distance running by the International Amateur Athletic Federation (IAAF) was in 1953 (IAAF Handbook, 1953, page 65). In the 1953 IAAF rules controlling marathon races, it states that "*refreshments shall (only) be provided by the organisers of a race after 15 km or 10 miles, and thereafter every 5 km or 3 miles. No refreshment may be carried or taken by a competitor other than that provided by the organisers...*" As water was the *only* drink made available to runners at the official refreshment stations, it was clear that the IAAF had little knowledge of the pioneering studies which had been conducted in the preceding two decades showing the benefits of ingesting CHO during prolonged exercise.

For the next quarter century there was a distinct scarcity of research in exercise physiology in general, probably, in part, due to the disruption resulting from the second World War (Noakes 1991). It was not until the 1960's when Scandinavian physiologists reintroduced the percutaneous needle muscle biopsy technique that studies of CHO utilisation during exercise again became popular (Ahlborg et al. 1967a, 1967b; Bergstrom et al. 1967, 1969; Bergstrom and Hultman 1966, 1967; Hermansen et al. 1967). Bergstrom and Hultman (1966) showed that during one-legged cycling, the glycogen concentration of the exercising muscles declined to very low levels at the point of exhaustion, whereas the glycogen levels in the non-exercising leg remained normal. In 1967 an investigation into the time-course of glycogen disappearance during prolonged heavy exercise showed that a progressive utilisation of muscle glycogen took place and that exhaustion occurred when

the working muscle glycogen content had "*almost totally disappeared*" (Hermansen et al. 1967). In the same year it was also found that diets containing varying quantities of CHO ingested for several days after exercise influenced the rate of glycogen re-synthesis and allowed the pre-exercise muscle glycogen content to be manipulated (Ahlborg et al. 1967a, 1967b). When muscle glycogen was low at the start of exercise, endurance capacity was reduced and fat metabolism during exercise was increased. In contrast, when exercise began with "*super compensated*" muscle glycogen stores, both the endurance capacity and the rate of CHO utilisation were increased (Ahlborg et al. 1967a). These studies demonstrated the crucial role of adequate starting muscle glycogen stores during prolonged exercise, and focused attention on pre-exercise nutrition and strategies aimed at maximising muscle glycogen content prior to exercise. The overriding assumption in these investigations was that muscle glycogen depletion *alone* caused fatigue during prolonged exercise.

This muscle glycogen depletion paradigm was further strengthened by the findings that dietary manipulations which increased muscle and liver glycogen concentration increased endurance performance, whereas those that reduced pre-exercise body glycogen stores impaired exercise capacity (Bergstrom et al. 1967). It was shown that when free-fatty acid (FFA) availability was reduced, the muscle relied more heavily on glycogen utilisation (Bergstrom et al. 1969; Pernow and Saltin 1971) which resulted in a more rapid rate of muscle glycogenolysis (Gollnick et al. 1981), conditions which resulted in a reduced endurance time (Gollnick et al. 1981; Pernow and Saltin 1971). The concept that muscle glycogen was the primary CHO source during prolonged exercise and that plasma glucose contributed little to the fuel needs of the working muscles during exercise therefore became firmly established (Conlee 1987).

By this time the IAAF had *slightly* modified their rules governing the provision of refreshments during marathon races. Whereas in 1953 "*refreshments could only be provided by the organisers of a race after 15 km or 10 miles,*" in 1967 the IAAF

handbook stated that "*refreshments could be provided after (only) 11 km or 7 miles*" (IAAF Handbook, Rule 155, page 103).

For the next decade, the idea that water rather than CHO replacement was more important during exercise gained the ascendancy (Wyndham and Strydom 1969; Costill and Saltin 1974). Wyndham and Strydom (1969) studied two groups of athletes competing in a 20 mile (32 km) road-race and found that those runners who became dehydrated by more than three percent during the race had elevated post-race rectal temperatures. Further, those runners who were the most dehydrated had the highest post-race temperatures. This finding led Wyndham and Strydom (1969) to speculate that the athletes' level of dehydration and their post-race temperatures were *causally* related, and that dehydration alone was the most important factor determining rectal temperature during prolonged exercise (Wyndham 1977; Wyndham and Strydom 1969).

Largely as a result of this single study (Wyndham and Strydom 1969) and a failure to note that rectal temperatures are more a function of metabolic rate than of dehydration (Costill 1977; Davies 1979; Davies et al. 1976; Greenhaff and Clough 1989; Wyndham et al. 1970; Noakes et al. 1991b), the concept that fluid replacement alone was of primary importance for optimising performance during prolonged exercise was promoted (Noakes 1991). These findings were later reinforced by the study of Costill and Saltin (1974), which showed that drinks with a high (6.25 - 37.5 g/100 ml) CHO content emptied more slowly from the stomach than water during exercise. These workers concluded that "*the replacement of water was important during prolonged exercise in the heat,*" and "*that only when competing in the cold could carbohydrate safely be included in solutions to be ingested during exercise.*" Accordingly, the CHO studies of the 1920's and 1930's were largely ignored (Noakes 1991) and water, in large volumes, was considered to be the optimum fluid replacement for ingestion during prolonged exercise.

At this time the IAAF, obviously became aware of the `dangers' of dehydration during long-distance events and changed their rules so that competitors during a marathon could

now have "*refreshments provided by the organisers of the race after approximately 5 km or 3 miles.*" In addition, rule number 165, clause 4 stated that "*the organisers shall provide sponging points where water only shall be supplied, midway between (two) refreshment stations*" (IAAF Handbook, 1977, page 98).

Thus, during the 1970's it was universally accepted that CHO ingestion during prolonged exercise was of little benefit to the performance of endurance athletes. Indeed, in the proceedings of the October 1976 New York Academy of Sciences sponsored conference "The Marathon: Physiological, Medical, Epidemiological, And Psychological Studies", considered at that time as the `state of the art,' there was not a *single* reference to CHO ingestion during prolonged exercise.

Several studies at this time, however, did *suggest* that CHO feedings during exercise could potentially delay fatigue. In 1972 for example, Green and Bagley (1972) fed subjects either a placebo or 230 g of a maltodextrin solution prior to and during a canoe race that lasted ~150 min. They observed that the CHO feedings resulted in the elevation of plasma glucose concentrations above pre-exercise levels both before and during the exercise bout; when fed the placebo, plasma glucose concentrations fell during exercise by 1.0-1.5 mmol/L. In this study CHO ingestion permitted the athletes to maintain their pace during the final 30 min of the race, whereas when they were fed the placebo, they were forced to reduce their pace by ~10-15%. A subsequent study by the same group (Brooke and Green 1974) examined the effect of the ingestion of glucose syrup on the ability to recover exercise capacity after previously exhausting exercise. Trained cyclists rode at 70% of VO_{2max} until "*exhaustion*", defined in this study as the work time until the fall in work rate reduced the RER to 0.73 (a non protein RER value of this magnitude means that 90.4% of energy utilisation is from fat oxidation and only 9.6% from CHO sources [Péronnet and Massicotte 1991]). At this point (~153 min) the subjects rested for 40 min and consumed either a glucose syrup (86 g), an equicaloric rice pudding/sucrose mixture, or a placebo. Subjects then cycled again at 70% of VO_{2max} until exhaustion reduced the RER again to 0.73. Plasma glucose concentrations were maintained during the second

exercise bout when subjects had been fed CHO rather than placebo. The work times to exhaustion after the CHO feedings were also significantly longer than after placebo (79.8, 58.2 and 28.8 min for the glucose syrup, the pudding/sucrose and the placebo trials respectively). The authors concluded that "*neural factors were probably involved in the differences between treatments.*" However, it is also possible that subjects would have had significantly reduced muscle glycogen contents in the ~2.5 hr of cycling *before* CHO feedings, and that the work enhancement observed *after* the CHO feedings was due to the increased availability of plasma glucose for muscle oxidation (Maughan 1991) which would help maintain RER values above 0.73.

Another study to demonstrate that CHO ingestion could potentially enhance endurance during prolonged moderate-intensity exercise was that of Brooke et al. (1975). These investigators had eight well trained cyclists simulate a 100 mile (160 km) race by riding their own bicycles mounted on a special ergometer at a work rate (76% of VO_{2max}) selected to exhaust the subjects in approximately four hr. Twenty min after commencing the ride and at subsequent 20 min interval during the ride, subjects ingested either 87 g of maltodextrins, a rice pudding plus sucrose mixture, or a placebo solution. When fed CHO, plasma glucose concentrations, the rate of CHO oxidation and "*work cut-off time*" were all enhanced. Indeed, time to exhaustion was significantly prolonged when subjects ingested either maltodextrin (214 ±40 min) or the rice pudding plus sucrose mixture (200 ±46 min) compared to the "*low energy*" placebo solution (180 ±53 min). These authors proposed that "*the intensity and duration of work is such that the muscle glycogen (stores) must (have been) reduced considerably, and the only way of maintaining such a high carbohydrate participation is by the use of blood glucose ..... to support muscle metabolism*". However, as noted by Coggan and Coyle (1991), these data should be interpreted with caution because the "*performance time*" of the subjects was defined by a decrease in RER *or* blood glucose concentration, *or* both, rather than by the subjects inability to maintain a predetermined work rate.

Despite these limitations, the study of Brooke et al. (1975) did *suggest* that CHO feedings during prolonged exercise could postpone the onset of fatigue by the maintenance of euglycaemia (i.e. a plasma glucose concentration of 4-5 mmol/L) and the concomitant high rates of CHO oxidation. Interestingly, these findings and this interpretation are generally supported by the more recent well controlled studies of Coggan and Coyle (1987) and Coyle et al. (1986).

Further evidence that CHO ingestion may indeed improve endurance performance was provided by the study of Ivy et al. (1979). These workers had trained cyclists ingest either ~80 g of maltodextrin or a sweetened placebo while trying to maximise work output during 120 min of cycling. Although CHO ingestion compared to the placebo did not increase the average power output during the 120 min of cycling, it did enable subjects to increase their work output during the last quarter of the exercise bout (Ivy et al. 1979).

In contrast to the studies of Brooke and co-workers (Brooke and Green 1974; Brooke et al. 1975; Green and Bagley 1972) and Ivy et al. (1979), Felig et al. (1982) reported no differences in the time to fatigue during cycling at 60-65% of VO_{2max} when *untrained* subjects ingested either 5 g/100 ml (40 g/hr) or 10 g/100 ml (80 g/hr) glucose solutions every 15 min throughout exhausting exercise. They observed that prolonged exercise of moderate intensity precipitated hypoglycaemia (< 2.5 mmol/L) in 37% of subjects but, despite correcting this hypoglycaemia, glucose ingestion failed to cause a consistent increase in exercise time to exhaustion. Further, the time to fatigue in subjects who ingested only water, and who did not become hypoglycaemic (165 ±11 min), was not different from that of subjects who did develop hypoglycaemia during exercise (142 ±15 min). These results prompted Felig et al. (1982) to state that "*exercise can be continued in the presence of hypoglycaemia, which does not support a role for glucose ingestion in improving performance during prolonged exercise.*" Finally, they concluded that "*the so-called phenomenon of 'hitting the wall' during marathon running is probably due to factors other than a low blood glucose concentration*". Although it was recognised that muscle glucose uptake could increase significantly during exercise (Ahlborg et al. 1974;

Jordfeldt and Wahren 1970; Wahren et al. 1971), especially when subjects ingested CHO (Ahlborg and Felig 1976), during the 1970's it was generally accepted that glucose ingestion contributed little to the total energy utilised during prolonged exercise (Costill and Miller 1980).

Despite some lone voices which suggested that these studies did not necessarily prove that the performance of *trained* athletes would not also be improved by the ingestion of CHO during prolonged exercise (Noakes et al. 1983), the question of whether water or CHO replacement should be emphasised during prolonged exercise was not fully resolved until the mid to late 1980's. At that time, commercial interests in America revived research into the value of CHO ingestion during exercise: whereas water has no marketable value, CHO added to water can be marketed, and sold to millions of athletes world-wide.

As a direct result of large-scale commercial intervention, the landmark studies of Coyle et al. (1983, 1986) and Coggan and Coyle (1987, 1988, 1989) marked the re-emergence of investigations of CHO metabolism during prolonged exercise and, in particular, the role of CHO ingestion. In 1983 Coyle et al. (1983) had ten trained subjects cycle until fatigue (defined as the time at which oxygen uptake decreased below 10%) at 74% of VO_{2max} while ingesting either a glucose polymer (1 g of maltodextrin/ kilogram [kg] bodymass in a 50 g/100 ml solution after 20 min of exercise, followed by 0.25 g of maltodextrin/kg bodymass in a 6 g/100 ml solution after 60, 90 and 120 min), or a placebo solution. The exercise time to fatigue for the cyclists was significantly greater with the CHO feedings (157 ±5 min) than with the placebo (135 ±6 min). This was the first well controlled experiment to *conclusively* demonstrate that CHO ingestion during prolonged moderate intensity exercise can delay fatigue. At the time, Coyle et al. (1983) concluded that "*carbohydrate administration during exercise may result in increased utilization of blood glucose with a proportional slowing of muscle glycogen depletion*". However, muscle glycogen concentrations were not actually determined in that study and this interpretation was subsequently shown to be incorrect (Coyle et al. 1986).

At about the same time in Scandinavia, Bjorkman et al. (1984) investigated the effects of glucose or fructose ingestion on the rate of muscle glycogenolysis and the capacity to perform prolonged moderate intensity exercise. These workers had eight healthy men cycle at 68% of VO_{2max} until exhaustion, while ingesting either glucose (17.5 g/20 min for a total of 105 g), fructose (17.5 g/20 min for a total of 87.5 g), or water (250 ml/20 min). Unfortunately, due to non-randomised experimental design, the average time to exhaustion increased with repeated exercise regardless of the type of drink ingested. However, when statistically corrected for this order effect, the time to exhaustion with glucose ingestion (137 ±13 min) was significantly longer (~20%) than with both fructose (114 ±12 min) and water (116 ±13 min) intake. Muscle glycogen content decreased similarly during all three trials.

The question of whether CHO ingestion during exercise slows the rate of muscle glycogenolysis was raised again by Coyle and co-workers in 1986 (Coyle et al. 1986). Utilising essentially the same experimental protocol as previously described (Coyle et al. 1983), this group studied the pattern of decline in muscle glycogen content in trained cyclists, with and without CHO feedings. Mean exercise time to fatigue was 33% longer (4.02 ±0.3 versus 3.02 ±0.2 hr respectively) when subjects were fed CHO (~70 g of maltodextrin in a 50 g/100 ml solution 20 min after the commencement of exercise, followed by 28 g as a 10 g/100 ml solution every 20 min thereafter) compared to when they received the placebo solution. The pattern of muscle glycogen depletion was similar during the first three hr of exercise with and without CHO feedings. Surprisingly, the additional hr of exercise, made possible by the CHO feedings, occurred without a further significant decline in working muscle glycogen content. Coyle et al. (1986) concluded that "*when blood glucose concentration was maintained, highly trained endurance athletes were capable of oxidizing carbohydrate sources other than muscle glycogen at high rates during the latter stages of prolonged strenuous exercise.*" The rates of plasma glucose oxidation, however, were not determined in that study.

The observation that CHO ingestion (or glucose infusion) does not reduce the rate of muscle glycogen utilisation during prolonged exercise of moderate-intensity has been confirmed by other investigators (Bosch et al. 1993a; Coyle et al. 1991; Fielding et al. 1985; Flynn et al. 1987; Hargreaves and Briggs 1988; Noakes et al. 1988). Only one well controlled experiment involving low-intensity (49% of VO_{2max}) exercise in the heat (33°C, 52% relative humidity) has shown that, compared to water ingestion, CHO supplementation (198 g in 2 hr) can reduce the rate of decline of muscle glycogen (Yaspelkis and Ivy 1991).

Subsequent studies by Coggan and Coyle further proved the efficacy of CHO feedings during exercise and, in particular, the important role of plasma glucose as an oxidisable substrate late in exercise (Coggan and Coyle 1987, 1988, 1989). They showed that, during prolonged exercise in fasted subjects when no CHO is consumed, plasma glucose concentration falls after approximately two hr, a time when muscle glycogen levels are already low. These workers proposed that CHO ingestion during prolonged, exhaustive cycling maintains plasma glucose at euglycaemic levels (4-5 mmol/L) during the latter stages of exercise and improves performance primarily by maintaining sufficiently high rates of CHO oxidation. This hypothesis had originally been proposed by Dill et al. (1932) some 50 years earlier.

Although the validity of the conclusions from some of these investigations for performance in the field has recently been questioned (Valeriani 1991), several independent laboratories using a variety of CHO solutions and exercise performance tests have independently confirmed the efficacy of CHO feedings during prolonged running and cycling exercise (Davis et al. 1988a, 1988b; Hargreaves et al. 1984; Ivy et al. 1983; Millard-Stafford et al. 1992; Murdoch et al. 1993; Murray et al. 1987, 1989a, 1989b, 1991; Neufer et al. 1987; Wilber and Moffatt 1992; Williams et al. 1990; Wright et al. 1991). Thus, CHO/fluid consumption is not only allowed, but presently advocated by the IAAF in all races of 10 km (6.2 miles) and longer (IAAF Handbook, pp. 125, 1990). At most endurance events beverages are provided by either the race sponsors, the race organisers, the coaches, or the

athletes themselves. The choice of beverage is, however, typically not based upon any scientific rationale and there still remains much mysticism as to what, and how much (if indeed anything) should be consumed to provide adequate CHO and fluid to replace endogenous energy stores and sweat losses during exercise.

The modern studies of Coyle et al. (1983, 1986) and Coggan and Coyle (1987, 1988, 1989) showing that CHO replacement should be emphasised during prolonged cycling (in the overnight fasted state) have led to a search for the ideal CHO solution for ingestion during exercise. Because the addition of high (i.e. > 15-20 g/100 ml) concentrations of CHO to fluid replacement beverages may impair water absorption, while inadequate CHO ingestion may slow CHO oxidation late in exercise (Coyle and Montain 1992; Hawley and Neuter 1986), recent attention has focused on optimising the rate of exogenous CHO delivery, via the blood, to the working muscle during prolonged exercise (Flynn et al. 1987; Massicotte et al. 1986, 1989; Mitchell et al. 1988, 1989a, 1989b; Moodley et al. 1992; Neufer et al. 1986; Rehrer et al. 1992; Sole and Noakes 1989). In particular, the factors which could potentially limit the rate at which ingested CHO can be oxidised by the working muscles have been investigated.

2.2 Gastric emptying of fluids during prolonged, moderate-intensity exercise

The rate of oxidation of ingested CHO solutions depends upon the rate at which they empty from the stomach, are absorbed from the small intestine, and are taken up by the muscles. Historically, it has been assumed that the rate of gastric emptying is *the* factor limiting the rate of CHO delivery to the muscles where its utilisation can exert a beneficial effect (Costill and Saltin 1974; Houmard et al. 1991; Maughan 1991; Wheeler and Banwell 1986). However, it has been noted that the rate of ingested CHO oxidation by the working muscles rises with an increase in the CHO content of the ingested fluid (Mosora et al. 1981; Moodley et al. 1992; Pallikarakis et al. 1986), a condition which, normally, would be expected to reduce the rate of gastric emptying of fluid, but not necessarily of CHO (Noakes et al. 1991a).

Many factors have been shown to influence the rate of gastric emptying of fluids including gastric volume (Marbaix 1898; Costill and Saltin 1974; Mitchell and Voss 1991; Noakes et al. 1991a; Rehrer et al. 1990), solute osmolality (Carnot and Chassevant 1905; Hunt and Pathak 1960; Rehrer et al. 1993), beverage temperature (McArthur and Feldman 1989; Sun et al. 1988), beverage carbonation (Ryan et al. 1991; Zachwieja et al. 1991, 1992), the addition of medium-chain triglycerides (Beckers et al. 1992) and the caloric content of the ingested beverage (Costill and Saltin 1974; Coyle et al. 1978; Fordtran and Saltin 1967). There has been considerable emphasis on the effect of solute osmolality and energy content on the gastric emptying of ingested solutions (Costill 1990; Hunt and Pathak 1960; Hunt and Stubbs 1975). In this regard, solutions containing as little as 2.5 g/100 ml of CHO have been shown to reduce the rate of gastric emptying (Costill and Saltin 1974; Coyle et al. 1978; Foster et al. 1980).

Thus, in the late 1980's the practical advice given to athletes was that they should ingest solutions with low (< 2.5 g/100 ml) CHO content at a rate of 100-200 ml every 2-3 km during prolonged (running) exercise (American College of Sports Medicine 1987). However, as recently noted by Coyle and Montain (1992), this recommendation has limited practical value for the majority of athletes because "*at the extremes it could be interpreted to suggest that slow runners (i.e. 10 km/hr) drink only 330 ml/hr whereas the fastest runners should be drinking up to 2000 ml/hr.*" Such high rates of fluid intake may be inappropriate for some runners (Frizzell et al. 1986; Irving et al. 1991; Noakes 1993; Noakes et al. 1985, 1990; Young et al. 1987) and are well in excess of the 0.45-0.62 L/hr normally voluntarily ingested by long distance runners (Noakes 1993; Noakes et al. 1988).

A number of recent studies have shown that gastric volume may be a more important determinant of gastric emptying during exercise than either solute energy content or osmolality (Mitchell et al. 1988, 1989b; Mitchell and Voss 1991; Moodley et al. 1992; Noakes et al. 1991a; Owen et al. 1986; Rehrer et al. 1989, 1990; Ryan et al. 1989; Sole and Noakes 1989). These studies have shown that the rates of gastric emptying for solutions with vastly different CHO contents are quite similar when they are ingested

repeatedly during exercise (Noakes et al. 1991a; Maughan and Noakes 1991; Rehrer et al. 1989, 1990). The pattern of emptying follows an exponential time-course (Hunt and Spurrell 1951), and falls rapidly as the volume remaining in the stomach decreases (Gisolfi and Duchman 1992; Leiper and Maughan 1988; Rehrer et al. 1989). Thus, the maximum rate at which CHO and water can be delivered to the intestine from an ingested solution is strongly influenced by the average volume of fluid in the stomach at any time, which, in turn, is influenced by the drinking pattern of the athlete. Although this is not a novel finding (Costill and Saltin 1974; Hunt and Knox 1969; Hunt and MacDonald 1954; Hunt and Spurrell 1951; Marbaix 1898; Minami and McCallum 1984) its importance has only recently been recognised (Noakes 1992; Noakes et al. 1991a; Rehrer et al. 1990). Thus, it is now apparent that different drinking patterns can produce similar rates of both CHO and water delivery from solutions with quite different CHO concentrations. Indeed, only by following repetitive drinking patterns are athletes able to provide CHO at the rates required to sustain performance during prolonged exhaustive exercise, in events such as the Tour de France (Brouns et al. 1989a, 1989b; Saris et al. 1989).

2.3 Carbohydrate digestion and absorption

Most of the CHO that is ingested by humans is in the form of plant starch which is comprised of ~20% amylose and ~80% amylopectin. Amylose is an unbranched alpha-1,4-linked polysaccharide chain of relatively low molecular weight. Amylopectin is a branched alpha-1,4 and alpha-1,6-linked polysaccharide molecule of larger molecular weight. In addition some CHO is also ingested in the form of di- and monosaccharides. Common disaccharide's are sucrose and lactose and common monosaccharides are glucose and fructose.

Since monosaccharides are the only CHO that can be absorbed from the intestine (Newsholme and Leech 1983), CHO digestion is basically a process of glycoside hydrolysis achieved through the action of a series of hydrolytic enzymes known collectively as glycosidases. The effect of their action is best illustrated by considering the hydrolysis of starch.

Digestion of starch is catalysed by the alpha-amylases that are present in saliva and in the pancreatic secretion that is delivered into the lumen of the duodenum. Alpha-amylases hydrolyse the alpha-1-4-links in the middle of the chain to produce maltose, maltotriose and alpha-1-6 branched limit dextrin's (Figure 2.1).

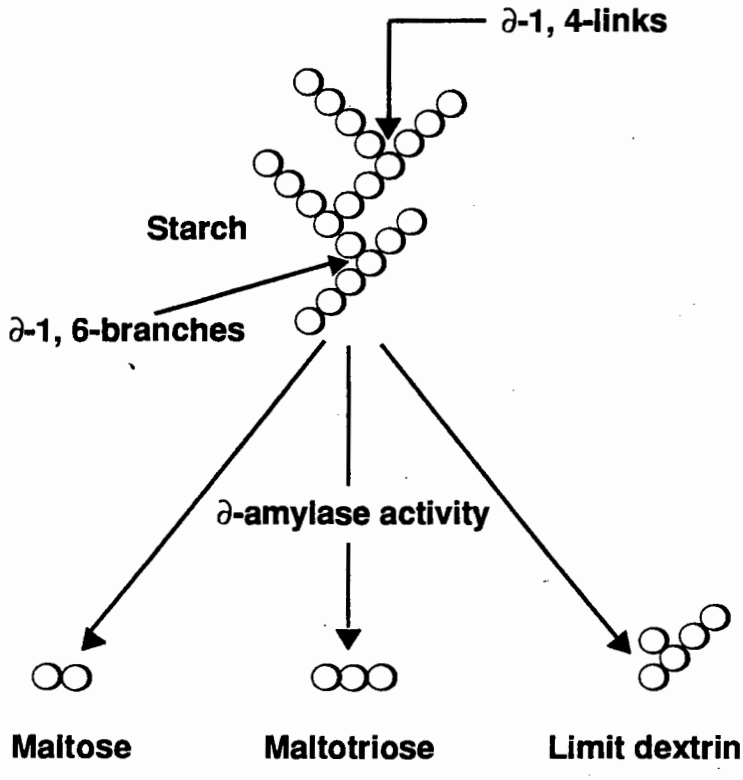

Figure 2.1. Digestion of amylopectin in starch.
Open circles represent C6 glucose units.

Maltose, maltotriose and branched chain limit dextrin's are then further digested by a number of different oligosaccharidases that are attached to the microvillae of the intestinal villae of the jejunum and most of the ileum. Included among these enzymes are:

i) ß-galactosidase, which cleaves lactose to galactose and glucose;
ii) sucrose alpha-D-glucohydrolase, which hydrolyses both the alpha-1-6 linkages of the branched limit dextrin's and sucrose to fructose and glucose, and
iii) exo-1,4-alpha D-glucosidase, which catalyses the sequential release of terminal glucose units from the non-reducing end of linear oligosaccharides.

Interestingly, the glucosidase enzymes are thought to be functionally linked to the glucose transporter in the luminal membranes of the columnar epithelial (absorptive) cells. Duodenal perfusion studies have shown that the rates of glucose absorption from the hydrolysis of maltose (Cook 1973; Fairclough et al. 1977; Gray and Santiago 1966; Sandle et al. 1982) and maltotriose (Jones et al. 1987) solutions are more rapid than from isocaloric glucose solutions. This suggests that liberation of monosaccharides by brush border enzymes achieves a greater local concentration at glucose transporter sites than that achieved by diffusion of free glucose through the `unstirred' water border (Crane 1975; Hanke et al. 1980).

Transport of glucose (and galactose) across the columnar epithelial cells occurs by a number of independent processes (Stevens et al. 1984). Amongst the transport mechanisms are two Na^+ dependent carriers on the luminal border (Figure 2.3a). One has a relatively low external affinity for glucose (and galactose) and a high capacity for $2Na^+$/glucose or galactose co-transport. The other has a much higher external affinity for glucose (or galactose) but a relatively low transport capacity. When glucose concentration in the gut increases after a meal, some glucose can also cross the brush border passively, that is, without being driven by the inward Na^+ gradient (Figure 2.3a).

Glucose entering the columnar epithelial cells is released into the bloodstream from the serosal border via a low affinity, very high capacity glucose transporter. This glucose transporter strongly resembles that present in other cell types, except that it is not insulin-sensitive. About 20% of glucose also enters the glycolytic pathway in the columnar epithelial cells and is released as lactate (Stanley et al. 1988). In order to complete the absorption of glucose, lactate must be reconverted into glucose, a process which is achieved in the liver via gluconeogenesis. The glucose produced by the liver is then released into the systemic circulation via the inferior vena cava.

Fructose, which is not actively absorbed in humans and when ingested in large quantities can produce gastrointestinal distress (Fruth and Gisolfi 1983), is transported across the brush border by a separate carrier (Figure 2.3b). Compared to the glucose transporters, the fructose carrier has a rather low overall capacity and is not dependent on Na^+. After an oral load of fructose, the portal blood carries first a fructose peak and then a peak of glucose derived from the fructose to glucose conversion in the columnar epithelial cells. As occurs after glucose ingestion, some of the ingested fructose is converted to lactate in the columnar epithelium. Fructose absorption is stimulated by the presence of glucose (Holdsworth and Dawson 1964).

Once hexose and triose molecules from CHO digestion appear in the systemic circulation, they become available for use as fuels by the working muscles. Techniques to monitor the oxidation of CHO are described in section 2.4.

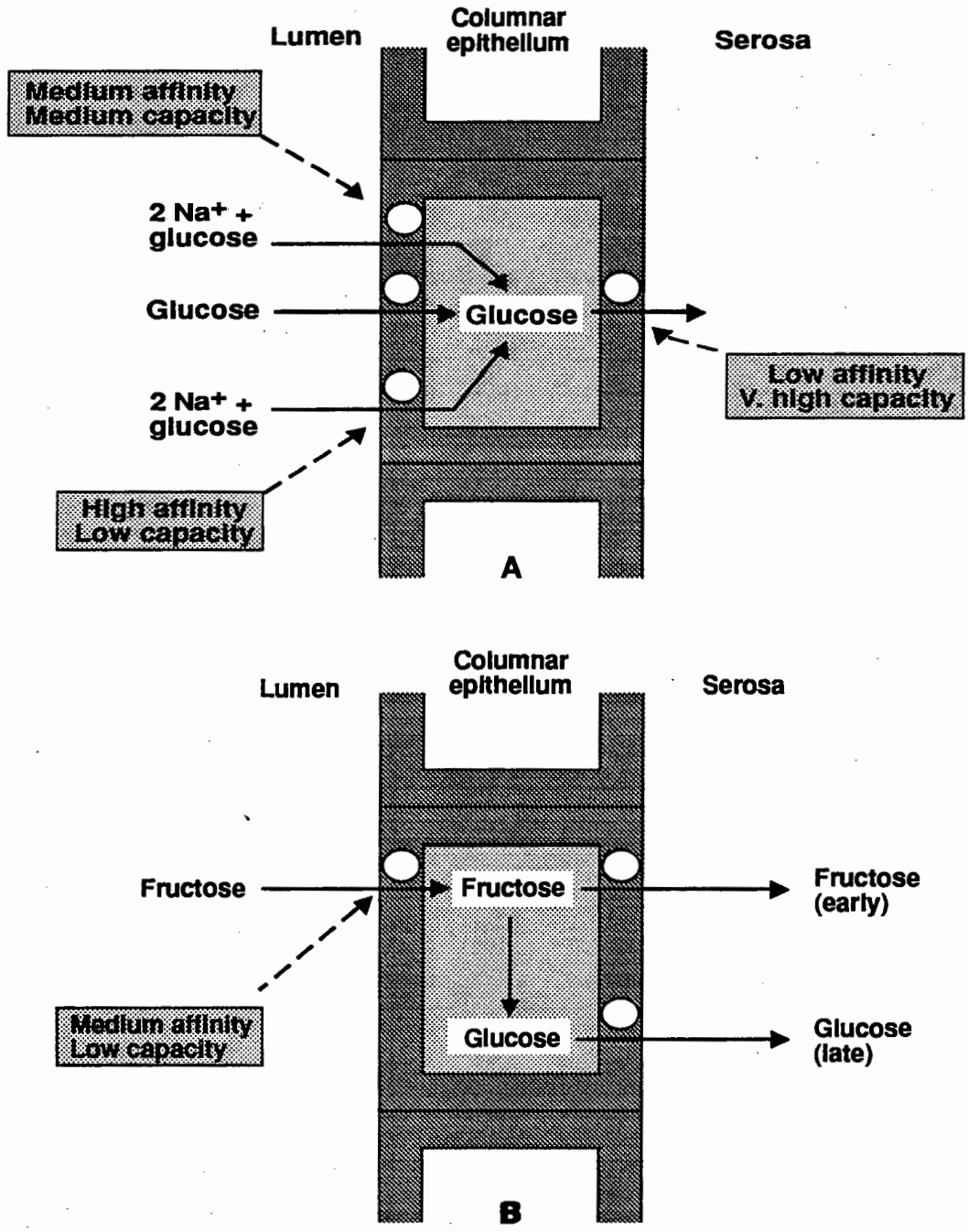

Figure 2.3. A scheme to illustrate **(A)** the glucose transporters and **(B)** the fructose transporters in the columnar epithelium of the small intestine. The presence of an 'unstirred water layer' complicates measurements of the binding affinities of these transporters for glucose. Unlike the transport of glucose **(A)** the transport of fructose **(B)** is not dependent on sodium.

2.4 Methods of quantifying ingested carbohydrate oxidation during prolonged, moderate-intensity exercise

2.4.1. Respiratory exchange ratio

Traditionally, attempts to quantify whole body substrate oxidation during prolonged, steady-state exercise have relied upon indirect calorimetry. For nearly a century it has been recognised that measurements of inspired oxygen consumption (VO_2) and expired CO_2 production (VCO_2) can yield information on the type and rate of fuel oxidation within the body (Lusk 1924, 1928). Such techniques are based upon the underlying assumption that VO_2 and VCO_2 measured in the expired air accurately reflect gas exchange at the tissue level (Ferrannini 1988; Frayn 1983; Péronnet and Massicotte 1991).

Estimates of the type of fuel oxidised are obtained from the RER, which is the ratio of CO_2 production to O_2 consumption (VCO_2/VO_2). When glucose is oxidised according to the equation:

$$\text{glucose } (C_6H_{12}O_6) + 6\ O_2 \longrightarrow 6H_2O + 6CO_2 \qquad \text{(Eq. 1)}$$

six moles of O_2 are consumed and six moles of CO_2 produced for each mole of hexose equivalent (180 g) oxidised. The RER value is thus 1.00. This value differs only slightly if CHO other than simple hexoses are oxidised (Frayn 1983).

Oxidation of a typical triglyceride, palmitoyl-stearoyl-oleoyl-glycerol (PSOG) proceeds according to the equation:

$$\text{PSOG } (C_{55}H_{104}O_6) + 78O_2 \longrightarrow 55CO_2 + 52H_2O \qquad \text{(Eq. 2)}$$

These reactions consume 78 moles of O_2 and produce 55 moles of CO_2 for each mole of triglyceride (861 g) oxidised. Thus, the RER value is 0.70.

RER values, however, have to be interpreted with caution. Firstly there is the uncertainty of the contribution of amino acid oxidation to VO_2 and VCO_2 during prolonged exercise (Wolfe et al. 1984a). Secondly, there is the question of the extent to which the rise in VCO_2 with increasing VO_2, especially during progressive exercise, is a respiratory compensation for the metabolic acidosis, displacing the plasma $H^+ + HCO_3$ $CO_2 + H_2O$ equilibrium to the right (Barstow et al. 1990; Newsholme and Leech 1983). Finally, an underlying assumption of indirect calorimetry is that the gas exchange resulting from metabolic processes such as gluconeogenesis, lipogenesis and ketogenesis, is negligible (Ferrannini 1988; Frayn 1983).

When steady-state conditions are attained during exercise, gas exchange measurements can be used to estimate the total CHO oxidised at any time, using the formula described by Consolazio et al. (1963):

Total CHOox = 4.55 VCO_2 - 3.21 VO_2 **(Eq. 3)**

where total CHOox is the overall carbohydrate oxidation in g/min; VCO_2 is the volume of CO_2 in the expired air measured in L/min, and VO_2 is the O_2 uptake during the same period measured in the same units.

2.4.2. Naturally labelled ^{13}C isotopes

RER values, however, provide no indication of the sources of glucose being oxidised (i.e. muscle or liver glycogen, gluconeogenesis, or glucose derived from CHO ingested before or during the experimental observation period). To measure specifically the rate of oxidation of ingested CHO, isotopes must be added to the food or drink, or specific foods can be ingested which contain a higher than normal proportion of a particular isotope (Lefèbvre 1985, 1986). One such isotope, 13-carbon (^{13}C), is in sugars from C_4 plants. In natural compounds, about 1.1% of carbon present is ^{13}C whereas, ~99% is in the form of ^{12}C (Gautier et al. 1993; Wolfe and George 1993). The enrichment of ^{13}C in the photosynthesis of C_4 plants, such as sugar cane, corn and sorghum, arises from the

indirect, rather than the direct, fixation of CO_2 into 3-phosphoglycerate via oxaloacetate in bundle-sheath cells (Lefèbvre 1985).

When a glucose tracer (^{13}C or ^{14}C) is used, the flux of carbon atoms from the tracer to expired CO_2 enables the rate of ingested CHO oxidation to be *estimated*. Investigations using naturally labelled ^{13}C glucose have been conducted by several groups (Coggan et al. 1990, 1991, 1992; Gerard et al. 1986; Jandrain et al. 1984, 1989; Krzentowski et al. 1984a, 1984b; Lefèbvre 1979, 1986; Lefèbvre et al. 1975, 1986; Massicotte et al. 1986, 1989, 1990, 1992; Pallikarakis et al. 1986; Péronnet et al. 1990; Pirnay et al. 1977a, 1977b, 1982; Rehrer et al. 1992; Romijn et al. 1992a, 1992b; Wolfe et al. 1984a, 1984b, 1984c, 1986, 1988). The general principle underlying the use of ^{13}C labelled substrates to quantify oxidation is that the rate of excretion of $^{13}CO_2$ is divided by the isotopic enrichment of the precursor (Wolfe and George 1993). Rates of ingested CHO oxidation can then be calculated from the VCO_2 and the $V^{13}CO_2/V^{12}CO_2$ ratio using the equivalent version of equation four (section 2.4.3).

Compared to the ^{14}C isotope procedure (described later in section 2.4.3), ^{13}C studies have the advantage that the isotope is naturally occurring and subjects are not exposed to radioactivity and so they can be repeatedly tested. There are, however, several disadvantages. One is the relatively low natural $^{13}C/^{12}C$ enrichment that necessitates corrections for the ^{13}C that is present in all energy-yielding substrates (Schoeller et al. 1977, 1980; Smith and Epstein 1971), particularly in North American subjects. Whereas in Western Europe the ^{13}C content of the diet is relatively low, in North America, cattle are fed maize, and hence beef and dairy products contain higher than normal $^{13}C/^{12}C$ ratios (Nakamura et al. 1982; Péronnet et al. 1992). This phenomenon is demonstrated by the higher $^{13}C/^{12}C$ isotopic ratios in CO_2 expired by North Americans versus Western Europeans (Schoeller et al. 1977).

As the ^{13}C enrichment of CHO is normally greater than that of fat (Jacobsen et al. 1970; Schoeller et al. 1980, 1984), any shift in the pattern of fuel utilisation will affect $^{13}CO_2$

production and thus complicate measurements of ingested CHO oxidation (Barstow et al. 1989; Coggan et al. 1990; Schoeller et al. 1984; Wolfe et al. 1984b). Uncertainties surrounding the degree to which CHO utilisation shifts from endogenous to ingested CHO during exercise itself also limits the accuracy of the correction of $^{13}CO_2$ measurements for ^{13}C background values (Péronnet et al. 1990; 1993; Robert et al. 1987; Wolfe et al. 1984b). Thus, studies which have attempted to measure the oxidation of ingested CHO by feeding exercising subjects with CHO that is naturally enriched with ^{13}C, *may* have overestimated the rate of oxidation (Péronnet et al. 1990). Comparisons among such data should, therefore, perhaps be regarded as more directional than absolute. Finally, there must be no influence of the bicarbonate kinetics on the observed CO_2 enrichment (i.e. whatever $^{13}CO_2$ is produced at the cellular level should be immediately and quantitatively reflected in the breath). This issue is discussed further in section 2.4.3.

2.4.3. ^{14}C Radioactive isotopes

An alternative method of measuring the rate of ingested CHO oxidation during exercise is to label the ingested CHO with the ^{14}C (or ^{3}H) radioactive isotope and use scintillation counting to monitor the appearance of ^{14}C in the expired CO_2. Depending on the isotope administered, rates of ingested CHO oxidation are determined using the following formula:

$$\textbf{Ex } CHO_{ox} = (^{14}CO_2 \cdot 6/((SA_{CHO})) \cdot VCO_2 \cdot 1.35 \qquad \textbf{(Eq. 4)}$$

In this equation, Ex. CHO_{ox} is the rate of ingested CHO oxidation in g/min; $^{14}CO_2 \cdot 6$ is the $^{14}CO_2$ dpm/mmol value multiplied by six, as there are six carbon atoms per molecule of monosaccharide absorbed into the blood; SA_{CHO} is the specific radioactivity of the ingested solution in dpm/mmol; VCO_2 is the volume of expired CO_2 in L/min; and 1.35 is the number of grams of hexose oxidised to produce one litre of CO_2.

This calculation requires steady-state labelling of glucose and expired CO_2, conditions which are difficult to achieve unless the exercise bout is relatively long (> 60-75 min).

Further, it is desirable to ingest (or infuse) the tracer after the start of exercise, and not at rest, as this minimises the possibility of incorporation of the label into liver and muscle glycogen stores (Sonne and Galbo 1985).

The advantage of using the ^{14}C isotope, rather than the ^{13}C isotope is that there is virtually no naturally occurring background level of ^{14}C that must be accounted for when calculating ingested CHO oxidation (Péronnet et al. 1992; Wolfe et al. 1984b). Additionally, the amount of ^{14}C which occurs in food, and thus in the endogenous substrate pools, is negligible when compared to the amount of tracer administered and recovered. Further, the appearance of ^{14}C in the plasma can be used to estimate the rates of ^{14}CHO appearance in the bloodstream which is an *indirect* measurement of the rate of CHO digestion and absorption. It is only possible to determine the appearance of ingested ^{13}C-labelled substrates in venous blood if the ^{13}C enrichment is very high, or during resting conditions (Normand et al. 1992). Of course, an obvious disadvantage of using ^{14}C is that it exposes the subject to radioactivity. The amounts of radioactivity, however, are minimal. Generally, less than 40 uCi/L is consumed (Costill et al. 1973; Moodley et al. 1992; Van Handel et al. 1980) which corresponds to a dose of approximately 0.02 rem. Permissible radiation doses for humans are 5.0 rem/yr or 3.0 rem for any three-month period (Wang 1969).

As recently pointed out by Coggan and Coyle (1991) and Wolfe and George (1993), studies using ^{14}C-glucose *may* still underestimate the extent to which plasma glucose is oxidised during exercise, because of a slow equilibration of $^{14}CO_2$ with the bicarbonate pool. Any $^{14}CO_2$ (or $^{13}CO_2$) produced at the cellular level must transit through the bicarbonate pool before exiting in the breath. The bicarbonate/carbonate pool in the body corresponds to ~130 L of CO_2. Of this amount, approximately 100 L of carbon dioxide is in the form of calcium carbonate found in bone (Irving et al. 1983). The bone carbonate pool constitutes a very slow exchanging pool, the mean residence time of a CO_2 molecule entering this pool being approximately three days (Péronnet et al. 1992). Because of the

skeleton acting as a CO_2 'sink' at rest, the recovery of $^{13}CO_2$ or $^{14}CO_2$ from the oxidation of labelled substrates is about 80% (Hoerr et al. 1989).

During exercise a slow equilibration of labelled CO_2 with the body bicarbonate pool is thought to result in an under-estimation of the rates of ingested ^{13}C and ^{14}C labelled substrate oxidation (Coggan 1991; Coggan and Coyle 1991; Wolfe and George 1993; Wolfe et al. 1984c). Lags in the appearance of $^{14}CO_2$ in the breath following ingestion of ^{14}C labelled substrates is a function of both their rate of oxidation and of the turnover kinetics of the CO_2-HCO_3^- pools (Winchell and Wiley 1970). Exercise increases the equilibration rate of the CO_2-HCO_3^- pool (Slanger et al. 1970), with the duration of this lag phase dependent on the intensity of steady-state exercise (Barstow et al. 1989; Pirnay et al. 1977a; Ravussin et al. 1979). At moderate to high (i.e. 2.5 L/min) rates of CO_2 production, the rapid turnover of the CO_2 in the body bicarbonate pool is thought to have minimal effect on the estimation of ingested CHO oxidation (Bosch et al. 1993c; Mazzeo et al. 1986).

Indeed, it can be predicted from the calculated flux of CO_2 through the body bicarbonate stores that a more or less complete equilibrium of the bicarbonate/CO_2 pool is attained in ~20-30 min (Bosch et al. 1993b). In a 70 kg male, body water content is ~40 L, of which 25 L are extracellular fluid containing 24 mmol/L bicarbonate, and 15 L are intracellular water containing ~10 mmol/L bicarbonate. Therefore, total body bicarbonate stores are ~775 mmol, which corresponds to 17.5 L of CO_2. As 80-85% of the CO_2 produced is carried to the lungs as bicarbonate, a typical work rate eliciting an exercising VCO_2 of 2.5 L would turn over 2.1 L of CO_2 from bicarbonate. Thus, most of the bicarbonate at such an exercise intensity should turn over in 17.5/2.1 L/min, i.e. ~8 min (Bosch et al. 1993b). In well trained subjects ingesting ^{13}C-CHO and exercising at 80-85% of VO_{2max}, ratios of $^{13}C/^{12}C$ in expired air have been shown to reach equilibrium within 15-20 min (Romijn et al. 1992).

In contrast, however, Coggan et al. (1990, 1991) have reported that a true plateau in $^{13}CO_2$ production is not reached until after 80-90 min of moderate intensity exercise (60-70% VO_{2max}). Others have also found that expired $^{14}CO_2$ lags behind the appearance of the ^{14}C labelled products in venous blood (Benade et al. 1973; Costill et al. 1983; Van Handel et al. 1980). Taken collectively, the findings of these studies suggest that the time taken to reach an equilibrium may be longer than earlier reported (Costill et al. 1973). However, it should be noted that the appearance of ^{14}C blood glucose, which is not affected by the bicarbonate pool, closely tracks the production of $^{14}CO_2$ (Bosch et al. 1993a). Thus, it is highly unlikely that any lag in the appearance of $^{14}CO_2$ could be attributed *solely* to the retention of $^{14}CO_2$ in the bicarbonate pool. Further, as emphasised by Massicotte et al. (1989), the potential lag in carbon dioxide expiration produced by the equilibration of $^{13}CO_2$ or $^{14}CO_2$ with the bicarbonate pool is similar from trial to trial and unlikely to vary greatly amongst individuals. As recently noted "*recognizing the limitations in quantifying the oxidation of a labelled substrate (given orally) does not eliminate the use of such compounds to make qualitative or comparative observations*" (Wolfe and George 1993).

2.5 Oxidation of glucose ingested during prolonged, moderate-intensity exercise

The first study to investigate the contribution of ingested U-^{14}C glucose to the energy demands of prolonged exercise in humans was that of Young et al. (1967). These workers examined the rate of plasma glucose turnover and oxidation in healthy male subjects during 4.5 hr of intermittent treadmill walking at approximately 33% of VO_{2max} after a single bolus injection of U-^{14}C-labelled glucose. The highest specific activity of the respiratory $V^{14}CO_2$ was observed 60 min after the administration of the isotope, and by the end of the exercise ~82% of the ingested U-^{14}C glucose had been recovered as $V^{14}CO_2$.

Later, Costill et al. (1973) studied glucose oxidation in seven men who ran or cycled at 60-72% of VO_{2max} for 30 min before, and 60 min after the ingestion of 32 g of U-^{14}C labelled glucose. The initial appearance of ^{14}C in the expired CO_2 occurred five to seven

min after ingestion, but the levels of radioactivity remained relatively low for the remainder of the exercise bout. Calculated rates of ingested CHO oxidation accounted for only five percent of total CHO oxidation during the 60 min of exercise. Costill et al. (1973) therefore concluded that while glucose feedings during prolonged exercise *may* conserve hepatic glycogen, they were "*of limited importance for muscle metabolism*". It has subsequently been shown that CHO ingestion during prolonged exercise does indeed reduce the rate of hepatic glycogenesis (Bosch et al. 1993a, 1993c), but glucose feedings are also an important energy source for muscle metabolism during prolonged moderate intensity exercise (see section 2.1).

In a later study from the same laboratory, low rates of ingested glucose oxidation were also reported (Van Handel et al. 1980). These workers had six well trained subjects cycle at 50% of VO_{2max} for 180 min on two separate occasions. After 120 min, subjects ingested either 10 g or 42 g loads of U-^{14}C-labelled glucose in 400 ml of water, and then cycled for a further 60 min. When subjects ingested the 10 g load the estimated contribution of ingested glucose to total circulating blood glucose reached 37% some 30 min after ingestion. Sixty min after the 42 g feeding, the contribution of ingested glucose to total circulating blood glucose reached 67%. However, by the end of exercise, less than 10% of either the 10 g or 42 g feedings had been recovered as expired $^{14}CO_2$. Van Handel et al. (1983) therefore concluded that "*as long as endogenous carbohydrate remains available, glucose feedings seem to be of limited importance for the maintenance of muscle metabolism in highly trained athletes.*" They also hypothesised that "*most of the ingested glucose (probably) remains in compartments within an unoxidized glucose pool*". The results of these latter two studies, however, are the exception as other workers using a variety of different experimental protocols have subsequently found much higher rates of ingested glucose oxidation during exercise.

2.5.1 Effects of feeding schedule

Pirnay et al. (1977a, 1977b) were the first group to show that a significant quantity of ingested glucose is oxidised during exercise (Table 2.1). They fed seven untrained, fasted subjects 100 g of ^{13}C-labelled glucose diluted in 400 ml of water; four subjects then walked on a treadmill for two hr and three subjects exercised for almost four hr. Measurements of expired $^{13}CO_2$ revealed that peak rates of ingested glucose oxidation (0.65 g/min) occurred 75-90 min after ingestion. After 120 and 225 min of exercise, 57 and 95 g respectively of the 100 g glucose load had been oxidised. Ingested glucose contributed 55% of total CHO oxidation after 90-120 min of exercise. At this time the oxidation of ingested glucose started to decline. After four hr of low-intensity exercise, almost the entire quantity (95 g) of the ingested glucose (100 g) had been oxidised. Thus, these workers concluded that "*it would be advisable to repeat glucose intake every 60-90 minutes for permitting long-duration muscular exercise*".

Pirnay et al's (1977a; 1977b) findings were subsequently confirmed by other groups (Jandrain et al. 1984, 1989, 1993; Krzentowski et al. 1984a; 1984b). All these groups showed that the peak rates of ingested glucose oxidation (0.56-0.65 g/min) occurred 75-90 min after ingestion of 100 g of glucose (Table 2.1). Further, the time taken to attain the peak rate of ingested glucose oxidation was found to be independent of when the glucose was ingested during exercise (Krzentowski et al. 1984a) or whether it was consumed as a dilute (439 mosmol/L) or concentrated (1,204 mosmol/L) solution (Jandrain et al. 1989).

Rates of ingested glucose oxidation also appear to be relatively unaffected by the use of different feeding schedules. Comparison of the single (50-100 g) glucose feeding studies (Decombaz et al. 1985; Guezennec et al. 1989; Jandrain et al. 1984, 1989; Krzentowski et al. 1984a; Pirnay et al. 1977b) to the multiple (97-220 g) glucose feeding studies (Massicotte et al. 1986, 1989, 1990, 1992), show that the amount of ingested glucose oxidised in the first hr of exercise, and the peak rates of ingested glucose oxidation are quite similar. With most of the feeding schedules, around 20 g of ingested glucose was

oxidised in the first hour and, in all cases, the peak rates of ingested glucose oxidation were between 0.5 and 0.9 g/min (Table 2.1).

Only following the repetitive ingestion of very large amounts of glucose (400 g), and after four hr of exercise have peak rates of ingested glucose oxidation been observed to rise above 1 g/min. Pallikarakis et al. (1986) studied six active males who walked uphill on a treadmill at a ~45% of VO_{2max} for 285 min. After 15 min 'adaptation' to exercise, subjects received either 200 g or 400 g of a 0.25 g/ml ^{13}C- labelled glucose solution in eight equal doses every 30 min. When subjects ingested the 200 g glucose load, the rate of ingested glucose oxidation rose progressively during the first 120 min of exercise to plateau at 0.66 g/min. In contrast, after subjects were fed the 400 g glucose load, rates of glucose oxidation continued to rise during the exercise period reaching values of 1.16 g/min at the end of the trial. The contribution of ingested CHO oxidation to total CHO oxidation was greater after the ingestion of the 400 g glucose load (39%) than after the 200 g feeding (22%). Thus, under the conditions of that study, increasing the ingestion of glucose from 25 g/30 min to 50 g/30 min increased both the peak rate of ingested glucose oxidation and the contribution of ingested glucose to the total CHO requirements of exercise (Pallikarakis et al. 1986).

Since repetitive feedings would be expected to accelerate the delivery of glucose from the stomach to the duodenum (Mitchell and Voss 1991; Noakes et al. 1991a), the similar peak rates of ingested glucose oxidation after single and multiple feedings of moderate glucose loads (50-200 g) suggest that ingested CHO oxidation may not be limited by gastric emptying, as was proposed (Costill and Saltin 1974; Houmard et al. 1991; Wheeler and Banwell 1986). Instead, studies of both gastric emptying and ingested glucose oxidation suggests that the oxidation of glucose in the early (60-80 min) stages of exercise is limited by either its rate of absorption from the intestine via the liver into the systemic bloodstream, or the capacity of the muscles to oxidise glucose (Moodley et al. 1992; Rehrer et al. 1992).

Table 2.1 Oxidation of ingested glucose during prolonged exercise.

Ingestion protocol (g in ml)	Exercise intensity (% VO_{2max})	Ing. CHO_{ox} in 1st hour (g)	Ing. CHO_{ox} total (g in min)	% of all CHO_{ox}	peak rate (g/min)	Reference
Single feedings						
50/200	45	12	43/240	18	0.48	Jandrain et al. (1989)
50/400	45	14	43/240	17	0.48	Jandrain et al. (1989)
50/600	45	15	49/240	18	0.47	Jandrain et al. (1989)
70/350	61	26[a]	26/60		0.45	Decombax et al. (1985)
100/400	40	10	54/120	41	0.63	Krentowski et al. (1984a)
100/400	45	29[b]	68/240	27	0.63	Jandrain et al. (1984)
100/400	50	21	57/180	43	0.65	Pirnay et al. (1977a)
100/400	60	15	67/120		0.56	Guezennec et al. (1989)
100/400	50	24	95/225	39	0.65	Pirnay et al. (1977b)
Multiple feedings						
97/6 x 232	50	18	56/120	28	0.50	Massicotte et al. (1990)
99/6 x 232	50	28	70/120	28	0.58	Massicotte et al. (1989)
140/9 x 225	50	21	106/180	38	0.83	Massicotte et al. (1986)
200/8 x 100	45	11	137/270	40	0.66	Pallikarakis et al. (1986)
400/8 x 100	45	22	227/270	68	1.16	Pallikarakis et al. (1986)

Ing. CHO_{ox}, ingested carbohydrate oxidation; [a], glucose was ingested one hour prior to exercise; [b], glucose was ingested three hours prior to exercise;. Multiple feedings (400/8 x 100), 400 g in 8 feedings of 100 ml per feeding.

Rehrer et al. (1992) studied eight trained male subjects who ingested 220 g of a 17.5 g/100 ml ^{13}C-labelled glucose during 80 min of cycling at 70% of VO_{2max}. The glucose was ingested together with 20 mEq/L of sodium chloride as a 600 ml loading bolus at the start of the 80 min exercise bout, followed by three 228 ml feedings after 20, 40 and 60 min. Rehrer et al. (1992) found that although 120 g of glucose was delivered to the duodenum, only 38 g (32%) was oxidised. Further, because ~100 g of glucose was still in the stomach at the end of exercise, only 17% of the ingested glucose load was oxidised after 80 min. The peak (0.78 g/min) rate of ingested CHO oxidation from the 15% glucose solution was observed during the last 15 min of exercise and is similar to values reported in other studies (Massicotte et al. 1986; Wagenmakers et al. 1990). Rehrer et al. (1992) concluded that "*gastric emptying is not absolutely limiting for exogenous carbohydrate oxidation*".

Similar results have also been reported by Moodley et al. (1992). They measured gastric emptying and ingested glucose oxidation in subjects who consumed 90 g of U-^{14}C labelled glucose as nine 100 ml feedings while cycling at 70% of VO_{2max} for 90 min. Again, whereas 49 g of glucose was delivered to the duodenum, only 16 g (33%) was oxidised. Thus, in both Rehrer et al's. (1992) and Moodley et al's (1992) studies, only a third of the glucose emptied from the stomach was oxidised during 80-90 min of moderate-intensity exercise. Moodley et al. (1992) speculated "*that most of the ingested carbohydrate probably remains in the intestine, at least during the first 90 minutes of exercise.*"

A question yet to be addressed is whether the low rates of ingested CHO oxidation reported by Rehrer et al. (1992) and Moodley et al. (1992) are due to the time taken for CHO digestion and absorption, or, to the lack of demand for CHO by the working muscles.

2.5.2. Effects of glycogen depletion and fasting

Evidence to suggest that ingested CHO oxidation may not be limited by the muscles demand for glucose comes from investigations which have examined the effects of glycogen depletion (Ravussin et al. 1979) and fasting (Massicotte et al. 1990) on rates of ingested CHO oxidation during prolonged exercise. These states of CHO depletion did not increase the reliance on ingested CHO during exercise.

Ravussin et al. (1979) studied ingested CHO oxidation during prolonged, low-intensity exercise in untrained, (muscle) glycogen depleted and control subjects. One hr after they had ingested 100 g of ^{13}C-labelled glucose in 300 ml of water, subjects cycled for 120 min at 40% of VO_{2max} (97-107 W) in either a glycogen depleted or normal (control) state. Under both conditions ingested glucose oxidation reached a peak after 75 min of exercise. Further, during the 120 min exercise bout, oxidation of ingested glucose was the same for control and glycogen depleted subjects (41 g versus 38 g respectively), although, as would be expected, these amounts accounted for vastly different contributions to total CHO oxidation (37% for controls versus 72% for glycogen depleted subjects).

Ravussin et al. (1979) concluded that "*depletion of the (muscle) glycogen stores does not improve the utilization rate of ingested glucose*". However, it should be noted that at such low intensity workloads (40% of VO_{2max}), fat, and *not* CHO oxidation accounted for most of the energy demands of exercise.

Massicotte et al. (1990) studied the effects of fasting (15 hr) on ingested glucose oxidation in ten subjects during two hr of cycling at 52-56% of VO_{2max}. Subjects ingested ~100 g of ^{13}C labelled glucose dissolved in 230 ml of water given as six feedings every 20 min throughout the exercise bout. The amount of glucose oxidised during exercise was the same under both experimental conditions (fed state 56 g; fasted 58 g).

The results of the studies of Ravussin et al. (1979) and Massicotte et al. (1990) are surprising, in that one might have expected glycogen depletion and fasting to have

increased plasma glucose oxidation and therefore ingested CHO oxidation. Thus, these two investigations suggest that either CHO digestion or absorption, *or* liver glucose release, *or* muscle glucose uptake may ultimately limits the oxidation of glucose ingested (before or) during exercise.

The results of these studies are summarised in Table 2.2.

Table 2. 2. The effects of glycogen depletion and fasting on ingested glucose oxidation during prolonged exercise.

Ingestion protocol (g in ml)	Exercise intensity (% VO_{2max})	Ing. CHO_{ox} in 1st hour (g)	Ing. CHO_{ox} total (g in min)	% of all CHO_{ox}	Condition	Reference
100/1 x 300	40	—	41/120	29	Control	Ravussin et al. (1979)
100/1 x 300	40	—	38/120	62	Depleted	Ravussin et al. (1979)
97/6 x 232	52	13	56/120	28	Control	Massicotte et al. (1990)
47/6 x 232	52	22	58/120	34	Fasted	Massicotte et al. (1990)

Ing. CHO_{ox}, ingested carbohydrate oxidation; depleted, muscle glycogen depletion; fasted, 15 hour overnight fast.

2.5.3. Effects of exercise intensity

During exercise of low intensity, the rate of skeletal muscle glucose oxidation may limit the rate of ingested glucose oxidation. To investigate the influence of workload on the utilisation of ingested glucose, Pirnay et al. (1982) studied four subjects who ingested 100 g of naturally enriched ^{13}C glucose in 400 ml of water and then exercised for 90 min at intensities ranging from 22-64% of VO_{2max}. Ingested glucose oxidation peaked after 75 min irrespective of exercise intensity, and then tended to plateau for the remainder of the experiment. There was a linear relationship between the amount of ingested glucose oxidised and the relative workload up to work-rates of 51% of VO_{2max} ($r = 0.81$, $P < 0.01$). However, at higher exercise intensities, the amount of ingested glucose oxidised failed to increase further (Table 2.3). These workers concluded that "*at high intensity (> 51% of VO_{2max}) levels of exercise the metabolic availability of glucose (ingested during exercise) cannot keep pace with the energy requirements. Whether this represents a 'true' block in the muscles ability to oxidise (the) ingested glucose or simply reflects an inability of the rate of gastrointestinal absorption to keep pace with the metabolic demands of the working muscle is not clear...*" Consequently, Pirnay et al. (1982) speculated that "*the rate of exogenous glucose oxidation may be regulated by local influences at the level of the muscle cells.*"

Bosch et al. (1993c) recently investigated the effects of CHO ingestion (a 10 g/100 ml U-^{14}C labelled glucose solution) on fuel substrate kinetics during three hr of cycling at 55% of VO_{2max}. Ingested CHO oxidation peaked at 0.91 g/min at the end of exercise which was similar to the peak rate of plasma glucose oxidation (0.98 g/min). These findings are comparable to the value reported for ingested CHO oxidation and plasma glucose oxidation during three hr of cycling at 70% of VO_{2max} (Bosch et al. 1993a).

Péronnet et al. (1992) have suggested that the single most important factor which determines the rate (g/min) of ingested ("*exogenous*") CHO oxidation is the *absolute* workload during exercise for that individual, expressed as VO_2 in L/min. They have shown that in a number of studies using ^{13}C-labelling when feeding different types of

CHO (glucose, fructose, sucrose, maltodextrins, glucose polymers and starch) with doses ranging from 50 up to 400 g, administered before or during exercise, that there is "*a good correlation between VO_2 and exogenous CHO oxidation rate (r = 0.617).*"

However, it is, perhaps, a gross oversimplification of glucose kinetics to try and estimate the oxidation rates of *all* ingested CHO solely from VO_2 values obtained during exercise.

Firstly, there is a large dispersion of the data points around their computed regression line (for example at a VO_2 of 2.0 L/min the rates of ingested CHO oxidation range from 0.3 g/min to over 0.8 g/min).

Secondly, a zero-order correlation coefficient of 0.617 between VO_2 (L/min) and the oxidation rate of ingested ("*exogenous*") CHO (g/min), while being statistically significant, is of dubious physiological importance and indeed shows that VO_2 (L/min) values can only explain 38% of the variation in the rates of ingested CHO oxidation (g/min).

Thirdly, utilising the regression equation of Péronnet et al. (1992), the average oxidation rate of *any* CHO would never exceed 0.63 g/min for a given VO_2 of 3.3 L/min. Clearly this is a large underestimation as both data from their own laboratory (Massicotte et al. 1986) and a number of other independent groups (Moodley et al. 1992; Pallikarakis et al. 1986; Rehrer et al. 1992; Saris et al. 1993) report rates of ingested CHO oxidation exceeding 0.63 g/min at either similar or *lower* exercise intensities than 3.3 L/min.

Finally, the relationship between the rate of ingested CHO oxidation and power output is based on studies which have employed solely ^{13}C labelling and, for the most part, have used moderate to low exercise intensities (50 to 60% of VO_{2max}) where fat and not CHO would provide most of the energy for oxidative metabolism.

Table 2.3 The effects of exercise intensity on ingested glucose oxidation.

	Ing. CHO_{ox}	
Exercise intensity (% VO_{2max})	**(g)**	**% of all CHO_{ox}**
22	16	31
39	33	30
51	41	30
64	44	22

Ing. CHO_{ox}, ingested carbohydrate oxidation. Subjects consumed 100 g of glucose in 400 ml of water. The duration of exercise was 75 minutes. Data are from Pirnay et al. (1982).

2.5.4. Effects of glucose load

Rehrer et al. (1992) investigated the effects of two different glucose loads on the rates of ingested CHO oxidation and gastric emptying during 80 min of cycling at 70% of VO_{2max}. Subjects ingested either 58 g or 220 g of ^{13}C-labelled glucose as a 600 ml loading bolus at the start of exercise and thereafter as three 228 ml feeding every 20 min for the first 60 min of exercise. At the end of exercise 55 g (95%) of the 58 g feeding had been delivered to the intestine, whereas 133 g (60%) of the 220 g load had been emptied. Somewhat surprisingly, the amount of ingested CHO oxidised during exercise was not statistically different between the 4.5 g/100 ml glucose solution (31 g) and the 17.5 g/100 ml drink (42 g). However, there was a significant beverage by time interaction effect (P

<0.01) when an analysis of variance was performed on the amounts of ingested CHO oxidised during each 15 min period of the 80 min exercise bout. This result indicated that as the exercise time increased, the contribution of ingested CHO to total CHO utilisation was greater with the 17.5 g/100 ml drink than with the 4.5 g/100 ml drink.

In contrast, Moodley et al. (1992) reported that the rate of ingested CHO oxidation during 90 min of cycling at 70% of VO_{2max} rose significantly when the concentration of ingested U-^{14}C-labelled glucose was increased from 7.5 g/100 ml to 15 g/100 ml. The higher rates of ingested CHO oxidation was the result of both an increased rate of intestinal CHO delivery and an increase in the proportion of the CHO delivered to the intestine that was oxidised.

2.6 Oxidation of carbohydrates other than glucose during prolonged, moderate-intensity exercise

2.6.1. Fructose

Since glucose ingestion prior to exercise inhibits lipolysis by increasing plasma insulin concentrations (Ahlborg and Felig 1976; Galbo et al. 1979), there has been some interest in the use of fructose as an alternate source of CHO. Compared to glucose ingestion, fructose ingestion produces a 20 to 30% lesser rise in plasma insulin concentrations (Koivisto 1978; Koivisto et al. 1981).

Decombaz et al. (1985) were the first workers to investigate the oxidation of ingested fructose using isotopic techniques. They compared the effects of the ingestion of 70 g of ^{13}C labelled fructose and glucose on ingested CHO oxidation during moderate intensity exercise. Subjects were fed either fructose or glucose in 350 ml of water one hr prior to a 60 min ride. For the first 45 min of exercise subjects cycled at 61% of VO_{2max}. They were then told to "*work as hard as possible*" for the next 15 min. Two hr after ingestion, 30 g (43%) of the 70 g fructose feeding had been oxidised to $^{13}CO_2$. This value was similar to the amount of ingested glucose oxidised (26 g) over the same period. The amount of work produced during the last 15 min of the exercise bout did not differ

between the two types of CHO. These findings indicate that fructose (and glucose) ingested *before* exercise were oxidised *during* exercise at the same rates.

Data from other studies, however, indicate that the rate of oxidation of ingested fructose is slower than that of ingested glucose during two to three hr of moderate intensity (52-55% of VO_{2max}) exercise (Guezennec et al. 1989; Jandrain et al. 1993; Massicotte et al. 1986, 1989), at least in the fed state. Massicotte et al. (1986) reported that the rates of ingested CHO oxidation from ^{13}C-labelled fructose solutions ingested as multiple feedings during 180 min of cycling at 50% of VO_{2max} were significantly lower than from isocaloric ^{13}C-labelled glucose solutions (glucose 106 g; fructose 79 g). This same group subsequently compared the oxidation of 100 g of ^{13}C-labelled fructose, ingested every 20 min during two hr of cycling at 53% of VO_{2max}, with the oxidation of equicaloric ^{13}C-labelled glucose and ^{13}C-labelled glucose polymer solutions. Again, they found that the oxidation of fructose during exercise (54 g) was significantly less than for glucose (72 g). Rates of ingested CHO oxidation from fructose were also lower than from the glucose polymer solution (54 g versus 65 g respectively).

Guezennec et al. (1989) compared the oxidation of naturally enriched ^{13}C fructose with glucose and corn starch ingested as one feeding (100 g in 400 ml of water) 60 min before two hr of cycling at 60% of VO_{2max}. Over the two hr of exercise, the oxidation of ingested fructose (54 g) was significantly less than both glucose (67 g) or corn starch (66 g). In agreement with Massicotte et al. (1986, 1989) and, more recently, Jandrain et al. (1993), these workers concluded that "*exogenous fructose is less available for oxidation than glucose (or corn starch)*."

In contrast, Massicotte et al. (1990) have recently reported that the initial rates of ingested fructose oxidation are only slower than those of glucose when subjects start exercise in the fed state. These workers compared the effects of a 15 hr fast on the oxidation of 100 g of fructose or glucose ingested as repetitive feedings (six feedings of 16 g/feeding in 232 ml of water/20 min of exercise) throughout 120 min of cycling at 52% of VO_{2max}. During

the first hr of exercise the amount of ingested fructose oxidised was threefold greater in fasted than in fed subjects. The utilisation of ingested fructose was, however, similar during the second hr of exercise. Over the two hr exercise bout, the energy contribution from ingested fructose represented 31% of total CHO oxidation compared to 20% in the fed state. Fasting did not influence the amount of ingested glucose oxidation under the conditions of this experiment. Thus, when hepatic gluconeogenesis is accelerated by fasting (Dohm et al. 1986), glucose formed from fructose in mainly the liver appears to be oxidised as rapidly as glucose absorbed directly from the gut.

2.6.2. Sucrose

Benade et al. (1973) were the first to describe the effects of sucrose ingestion during prolonged exercise. They had four trained subjects cycle for six hr (50 min exercise followed by ten min rest each hr) at 44-50% of VO_{2max}. After four hr subjects ingested 100 g of U-^{14}C-labelled sucrose in 400-500 ml of water in one feeding. Peak rates of ingested sucrose oxidation were observed approximately 70 min after ingestion and accounted for 44% of total CHO oxidation at this time. Two hr after ingestion, 44 g of the 100 g sucrose feeding had been recovered as expired $^{14}CO_2$.

Utilising stable isotope techniques, Gerard et al. (1986) investigated the effects of sucrose ingestion (100 g in 400 ml of water given as a single feeding 15 min after the start of exercise) during four hr of walking at a speed (4-5 km/hr) requiring approximately 50% of VO_{2max}. They reported that 93 ±4 g of ingested sucrose was oxidised during the exercise bout. Further, they showed a significant "*sparing of endogenous CHO*" (Gerard et al. 1986). Muscle glycogen utilisation was, however, not directly determined.

Recently, Moodley et al. (1992) investigated the effects of ingesting varying concentrations of sucrose (7.5 g/100 ml, 10 g/100 ml and 15 g/100 ml) upon rates of gastric emptying and ingested CHO oxidation. These drinks, which were U-^{14}C labelled, were ingested repeatedly (nine 100 ml feedings every ten min) during 90 min of exercise at 70% of VO_{2max}. They found that although gastric emptying fell as the sucrose content of

the drink increased, CHO delivery to the intestine and ingested CHO oxidation increased linearly with increasing CHO concentration (Moodley et al. 1992). As only 15-20 g (26-34%) of the CHO delivered to the intestine was oxidised, these workers concluded that "*the low rates of exogenous sucrose oxidation were not due to a lack of demand by the working muscles but rather, must have resulted from low rates of absorption from the intestine*" (Moodley et al. 1992).

2.6.3. Glucose polymers

Glucose polymer ingestion during prolonged exercise has been thought to be preferable to the ingestion of equivalent concentrations of glucose, fructose or sucrose (Foster et al. 1980; Ivy et al. 1983; Sole and Noakes 1989). The ~80% lower osmotic pressure of glucose polymer solutions than of isocaloric glucose solutions was assumed to increase the rate of gastric emptying and to decrease the movement of water from the plasma into the intestinal lumen (Foster et al. 1980; Wheeler and Banwell 1986). Ingestion of maltodextrins also results in lower rates of gastric secretion (Foster et al. 1980; Owen et al. 1986). Although results from studies which have measured rates of gastric emptying from glucose polymer solutions are variable (Foster et al. 1980; Moodley et al. 1992; Sole and Noakes 1989; Wheeler and Banwell 1986), there are no reports of glucose polymer solutions being emptied more slowly than comparable glucose solutions when ingested in sufficient volumes (Maughan and Noakes 1991).

Recent studies however, have raised the question of whether the ingestion of glucose polymer solutions offer any advantage over the ingestion of glucose solutions in terms of ingested CHO oxidation or water balance (Massicotte et al. 1989; Rehrer et al. 1989; 1992). Massicotte et al. (1989) compared the oxidation of 7 g/100 ml solutions of ^{13}C labelled glucose polymer with glucose and fructose. Drinks were ingested as repetitive feedings (six 16 g feedings in 235 ml of water every 20 min) throughout 120 min of cycling at 53% of VO_{2max}. They found that the oxidation rates of ingested glucose and glucose polymers solutions were similar throughout the two hr exercise bout (70 versus 64 g respectively), and both were higher than rates of ingested fructose oxidation (53 g).

Massicotte et al. (1989) concluded that "*despite a lower osmotic pressure, glucose ingested in the form of a glucose polymer does not appear to be delivered more quickly and to be oxidised in larger amounts than when ingested in the form of an isocaloric free glucose solution*".

Rehrer et al. (1992) studied both the rates of gastric emptying and ingested CHO oxidation in eight trained subjects who cycled at 70% of VO_{2max} for 80 min while drinking 17 g/100 ml ^{13}C-labelled glucose or glucose polymer solutions. They found no significant differences in the rates of gastric emptying (glucose 781 ml/80 min; glucose polymer 864 ml/80 min), or the calculated intestinal CHO delivery (glucose 133 g; glucose polymer 147 g). The amount of ingested CHO oxidised during the 80 min exercise bout was also similar for both drinks (glucose 42 g; glucose polymer 39 g). Thus, the results of Rehrer et al. (1992) show that under the experimental conditions they studied, a 17 g/100 ml glucose polymer solution did not confer any advantages in terms of rates of gastric emptying, intestinal CHO delivery, or ingested CHO oxidation over an equicaloric free glucose solution. This again suggests that the gastric volume resulting from repetitive drinking during exercise is a more important determinant of gastric emptying than is solute osmolality (Noakes et al. 1991a).

The only study to find differences between the rates of ingested CHO oxidation for glucose polymer and glucose solutions was that of Moodley et al. (1992). They found that the rates of ingested CHO oxidation from 11 and 22 chain-length glucose polymer solutions ingested as repetitive (nine 100 ml) feedings during 90 min of cycling at 70% of VO_{2max} were greater than from isocaloric glucose solutions. With the initially low gastric volumes, there was a faster rate of gastric emptying of the glucose polymer solution than the glucose solution resulting in slightly higher rates of intestinal CHO delivery.

2.6.4. Starch

To date few studies have examined the oxidation of starch ingested before or during prolonged exercise. Guezennec et al. (1989) studied six subjects who ingested either 90 g of naturally labelled ^{13}C corn starch or 100 g of ^{13}C labelled glucose or fructose 60 min before two hr of cycling at 60% of VO_{2max}. They found that there was no difference in ingested CHO oxidation between the corn starch and glucose (66 g versus 67 g respectively) and concluded that "*corn starch does not offer any advantage over glucose for a pre-exercise meal before the beginning of prolonged exercise of moderate intensity*".

Jarvis et al. (1992) investigated the oxidation of uniformly labelled ^{13}C starch (45 g of potato starch, 0.8 g of corn starch, 23.7 g of sucrose in 400 ml of water) in five active males who walked for four hr on a 10% uphill graded treadmill set at a speed which elicited approximately 40% of the subjects VO_{2max}. Compared to a liquid glucose meal (70 g in 400 ml of water) ingested under the same regimen, the recovery of ^{13}C as breath $^{13}CO_2$ was higher for glucose (41.2 ±5.4% of dose/hr) than for the starch preparation (36.4 ±5.3% of dose/hr). These workers suggested that the amylose content of the potato starch (20-25%) may have "*had an effect on the slower assimilation (of the starch) compared to the glucose*".

Guezennec et al. (1993) investigated the oxidation of various starch preparations (100% crude amylopectin starch; 100% gelatinised amylopectin starch; 70% amylose-30% amylopectin crude starch; 70% amylose-30% amylopectin gelatinised starch, and control, i.e. glucose), ingested one hr prior to 120 min of cycling at 60% of VO_{2max}. At the end of exercise, ingested gelatinised amylopectin and glucose were oxidised to the same extent (85% versus 82% of the amounts ingested respectively), but the corresponding rates of oxidation of ingested crude amylopectin (49%) and crude amylose (38%) starches were significantly lower.

Recently, Saris et al. (1993) compared the oxidation of two naturally enriched ^{13}C starches ingested by eight subjects cycling for 150 min at 60% of PPO. The starches were a 15 g/100 ml soluble partly hydrolysed corn starch and an isocaloric insoluble corn starch. The peak rates of ingested starch oxidation were 1.10 and 0.81 g/min at the end of exercise for the soluble and insoluble starches respectively. The amount of starch that was oxidised during exercise was significantly greater when subjects ingested the soluble starch (126 g/150 min) compared to the insoluble starch (75 g/150 min). The results of this study suggest that, compared to an insoluble isocaloric starch, 15 g/100 ml solutions of soluble starch are oxidised at a higher rates throughout 150 min of moderate intensity cycling (Saris et al. 1993).

The results of studies described in section 2.6, which examined the rates of oxidation of CHO other than glucose when ingested by subjects during moderate-intensity exercise, are summarised in Table 2.4.

Table 2.4. Oxidation of carbohydrates other than glucose during prolonged exercise.

Ingestion protocol (g in ml)	Exercise intensity (% VO_{2max})	Ing. CHO_{ox} in 1st hour (g)	Ing. CHO_{ox} total (g in min)	Ing. CHO_{ox} % of all CHO_{ox}	Ing. CHO_{ox} peak rate (g/min)	Reference
Fructose						
70/1 x 350	61	26[a]	26/60	—	0.53	Decombaz et al. (1985)
99/6 x 235	53	21	53/120	20	0.44	Massicotte et al. (1989)
140/9 x 225	50	14	79/180	31	0.43	Massicotte et al. (1986)
150/6 x 100	45	—	57/180	31	0.66	Jandrain et al. (1993)
Sucrose						
90/9 x 100	70	8	16/90	10	0.45	Moodley et al. (1992)
Glucose polymer						
99/6 x 235	53	24[b]	64/120	26	0.53	Massicotte et al. (1989)
90/9 x 100	70	11	24/90	13	0.80	Moodley et al. (1992)
90 x 100	70	7[c]	26/90	15	0.70	Moodley et al. (1992)
220/600 + 3 x 228	70	18	39/80	18	0.73	Rehrer et al. (1992)
Starch						
300/2000	67	[d]	75/150	20	0.81	Saris et al. (1993)
300/2000	67	[e]	126/150	33	1.10	Saris et al. (1993)

Ing. CHO_{ox}, ingested carbohydrate oxidation; [a], fructose was ingested one hour prior to exercise; [b], 11-chain-length glucose polymer; [c], 22-chain-length glucose polymer; [d], insoluble starch; [e], soluble starch

2.7 Summary

CHO supplementation during continuous exercise of moderate to high intensity does not slow muscle glycogenolysis (Bosch et al. 1993a; Coyle et al. 1986; Fielding et al. 1985; Flynn et al. 1987; Hargreaves and Briggs 1988; Noakes et al. 1988) but, instead, maintains plasma glucose concentrations at euglycaemic levels (i.e. 4-5 mmol/L) at a time (about three hr) when plasma glucose levels would otherwise decline to less than 3.5 mmol/L in overnight fasted subjects (Coggan and Coyle 1991; Coyle 1991). Thus the duration of exercise at intensities of ~70-80% of VO_{2max}, that would otherwise be limited by low liver glycogen levels, is increased by the ingestion of CHO solutions during exercise. Further, if prolonged moderate exercise is followed by a high intensity time trial or sprint test, performance during this second work bout is enhanced, if CHO drinks have been ingested *during* the prolonged exercise bout (Maughan 1991).

The peak rate of ingested CHO oxidation during prolonged continuous exercise occurs 75-90 min after ingestion. Up to this time, different feeding schedules do not appear to greatly influence rates of ingested CHO oxidation. Irrespective of whether subjects ingest CHO as single or multiple feedings, around 20 g of CHO is oxidised in the first hr of exercise (Decombaz et al. 1985; Guezennec et al. 1989; Jandrain et al. 1984, 1989; Krzentowski et al. 1984a; Massicotte et al. 1986, 1989, 1990, 1992; Pirnay et al. 1977a, 1977b).

Ingested CHO oxidation does not appear to be limited by the muscles demand for glucose. Manipulation of either muscle or liver glycogen stores by exercise (Ravussin et al. 1979) or fasting (Massicotte et al. 1990) does not increase the reliance on ingested CHO during subsequent exercise.

Neither does exercise intensity appear to affect ingested CHO oxidation. Although ingested glucose oxidation increases in a linear fashion at work rates corresponding to 22-51% of VO_{2max} (Pirnay et al. 1982), at higher exercise intensities ingested glucose utilisation fails to increase further (Bosch et al. 1993c; Pirnay et al. 1982).

The peak rates of ingested oxidation of a variety of CHO's (i.e. glucose, fructose, sucrose, glucose polymers, and soluble starch) are limited to ~1 g/min during the latter stages of exercise (Massicotte et al. 1989; Moodley et al. 1992; Pallikarakis et al. 1986; Rehrer et al. 1992; Saris et al. 1993). However, when muscle glycogen content is low, and plasma glucose becomes the major source of CHO oxidation (Coggan & Coyle 1991), it has been estimated that plasma glucose can be oxidised at rates approaching 2 g/min and provide up to 75% of the total CHO oxidation (Coyle et al. 1986). Thus it has been recommended that CHO must be provided at a rate of *at least* 1 g/min to maintain euglycaemia and the concomitant rates of CHO oxidation necessary to prevent fatigue (Coggan & Coyle 1991; Noakes 1992).

Few studies have been conducted to systematically determine the gastric volume and drinking regimen that will result in the maximal rates of gastric emptying during prolonged exercise. Further, the most effective CHO solution for ingestion during exercise has yet to be formulated. In addition, the factors which limit the rate at which ingested (or infused) CHO (or glucose) can be oxidised by the working muscles during prolonged, moderate-intensity exercise (i.e. the rate of gastric emptying of an ingested fluid, the rate of digestion and subsequent transport of glucose into the systemic blood supply, and the rate of muscle glucose oxidation), remain to be established.

CHAPTER THREE

METHODOLOGY

3.1 Subjects and preliminary testing

3.1.1 Subject selection and training background

Thirty-four male cyclists or triathletes served as subjects in this series of studies, which were all approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. The nature and risks of the experimental procedures were explained in detail to all subjects prior to their participation, and their written informed consent was obtained in accordance with the guidelines outlined by the American College of Sports Medicine (1992). All subjects had been involved in regular endurance-training for at least three years prior to participation in a study. Because of the exposure to radioactivity in these investigations, subjects usually only participated in one set of experiments. The maximum radiation dose received by each subject was ~10 mrem. The radiation dose that is regarded as safe in this country is 500 mrem/yr or 130 mrem/13 wk (Bosch 1993a).

3.1.2 Determination of maximal oxygen uptake (VO_{2max})

All subjects were tested for VO_{2max} and peak sustained power output (PPO) seven to ten days prior to their participation in a study. Each athlete performed a maximal test on a Lode electronically braked cycle ergometer (Groningen, Holland), modified with clip-on pedals and triathlon low-profile handlebars. Power output on this ergometer is constant and independent of pedalling rate between 40 and 100 revolutions/min. For all tests subjects cycled at 90-100 revolutions/min. Maximal tests were undertaken the day after the subjects had either rested completely, or trained very lightly. Subjects reported to the laboratory between 1500-1800 hr and after they had urinated, their bodymass was recorded to the nearest 0.1 kg. The saddle height and handlebar position of the ergometer was adjusted to the subjects requirements, and the subject warmed-up at a self-selected intensity and duration. When the subject was ready to commence the test, the work rate was immediately adjusted to 3.33 Watts (W)/kg body mass. This initial work rate was maintained for 150 seconds (sec) and thereafter increased by 50 W for a further 150 sec. After the second stage the exercise intensity was increased by a further 50 W for 150 sec, and thereafter by 25 W every 150 sec until volitional fatigue, which always coincided with

either a drop in cadence of > 10 revolutions/min, an RER > 1.10, or both. Subjects remained seated throughout the test and received strong verbal encouragement from the investigator.

The PPO (W) attained by each subject was defined as the highest exercise intensity completed. If an exercise intensity was not completed for the full 150 sec, then PPO (W) was determined from the following equation (Hawley and Noakes 1992; Kuipers et al. 1985):

$$\mathbf{PPO = W_{final} + ((t/150) \cdot 25\ W)} \qquad \textbf{(Eq. 5)}$$

where PPO refers to the peak sustained power output (W); W_{final} was the last exercise intensity (W) the subject completed for 150 sec; t was the number of seconds for which the final, uncompleted exercise intensity was sustained; and 25 was the final work rate increment (W).

During gas collection subjects wore a nose clip and a counterbalanced head support, while breathing through a one-way Rudolph valve (No. 2700, Hans Rudolf, Kansas City, KA, United States of America) secured in the mouth. Expired air was passed though a 15 L mixing chamber and a condensation coil to Ametek N-22M oxygen and CD-3A carbon dioxide analysers (Applied Electro-Chemistry, Amatek, Pittsburgh, PA, United States of America). Before each trial the gas meter (Collins, Braintree, MA, United States of America) was calibrated with a Hans Rudolph 5530 three L syringe, and the analysers were set with air and a 4% CO_2-16% O_2-80% N_2 gas mixture. Outputs from the instruments were processed by an on-line IBM PC computer, which calculated VO_2, CO_2 production (VCO_2) and RER using conventional equations (Jones and Campbell 1982).

The results of this initial test were used to determine the work rate that corresponded to 63% of each subjects PPO. Exercise of submaximal intensity can be expressed as either a percentage of VO_{2max} (Astrand and Rodahl 1986), or as a percentage of PPO (Saris et

al. 1993; Wagenmakers et al. 1991). Since the day to day variation in PPO is less than the variation in VO_{2max} (Kuipers et al. 1985), and since endurance can vary greatly among individuals with a similar VO_{2max} (Coyle et al. 1988), the experimental rides were conducted at a standardised submaximal work rate equal to 63% of each subject's PPO (approximately 68-72% of VO_{2max}).

3.1.3 Determination of body composition

So that measurements of glucose kinetics (described subsequently in sections 3.2.6 and 3.2.9) could be expressed in terms of fat free mass (FFM), the percentage body fat of the subjects was assessed by conventional techniques (Durnin and Wormersley 1974). The anatomical sites used were the triceps, biceps, sub scapular and supra iliac skin folds. Body composition was measured just before the subjects undertook their maximal test.

3.1.4 Experimental trials.

In the studies described in Chapters Four and Five, where subjects were required to ingest two U-^{14}C labelled CHO solutions, each subject undertook two 90 min continuous rides at a work rate of 63% of PPO. Twenty-four hr prior to these experimental rides, the subjects rode in the laboratory for 60 min at 63% of PPO in an attempt to standardise their leg muscle glycogen stores at the start of the following days' trial. On the morning of an experiment, the subject arrived at the laboratory in a 12 hr post-absorptive state.

These experimental rides were always separated by a minimum of seven days and a maximum of 14 days. Where necessary, the order of trials was random and subjects were kept blind as to the nature of the experiment. Between trials, subjects maintained their usual training programmes and diets. During all rides, subjects were cooled with an electric fan while the laboratory was maintained at a constant ambient temperature of 22-23^{o}C.

The studies described in Chapters Six and Seven involved both the ingestion of U-^{14}C labelled glucose (or water), combined with a continuous infusion of tritiated (2-^{3}H)

glucose. Thus, in order to minimise the exposure to radioactivity from both U-^{14}C and 2-^{3}H glucose in these studies, subjects were divided into two groups and each subject performed only one 125 min experimental ride. On the morning of these experiments, the subject arrived at the laboratory between 0700-1000 hr a ***minimum*** of three hr after a standardised breakfast of ~85 g of CHO. This breakfast was similar in size and composition to that which an athlete would normally ingest before training or competition.

3.2 Sample collection and analyses

3.2.1 Test solutions and ingestion regimen

In those experiments where subjects consumed CHO containing beverages during exercise, test solutions were labelled with 0.17 uCi/g of a uniformly labelled fourteen-carbon (U-^{14}C) tracer (Amersham International, Buckinghamshire, United Kingdom) so that ingested CHO oxidation could be determined from equations six and seven (described in section 3.2 5).

When subjects ingested artificially coloured and flavoured water, rates of plasma glucose oxidation were measured using tracer amounts (0.17 uCi/g) of a U-^{14}C glucose label (Amersham International) added to the water. Subjects, therefore, usually ingested a total of between 30 and 41 uCi of ^{14}C-radioactivity. The tracer was ingested, rather than infused, because plasma glucose oxidation was calculated from simultaneous measurements of expired $^{14}CO_2$ and plasma specific radioactivity measurements (described in section 3.2.9). Such measurements are unaffected by the route of administration of the ^{14}C glucose tracer.

As a large intragastric volume has been shown to accelerate gastric emptying (Noakes et al. 1991a; Rehrer et al. 1990), athletes ingested an initial bolus feeding (400 ml) of the solution to be tested that day during a five min warm-up which immediately preceded each ride. Every ten min thereafter, the subject ingested a further 100 ml of the same test solution until the completion of the exercise bout. All CHO drinks were (a) given at a concentration of 15 g/100 ml, (b) kept at a temperature of 12-15^{o}C, and (c) contained 25

mg/L of a non absorbable marker, phenol red, which was used for subsequent analyses of gastric emptying (described in section 3.2.2). In those experiments where subjects consumed artificially flavoured water with tracer amounts of a U-^{14}C glucose label, they followed the same ingestion regimen.

The osmolality of several of the test solutions was measured using the freezing-point depression method (Osmette A, Precision Systems, Newton, MA, United States of America).

3.2.2 Calculation of gastric emptying and intestinal carbohydrate delivery

Immediately upon completion of an exercise bout during which a CHO containing solution had been ingested, subjects dismounted the ergometer and reclined on a couch. A number 14 French Levine nasogastric tube was then passed into the stomach and the gastric contents were aspirated with a 50 ml syringe. The volume of the aspirate was recorded and phenol red concentrations subsequently determined using conventional standardised methods (Foster et al. 1980; Ivey and Schedl 1970; Schedl et al. 1966; Sole and Noakes 1989). Complete emptying of the stomach was ensured by subsequently injecting 100 ml of distilled water down the nasogastric tube and re-aspirating to confirm the absence of phenol red dye (Moodley et al. 1992). The volume of the test solution recovered was subtracted from the amount originally ingested to calculate the volume emptied from the stomach. The amount of CHO delivered to the intestine from a test solution was calculated from the product of the volume emptied and the 15 g/100 ml CHO concentration.

3.2.3 X-ray powder diffraction analyses and scanning electron microscopy

In order to compare the structural order of the starch preparations ingested by some subjects, starch samples were subjected to *X*-ray diffraction analysis using a Philips PW 1050/70 automatic *X*-ray powder diffractometer (Philips Pty Limited, Eindhoven, The Netherlands) and CuK0 radiation (Wilson 1970). *X*-ray diffraction spectra were recorded while scanning the samples through a 2Ø range of 10-50^{o}.

The particle sizes of several of the CHO preparations was also assessed by scanning electron microscopy. Grains of a sample were sprinkled onto separate aluminium stubs, the faces of which had been painted with a thin coat of glue and graphite. The stubs were then coated with ~100 nm of carbon at a pressure of ~1.3 mPa in a Blazer's vacuum coater equipped with a planetry sample rotator. Specimens tilted at 35°C to the collector were examined using a Cambridge S200 Scanning Electron Microscope (Cambridge Instruments, London, United Kingdom) operating at a beam voltage of 5 kV.

3.2.4 VO_2, VCO_2, RER, $V^{14}CO_2$ and ratings of perceived exertion measurements

Steady-state measures of gas exchange (VO_2, VCO_2, RER) were taken for five to six min at regular (20 min) intervals during exercise. Immediately prior to gas collection, ratings of perceived exertion (RPE) were obtained using the 20 point Borg scale (Borg 1975), and stomach fullness was rated on a five-point scale with zero corresponding to a feeling of "*no fullness*" and five indicating "*extreme fullness.*"

In order to determine the rates of ingested CHO oxidation and plasma glucose oxidation (described in section 3.2.5), expired CO_2 was also trapped during the gas exchange measurements by passing air from the gas analyser vent through a solution containing 1 ml of hyamine hydroxide (United Technologies, Packard, Illinois, United States of America), 1 ml of 96% ethanol (SAARCHEM, Krugersdorp, Republic of South Africa) and 1-2 drops of phenolphthalein (SAARCHEM, Krugersdorp, Republic of South Africa). The expired air was bubbled through this solution until the phenolphthalein indicator changed colour from pink to clear. At this end point exactly 1 mmol of CO_2 was trapped (Scherrer et al. 1978). Ten ml of liquid scintillation cocktail (Ready Gel, Beckman Instruments, Fullerton, California, United States of America) was then added to the solution and $^{14}CO_2$ radioactivity in disintegrations/min (dpm) counted in an Insorb 460C Automatic Liquid Scintillation counter (United Technologies, Packard, Illinois, United States of America). All counts were corrected for differences in quench and background.

3.2.5 Ingested and total carbohydrate oxidation

While $V^{14}CO_2$ measurement were made, steady-state VO_2 and VCO_2 values were also obtained so that rates of ingested CHO oxidation could be determined by using one of the following equations:

$$\textbf{Ing CHOox} = (^{14}CO_2 \cdot 6 / SA_{CHO}) \cdot VCO_2 \cdot 1.35 \qquad \textbf{(Eq.6)}$$

$$\textbf{Ing CHOox} = (^{14}CO_2 \cdot 12 / SA_{CHO}) \cdot VCO_2 \cdot 1.35 \qquad \textbf{(Eq.7)}$$

In these equations, Ing CHO_{ox} is the rate of ingested CHO oxidised in grams/min; $^{14}CO_2 \cdot 6$ (or 12) is the $V^{14}CO_2$ dpm/mmol value multiplied by either six (glucose) or 12 (maltose), as there are either six (or 12) carbon atoms per molecule of glucose (or maltose) oxidised; SA_{CHO} is the specific radioactivity of the ingested CHO solution in dpm/mmol; VCO_2 is the volume of expired carbon dioxide in L/min; and 1.35 is the number of grams of glucose oxidised to produce one L of carbon dioxide.

Overall CHO oxidation in g/min was calculated from the equation of Consolazio et al. (1963) described in Chapter Two (section 2.4.1). Total CHO oxidation during exercise was estimated from the area under the CHO oxidation versus time curve for each subject. Rates of CHO oxidation were converted from g/min values to umol/min kg FFM units by dividing the values by the molecular weight of glucose (180) in ug and the subject's FFM. Rates of fat oxidation were converted from g/min values to umol/min kg FFM units by dividing the values by the molecular weight of palmitate (237) in ug and the subject's FFM.

The total energy demands of exercise (i.e. the sum of CHO and fat oxidation in kcal/min) was derived from the g/min rates of CHO and fat oxidation multiplied by 4.1 and 9.3 kcal/g respectively

3.2.6 Measurement of glucose turnover during exercise

Glucose turnover in the glucose infusion experiments described in Chapters Six and Seven was estimated from the isotope dilution of a continuous infusion of 2-^{3}H glucose tracer (Amersham International, Buckinghamshire, United Kingdom) administered through an 18 gauge Jelco cannula (Johnson and Johnson, Halfway House, Transvaal, Republic of South Africa) placed into an antecubital vein in the subjects left arm. ^{3}H in the 2-carbon position of glucose was chosen because a large proportion of the label in the 2 position is lost through a cycling between hepatic glucose 6-phosphate and fructose 6-phosphate (Katz and Dunn 1967), thus minimising ^{3}H incorporation into hepatic glycogen during the subsequently described pre-exercise infusion of 2-^{3}H glucose. Avoiding this potential error (likely to be less than ten percent [Radziuk et al. 1978b]) was considered to be more important than the larger underestimation of hepatic glucose appearance that is thought to occur when 3-^{3}H glucose (Bell et al. 1986) and 6-^{3}H or 6,6-$^{2}H_2$ glucose tracers are used (McMahon et al. 1989).

The 2-^{3}H-glucose was infused at a constant rate of 0.29 ml/min (25 uCi/hr) via a calibrated auto-syringe (Travenol Laboratories Incorporated, Hooksett, New Hampshire, United States of America) for 60-75 min prior to the commencement of exercise. A previous study from this laboratory has shown that a 60 min infusion is sufficient to achieve a constant plasma glucose specific radioactivity (Bosch et al. 1993a).

Ra and Rd were determined from the non-steady state equations of Steele (1959):

$$\mathbf{Ra = (I - (pV \cdot Glu \cdot dSA / dt)) / SA} \qquad \textbf{(Eq. 8)}$$

$$\mathbf{Rd = Ra - (pV \cdot dGlu / dt)} \qquad \textbf{(Eq. 9)}$$

where Ra and Rd are the rates of liver plus infused glucose appearance and disappearance in mmol/min; I is the rate of infusion of 2-^{3}H glucose in dpm/min/kg; p is the pool fraction, taken as 0.75; V is the glucose distribution volume (L) taken as 19.6% of body mass (Jenkins et al. 1985) which is constant throughout exercise; Glu is the mean of

successive plasma glucose concentrations in mmol/L; dSA/dt is the change in plasma 2-^{3}H specific activity in dpm/mmol over the sample interval (normally 20 min); SA is the dpm/mmol glucose specific activity in successive samples; and dGlu is the mmol/L/min change in glucose concentration.

The pool fraction in which changes in glucose concentration and specific activity occur can vary from 0.5 to 0.75 (Jenkins et al. 1985; Radziuk et al 1978b). In experiments which have been performed at rest and in which rapid changes in the rate of glucose infusion cause immediate perturbations in blood glucose concentration, a pool fraction of 0.5 to 0.65 might be chosen (Jenkins et al. 1985). However, a glucose pool fraction of 0.75 was deemed to be more appropriate for the current investigation where changes in blood glucose concentration were minimal after the first 5 min of exercise (Cowan and Hetenyi 1971; Radziuk et al. 1978b).

Liver glucose production during glucose infusion experiments was determined from the differences between the total rates of glucose turnover (determined by isotope dilution) and the glucose infusion rates. The infusion rates during the euglycaemic and hyperglycaemic glucose clamps were averaged for the first 25 min and then over successive 20 min intervals until the completion of the 125 min ride.

3.2.7 Unlabelled glucose infusion procedures

The glucose infusion procedures utilised in the experiments described in Chapters Six and Seven were based on the negative feedback principle `clamp' technique described by DeFronzo et al. (1978, 1979). Glucose (25 g/100 ml dextrose, Adco Pharmaceutica, Adcock Ingram Laboratories, Johannesburg, Republic of South Africa) was infused intravenously via a calibrated auto syringe at a rate necessary to maintain plasma glucose at the desired concentration. In order to achieve these plasma glucose concentrations, a 1 ml blood sample was taken and rapidly analysed for glucose concentration (Reflolux II, Haemo-Glukotest 20-800R, Mannheim Boehringer, Germany) immediately before, and then at five minute intervals throughout the exercise bout. With this information the rate

at which the infusion pump administered glucose was adjusted throughout the experiments to maintain blood glucose concentrations at either euglycaemic (i.e. 5 mmol/L) or hyperglycaemic (i.e. 10 mmol/L) levels.

3.2.8 Plasma glucose and plasma lactate specific activity

Plasma samples (1 ml) for the separation of glucose and lactate were adjusted to a pH of ~4.0 with 60 ul of 2 M H_3PO_4, placed in sealed tubes and deproteinised by heating for ten min at 70°C in a shaking water bath. They were then cooled on ice for a further ten min before the seals of the tube were removed and the inside of the tube was rinsed with 1 ml of distilled water (raised to pH 8.0 by the addition of traces of NaOH) to wash down any lactate which may have condensed onto the side of the tube. The pH of the samples was then adjusted to 7.0 with ~40 ul of 3M K_2CO_3 and centrifuged at 5,000 revolutions/min for ten min. Following centrifugation, the supernatant was stored, the pellet re suspended in 0.75 ml of H_2O, re centrifuged, and the supernatant was added to that previously saved. This process of washing with 0.75 ml of H_2O and re centrifuging was repeated a further three times.

Separation of plasma glucose from plasma lactate was achieved by passing the combined supernatants through 1.0 x 0.5 cm anion exchange columns containing sephadex (Bakerbond SAX, Cape Town, Republic of South Africa) that had been conditioned with several void volumes of distilled water, also raised to a pH of ~8.0 by the addition of traces of NaOH. Glucose appeared in the void volume and was fully eluted with 3 ml of distilled H_2O. Lactate was eluted into a second vial with 2 ml of 1M $CaCl_2$ (adjusted to a pH of 2.0 with HCl). Passage of the solutions through the exchange column was accelerated to approximately one ml/min with a vacuum processor. The eluates were collected into scintillation vials and evaporated to near dryness at 70°C for approximately 20 hr in order to (i) minimise the presence of $^3H_20$ from the metabolism of 2-^{3}H glucose in the glycolytic pathway to < two per cent and, (ii) reduce the water/liquid scintillation cocktail ratio during subsequent counting. Each time plasma glucose and lactate samples were separated, a non-labelled plasma sample was 'spiked' with a measured quantity of 2-

^{3}H and U-^{14}C glucose and run simultaneously to correct the experimental dpm values for the percent recovery, so that plasma glucose specific radioactivity's could be calculated using the concentration of glucose measured in the plasma. Such recoveries were > 88% for the U-^{14}C glucose samples and > 96% for 2-^{3}H glucose samples.

3.2.9 Rates of plasma glucose oxidation

After the separation of plasma glucose and plasma lactate, it was found that the plasma lactate counts were not significantly different from background counts. Rates of plasma glucose oxidation, therefore, did not need to be corrected for ^{14}C lactate oxidation and were determined with the following formula:

$$\mathbf{Rox = (SACO_2 \ / SA_{glu}) \cdot VCO_2} \qquad \textbf{(Eq. 10)}$$

where Rox is the rate of plasma glucose oxidation in umol/min/kg FFM; $SACO_2$ is the specific activity of expired $^{14}CO_2$ in dpm/mmol; SA_{glu} is the corresponding specific activity of the plasma glucose in dpm/mmol; and VCO_2 is the volume of expired carbon dioxide in umol/min/kg FFM calculated from the L/min VCO_2, the subjects FFM and the 22.4 L/mol gas volume. Since the complete conversion of one molecule of U-^{14}C glucose to six molecules of $^{14}CO_2$ decreases the dpm/mmol specific activity by a factor of six, the carbon dioxide value does not need to be divided by six to allow for six CO_2 molecules arising from the oxidation of one glucose molecule.

3.2.10 Plasma glucose and plasma insulin concentrations

Before all experimental trials, a flexible 22 gauge catheter was placed in a right forearm vein for blood sampling. Blood samples were obtained at rest, five min after the commencement of exercise and then at regular (usually 20 min) successive intervals throughout the ride. Ten ml of blood was drawn, of which half the sample was placed into pre-chilled tubes containing lithium heparin for subsequent determination of plasma glucose and plasma insulin concentrations, and the appearance of the ^{14}C label in the plasma. The rest of the blood was added to tubes containing potassium oxalatate and

sodium fluoride for separation of glucose and lactate counts (described previously in section 3.2.8). After each blood sample had been taken, the cannula was immediately flushed with 2-3 ml of sterile saline (plus 5 iU/ml of heparin). All samples were kept on ice until centrifuged at 2,500 revolutions/min at 5°C upon completion of the trial. Plasma samples were then stored at -20°C for later analyses.

Plasma glucose concentrations were determined by an automated glucose analyser (LM3 Glucose Analyser, Analox Instruments, London, United Kingdom). The measurement of plasma glucose concentration by this analyser method is based upon the glucose oxidase method described by Hyvarinen and Nikkila (1962). To ensure reliability, a known (7.0 mmol/L) glucose standard was run after the analyses of the plasma samples of each subject (normally seven samples), and, if necessary, the analyser re calibrated. Glucose determinations were performed in duplicate with an intra-assay variation of less than four per cent.

Plasma insulin concentrations were determined, in duplicate, by radio immunoassay procedures (Phadeseph Insulin RIA package, Pharmacia Diagnostics AB, Uppsala, Sweden) according to the techniques of Goetz and Greenberg (1961). The intra-assay variation was < 3%.

3.2.11 Statistical analyses

Data were analysed by either a one-way or two-way (treatment-by-time) analysis of variance (ANOVA) for repeated measures. Significant differences between means were located by the Schéffe's post-hoc test. Where appropriate, between treatment means were compared using a paired *t*-test. A value of $P < 0.05$ was regarded as significant. All values are reported as mean ±SEM.

CHAPTER FOUR

EXOGENOUS CARBOHYDRATE OXIDATION FROM GLUCOSE AND MALTOSE SOLUTIONS INGESTED DURING PROLONGED, MODERATE-INTENSITY EXERCISE

4.1 Introduction

The primary end-products of CHO digestion by pancreatic alpha-amylase in the duodenum are maltose, maltotriose, and alpha-1,6 branch limit dextrin's. Maltose and maltotriose are then broken down further by a number of different oligosaccharides that are attached to the microvillae of the intestinal villae of the jejunum and the ileum. These glucosidase enzymes are thought to be functionally linked to the glucose transporters in the luminal membrane of the columnar epithelial (absorptive) cells (section 2.3)

Human duodenal perfusion studies undertaken in resting subjects have suggested that the rates of glucose absorption from the hydrolysis of maltose (Cook 1973; Fairclough et al. 1977; Gray and Santiago 1966; Sandle et al. 1982) and maltotriose (Jones et al. 1987) solutions are faster than from equicaloric glucose solutions.

Accordingly, this study compared the rates of gastric emptying and oxidation of ingested 15 g/100 ml solutions of glucose and maltose consumed during exercise to see if maltose might be a better CHO for ingestion during endurance activities. This is the first investigation of the rates of gastric emptying and oxidation of a maltose solution ingested by humans during exercise.

4.2 Results

4.2.1 Subjects characteristics

Six male endurance-trained cyclists participated in this study. Their mean (±SEM) age, height, mass and VO_{2max} was 25.7 ±1.5 yr, 1.81 ± 0.01 metres (m), 70.9 ± 1.6 kg and 4.56 ±0.12 L/min respectively.

4.2.2 Ratings of perceived exertion and stomach fullness

There were no significant differences for RPE or stomach fullness either between trials or over time for the two 90 min rides. RPE averaged 12.2 ±0.5 and 11.9 ±0.4 units during the glucose and maltose trials respectively. Stomach fullness averaged 1.7 ±0.4 units during the glucose trial compared to 1.9 ±0.4 units during the maltose experiment.

4.2.3 Steady-state gas exchange, RER and total carbohydrate oxidation

Table 4.1 shows the steady-state gas exchange data, RER values and total calculated CHO oxidation during the two 90 min experimental trials. There were no significant differences either between trials or over time for any of these variables.

Table 4.1 Steady-state gas exchange data and total calculated carbohydrate oxidation during the two 90 minute rides after the ingestion of 15 g/100 ml solutions of glucose and maltose.

	VO_2 (L/min)	VCO_2 (L/min)	RER	Total CHO_{ox} (g)
Glucose	3.20	2.95	0.92	246.1
	0.14	0.11	0.01	10.0
Maltose	3.18	2.94	0.92	249.8
	0.16	0.13	0.01	10.8

VO_2, oxygen consumption; VCO_2, carbon dioxide production; RER, respiratory exchange ratio; Total CHO_{ox}, total carbohydrate oxidation. Values are mean ±SEM of measurements taken at succesive 20 minute intervals during the experimental trials.

4.2.4 Gastric emptying, intestinal carbohydrate delivery and total ingested carbohydrate oxidation

Table 4.2 lists the volumes of each drink that were emptied from the stomach, the amount of CHO that was delivered to the intestine from the two test solutions, and the amount of ingested CHO that was oxidised during the experimental trials. Despite the fact that the osmolality of maltose would be roughly half that of glucose, there were no significant differences for any of these variables between the two rides.

Table 4.2 Gastric emptying, intestinal carbohydrate delivery and ingested carbohydrate oxidation during the two 90 minute rides after the ingestion of 15 g/100 ml solutions of glucose and maltose.

	Drink emptied (ml)	Intestinal CHO delivery (g)	Ingested CHO_{ox} (g)
Glucose	655.2	98.3	49.3
	38.2	5.7	3.6
Maltose	690.2	103.5	50.9
	48.7	7.3	3.3

Intestinal CHO delivery, intestinal carbohydrate delivery calculated from the product of the volume of the drink emptied and the carbohydrate content of the solution; Ingested CHO_{ox}, total ingested carbohydrate oxidation. The total volume of the ingested drink was 1.2 L, consumed as a 400 ml pre-exercise loading bolus plus eight feedings of 100 ml/10 min. Values are mean ±SEM.

4.2.5 Plasma glucose concentrations and appearance of U-^{14}C labelled carbohydrate in the plasma

Plasma glucose concentrations were also not significantly different between trials (5.2 ± 0.2 and 5.1 ±0.1 mmol/L; range 4.9-5.4 mmol/L for glucose and maltose respectively). Neither were the rates of appearance of the U-^{14}C label in the plasma from ingested glucose or maltose (Figure 4.1). The peak counts in the plasma were attained between 60-90 min of exercise and were not significantly different between the two ingested CHO solutions (glucose 698 ±39 dpm/ml; maltose 716 ±38 dpm/ml).

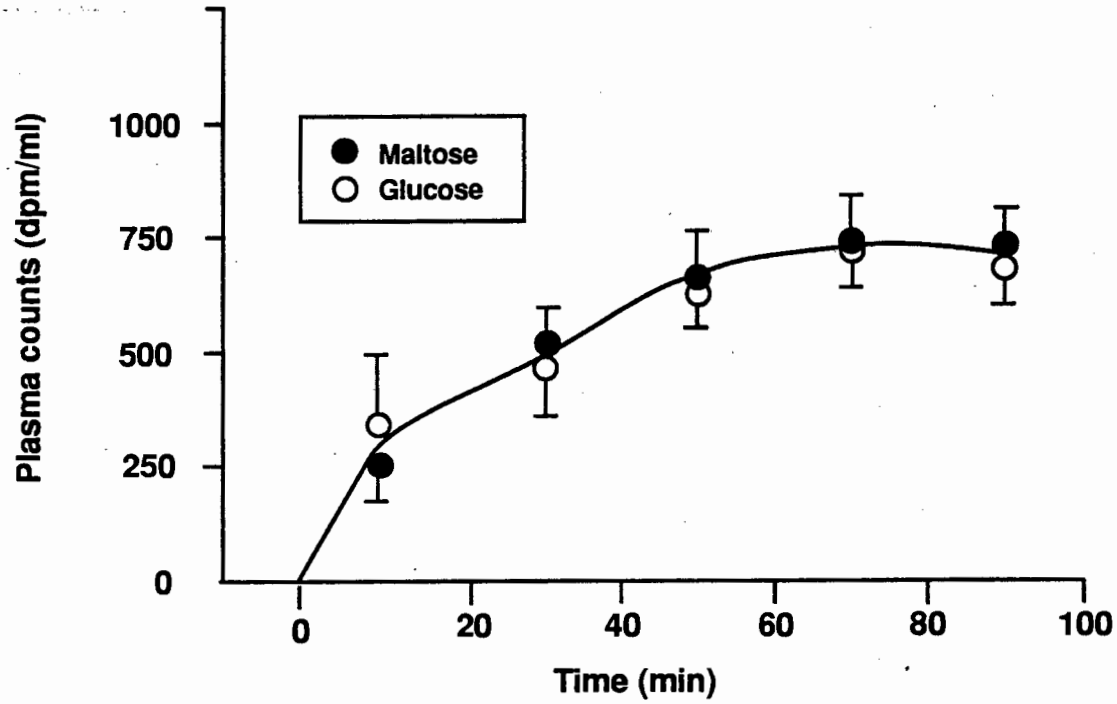

Figure 4.1. The appearance in plasma of the U-^{14}C label from 15 g/100ml solutions of glucose and maltose ingested repeatedly during 90 minutes of exercise. Values are mean ±SEM.

4.2.6 Rates of ingested carbohydrate oxidation

The rates of ingested CHO oxidation for the two test solutions are displayed in Figure 4.2. As would be expected from the similar rates of appearance of U-^{14}C label in the plasma from ingested glucose and maltose (Figure 4.1), their rates of oxidation and the time taken to attain the peak rates of oxidation (~75 min), were not significantly different with the ingestion of the two drinks. The peak rate of glucose oxidation was 0.9 ±0.04 g/min and the corresponding value for maltose was 1.0 ±0.04 g/min.

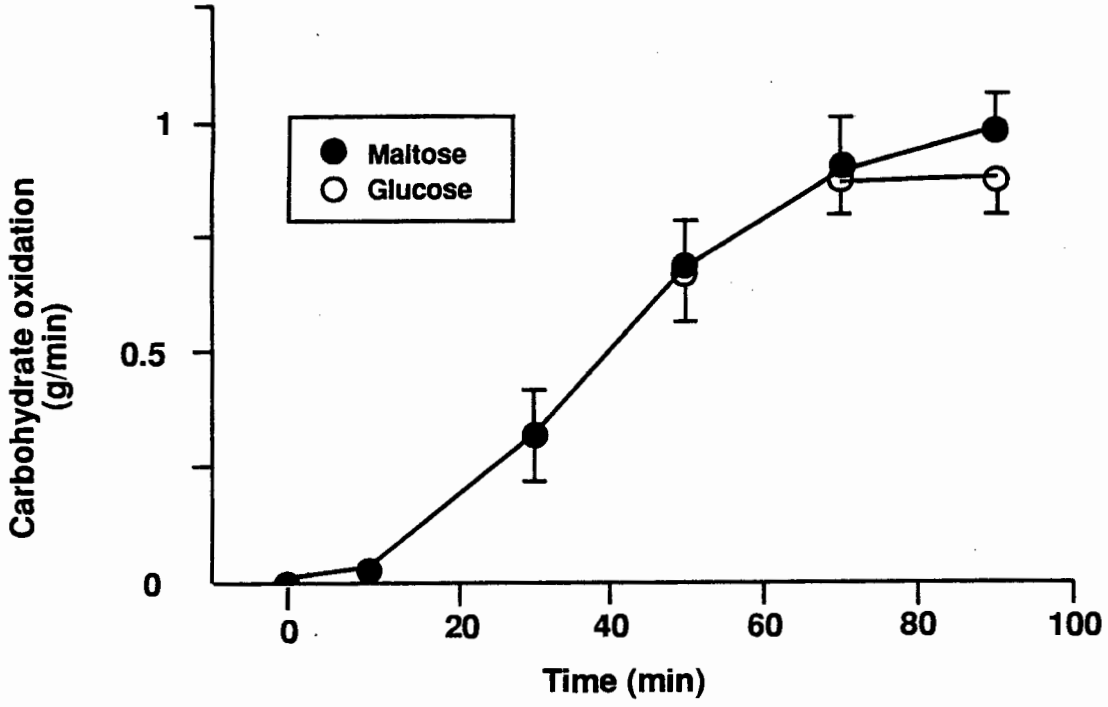

Figure 4.2. The rates of ingested carbohydrate oxidation from 15 g/100ml solutions of glucose and maltose ingested repeatedly during 90 minutes of exercise. Values are mean ±SEM

The 49.3 ±3.7 and 50.9 ±3.3 g of ingested CHO oxidation derived from glucose and maltose respectively accounted for 19 ±4.7% and 20 ±5.2% of total CHO oxidation during exercise. The amount of ingested CHO oxidised, when expressed as a percentage of the intestinal CHO delivered was the same for both solutions (glucose, 50%; maltose, 49%).

4.3 Discussion

The first finding of this study was that despite the presumed differences in osmotic pressure, the rates of gastric emptying for isocaloric (15 g/100 ml) solutions of maltose and glucose were similar (Table 4.2). The rates of gastric emptying and intestinal CHO delivery of glucose in the present study are also much the same as those reported previously for both glucose (Rehrer et al. 1992) and glucose polymer solutions (Moodley et al. 1992; Rehrer et al. 1992). The similar rates of gastric emptying for equicaloric but not equiosmotic solutions of glucose and maltose suggests that the drinking pattern of an athlete during exercise maybe a more important determinant of gastric emptying than the osmolality of the ingested solution (Rehrer et al. 1990; Noakes et al. 1991a).

In the current study the amount of CHO emptied from the stomach was far greater than the amount oxidised. Subjects ingested a total of 180 g of CHO during exercise of which just over half (glucose 55%; maltose 57%) was delivered to the intestine, and only a quarter (glucose 27%; maltose 28%) was eventually oxidised. Thus, the rate of gastric emptying cannot possibly limit the rate of CHO absorption and subsequent oxidation from soluble CHO repeatedly ingested during the early (up to 90 minutes) stages of moderate intensity exercise.

Initially the rate at which CHO diffuses from the lumen to the glucose transporters in the epithelial lining of the intestinal villi may limit its rate of absorption. The presence of an `unstirred' water layer is known to create a diffusion barrier (Hanke et al. 1980). However, despite a progressively rising CHO concentration in the intestine, resulting from the increasingly large amount of CHO ingested, the rate of ingested CHO oxidation plateaued at approximately 1 g/min. This suggests that there is an upper limit to the rate of ingested CHO oxidation.

The similar rates of `absorption' into the systemic circulation of 15 g/100 ml solutions of maltose and glucose found in the present investigation are in contrast to human intestinal perfusion studies which have shown that glucose absorption occurs more rapidly from

solutions of maltose (Cook 1973; Fairclough et al. 1977; Gray and Santiago 1966; Sandle et al. 1982), and glucose oligomers with a chain-length of three to six glucose units (Jones et al. 1983, 1987) than from isocaloric glucose solutions. If glucose absorption is indeed faster from short-chain glucose oligomers than from isocaloric free glucose (Jones et al. 1987), then one would expect water absorption also to be greater. In contrast, Gisolfi et al. (1992) have recently reported similar rates of water absorption from solutions of glucose, sucrose and a corn-syrup with an average chain-length of three glucose units, the latter being comparable to the oligomer mixture of Jones et al. (1987).

The short-chain glucose polymers are rapidly hydrolysed by the action of pancreatic alpha-amylase and a group of enzymes located on the enterocyte brush border (Kenny and Maroux 1982). Thus the most probable explanation for the increased glucose absorption from maltose found in intestinal perfusion studies performed on resting individuals (Cook 1973; Fairclough et al. 1977; Gray and Jones et al. 1983, 1987; Santiago 1966; Sandle et al. 1982) is that the liberation of monosaccharide by brush border enzymes achieves a greater local glucose concentration at the glucose transport sites than those achieved by the relatively slow diffusion of free glucose through the `unstirred' water layer (Crane 1975: Hanke et al 1980). In this regard, it is tempting to speculate that the motion of exercise may `stir' the water layer and, hence, reduce the diffusion barrier for the movement of glucose from the gut lumen to the glucose transport sites.

Because of the practical difficulties involved, there have been few attempts to measure intestinal absorption during exercise. As with gastric emptying, most of the information on the regulation of intestinal transport is derived from studies undertaken on resting individuals in which a short segment of the proximal intestine is perfused; the results of such experiments may not be applicable to the exercise setting.
As would be expected from the similar rates of appearance of the ^{14}C label in the plasma (Figure 4.1), ingested CHO oxidation from maltose and glucose was also similar (Figure 4.2), as was total CHO oxidation (Table 4.2). Both maltose and glucose ingestion resulted in identical rates of ingested CHO oxidation, accounting for approximately 20%

of total CHO oxidation during 90 min of continuous cycling. The peak rate of ingested CHO oxidation for glucose (0.9 ±0.04 g/min; Figure 4.2) was similar to that reported by other groups employing multiple glucose feedings during exercise (Massicotte et al. 1986, 1989, 1992; Pallikarakis et al. 1986; Rehrer 1992).

In summary, as the rates of ingested CHO oxidation from 15 g/100 ml solutions of maltose and glucose are similar to values previously reported for other mono-, di- and oligosaccharides (Massicotte et al. 1986; Moodley et al. 1992; Pallikarakis et al. 1986; Rehrer et al. 1992; Saris et al. 1993), and as the rates of CHO delivery to the intestine exceed by over 100% the rates of ingested CHO oxidation, it would appear that neither the rates of gastric emptying, nor of CHO digestion limit the rate of utilisation of simple CHO solutions ingested during exercise. Rather, the rate-limiting step must be either the absorption and transport of glucose into the systemic blood supply or the rate of working muscle glucose uptake and oxidation.

CHAPTER FIVE

EXOGENOUS CARBOHYDRATE OXIDATION FROM STARCH AND GLUCOSE POLYMER SOLUTIONS INGESTED DURING PROLONGED, MODERATE-INTENSITY EXERCISE

5.1 Introduction

Historically it has been assumed that the rate of gastric emptying is *the* primary factor limiting the rate of CHO delivered to the blood and the working muscles (Costill 1990; Costill and Saltin 1974). However, the results from the previous investigation (Chapter Four) show that after the ingestion of 15 g/100 ml solutions of maltose and glucose, the amount of CHO emptied from the stomach far exceeds the amount oxidised. Thus, gastric emptying cannot limit the rate of CHO absorption and subsequent oxidation from CHO solutions ingested during the early (90 min) stages of exercise.

Instead, the similar rates of ingested CHO oxidation from glucose and maltose solutions (only the latter of which undergoes any intestinal digestion), suggests that the rate limiting step for ingested di-, and probably oligosaccharide utilisation by the active muscles exists distal to intestinal digestion, and could be either the transport of glucose into the systemic blood supply or the rate of muscle glucose oxidation.

Recently Moodley et al. (1992) reported that the rate of ingested CHO oxidation from a 10 g/100 ml, ten and 22 chain-length glucose polymer solutions was greater than that from an isocaloric glucose solution. This finding may have occurred because the rate of gastric emptying was faster for the glucose polymers than for the glucose solution, or because of a more rapid transport of glucose into the blood resulting from significantly greater glucose absorption from short chain glucose oligomers than from free glucose, as has been proposed (Jones et al. 1983, 1987).

With CHO's of increasing complexity, however, rates of digestion might become limiting and slow the rate at which ingested CHO can be utilised by the working muscles during exercise. Accordingly, this study examined the influence of glucose polymer chain length on the rates of gastric emptying and ingested CHO oxidation from a 22 chain-length glucose polymer solution, and `soluble' starch, the longest glucose polymer that can be ingested in liquid suspension form.

5.2 Results

5.2.1 Subjects characteristics

Six male competitive triathletes or cyclists participated in this study. The mean (±SEM) age, height mass and VO_{2max} of the subjects was 27 ±1.5 yr, 1.82 ±0.02 m, 82.1 ±2.2 kg, and 4.9 ±0.2 L/min, respectively.

5.2.2 Metabolic responses to exercise

Table 5.1 shows the steady-state gas exchange data and the total CHO oxidation during the two 90-min rides. There were no significant differences either between trials or over time for VO_2, VCO_2, RER, and calculated CHO oxidation.

Table 5.1 Steady-state gas exchange data and total calculated carbohydrate oxidation during the two 90 minute rides after the ingestion of 15 g/100 ml glucose polymer and 'soluble' starch drinks

	VO_2 (L/min)	VCO_2 (L/min)	RER	Total CHO_{ox} (g)
Glucose polymer	3.28 0.10	3.01 0.10	0.91 0.02	266.8 17.1
'Soluble' starch	3.29 0.10	3.02 0.08	0.91 0.01	263.6 11.8

VO_2, oxygen consumption; VCO_2, carbon dioxide production; RER, respiratory exchange ratio; Total CHO_{ox}, total carbohydrate oxidation. Values are mean ±SEM of measurements taken at successive 20 minute intervals during the experimental trial.

5.2.3 Gastric emptying, intestinal carbohydrate delivery and solute osmolality

Table 5.2 lists the volume of each drink that was emptied during the two 90-min rides and the calculated amount of CHO delivered to the intestine from these solutions. Again, there were no differences in any of these variables between trials. The osmolality of the glucose polymer solution was 62 mosM while that of the `soluble' starch rapidly became zero as the starch precipitated.

Table 5.2 Gastric emptying and intestinal carbohydrate delivery during the two 90 minute rides after the ingestion of 15 g/100 ml glucose polymer and 'soluble' starch drinks

	Total drink emptied (ml)	Intestinal CHO delivery (g)
Glucose polymer	813.0	122.0
	53.2	8.0
'Soluble' starch	919.0	138.0
	47.2	7.1

Intestinal CHO delivery, intestinal carbohydrate delivery calculated from the product of the volume of the drink emptied and the carbohydrate content of the solution. The total volume of the ingested drink was 1.2 L, consumed as a 400 ml pre-exercise loading bolus plus eight feedings of 100 ml/10 min. Values are mean ±SEM.

5.2.4 Ratings of perceived exertion and stomach fullness

There were also no significant differences between trials or over time for RPE. In the glucose polymer trial, RPE rose gradually from 10.5 ±0.8 after ten minutes of exercise to 13.5 ±0.6 after 89 min of exercise. In the `soluble' starch trial, RPE rose from 10.9 ±0.7 after ten minutes of exercise to 13.9 ±0.7 at the end of 90 min of exercise. The subjective ratings of stomach fullness showed a pattern similar to those of RPE. Ratings of stomach fullness rose slightly from 1.0 ±0.3 after ten minutes of exercise to 2.2 ±0.6 at the end of the 90 min ride in the glucose polymer trial, and from 1.1 ±0.3 to 2.0 ±0.2 for the same time periods in the `soluble' starch trial. These values were also not significantly different.

5.2.5 Plasma glucose and plasma insulin concentrations

Table 5.3 shows the plasma glucose and plasma insulin concentrations during the two experimental rides. Plasma glucose concentrations at rest in the glucose polymer and `soluble' starch trials were 5.2 ±0.2 and 4.9 ±0.3 mmol/L, respectively. Throughout both trials glucose concentrations remained elevated (5.5-5.7 mmol/L for glucose polymer versus 4.6-6.0 mmol/L for `soluble' starch) but were not significantly different. Plasma insulin concentrations at rest were 4.9 ±0.3 and 5.3 ±0.7 uU/ml for glucose polymer and `soluble' starch respectively, and remained relatively constant throughout the 90 min of exercise (Table 5.3). Although plasma insulin concentrations were slightly lower during the `soluble' starch trial than in the glucose polymer trial, these differences were not significant.

Table 5.3 Plasma glucose and plasma insulin concentrations during the two 90 minute rides after the ingestion of 15 g/100 ml glucose polymer and 'soluble' starch drinks.

Exercise duration (min)	Rest	10	30	50	70	89
Glucose (mmol/L)						
GP	5.2	5.6	5.7	5.6	5.5	5.5
	0.2	0.3	0.2	0.1	0.2	0.2
SS	5.4	4.8	5.0	5.2	5.1	5.5
	0.2	0.4	0.3	0.2	0.2	0.2
Insulin (uU/ml)						
GP	4.9	5.5	6.0	4.6	5.4	5.2
	0.3	0.5	0.8	0.5	0.4	0.6
SS	5.3	4.7	4.7	4.4	4.1	3.9
	0.7	0.5	0.3	0.4	0.3	0.1

GP, glucose polymer; SS, 'soluble' starch. Values are mean ±SEM.

5.2.6 Appearance of U-^{14}C labelled carbohydrate in the plasma

In contrast to plasma glucose and plasma insulin concentrations, which did not differ between trials, the appearance of the U-^{14}C label in the plasma from the ingested `soluble' starch occurred more rapidly than from the glucose polymer solution (Figure 5.1; P <0.001).

With glucose polymer ingestion, U-^{14}C counts increased from 410 ±94 dpm/ml after ten minutes of exercise to 810 ±111 dpm/ml at the end of the exercise bout, whereas after ingestion of `soluble' starch, the corresponding counts were 960 ±152 and 1,540 ±234 dpm/ml (Figure 5.1; P <0.001).

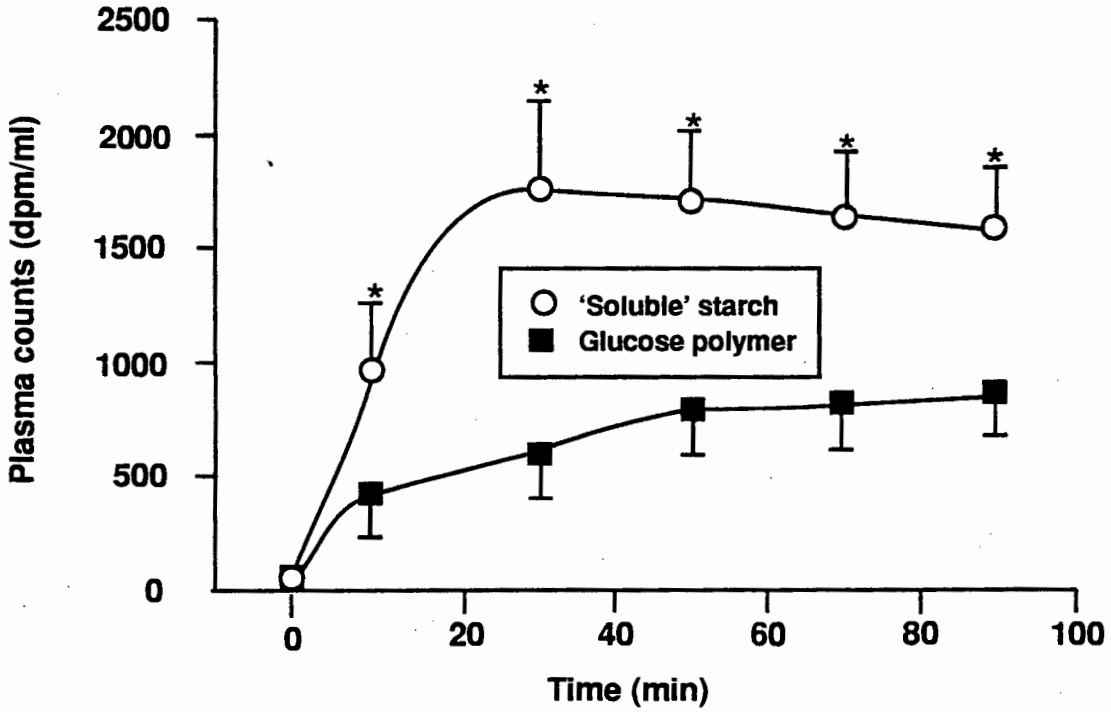

Figure 5.1. The appearance in plasma of the U-^{14}C label from the 'soluble' starch preparation and glucose polymer solution. * 'Soluble' starch significantly greater than glucose polymer, P <0.001. Values are mean ±SEM.

5.2.7 Rates of ingested carbohydrate oxidation

The rate of ingested CHO oxidation for the ingested glucose polymer solution increased from 0.03 ±0.01 g/min after ten min to 0.9 ±0.08 g/min at the end of exercise. When the amount of ingested CHO oxidised was expressed as a percentage of the intestinal CHO delivered, only 40.9 ±8.1% of the CHO delivered to the intestine was oxidised for the glucose polymer solution. In absolute terms the 49.6 g of CHO oxidation derived from the ingested glucose polymer solution accounted for 19% of the total CHO oxidation.

Unfortunately the rates of ingested CHO oxidation from the `soluble' starch suspension could not be determined accurately due to differences between the physical structure and composition of the U-^{14}C tracer and the ingested starch preparation.

A retrospective analysis of the composition of the commercially available U-^{14}C starch label with that of the unlabelled ingested starch revealed that whereas the `soluble' starch label was 100% amylopectin, the ingested starch preparation contained 24% amylose and only 76% amylopectin.

X-ray powder diffraction analyses of the ingested `soluble' starch and a 100% amylopectin starch preparation similar to the U-^{14}C starch label used in this investigation confirmed that there were major physical differences between the two samples. The presence of sharp, well defined peaks for the amylose-amylopectin maize starch (Figure 5.2b) indicates a regular crystaline structure (Byrn 1982). In complete contrast, the diffuse spectrum obtained for the 100% amylopectin starch (Figure 5.2a) indicates that either the average particle size is less than 10 um, or that the substance is amorphous.

That the amylopectin particles were indeed amorphous was confirmed by subsequent electron microscopy of the preparations. First, the particles were > 30 um in diameter and second, they had variable morphology with no well defined crystal faces (Figure 5.3a). In contrast, the particles of the amylose-amylopectin preparation were < 20 um in diameter and were comprised of oval deposits, spherules and chunks with well defined crystal faces (Figure 5.3b).

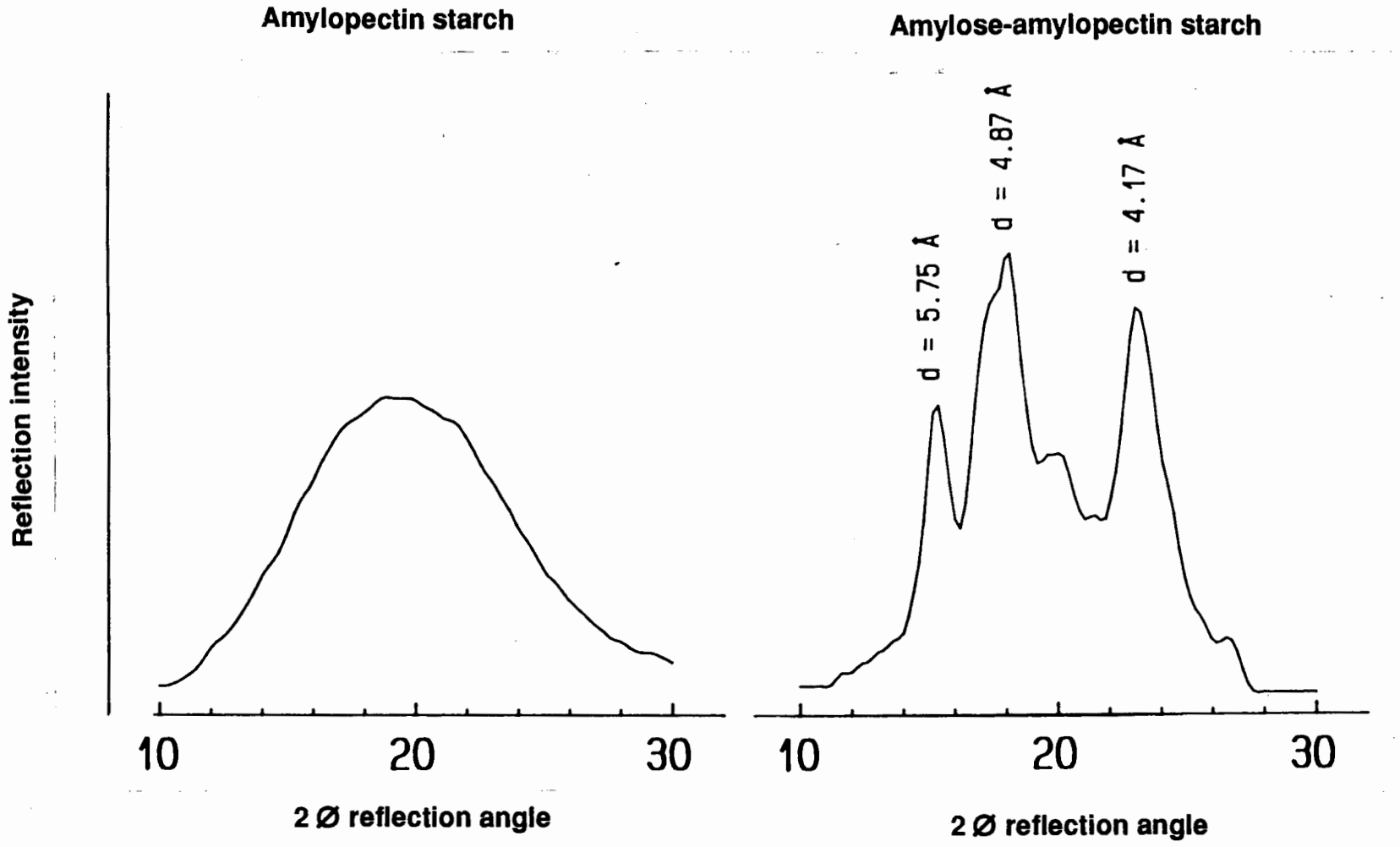

Figure 5.2. *X*-ray diffraction spectra for **(A)** the 100% amylopectin starch, and **(B)** the amylose-amylopectin starch.

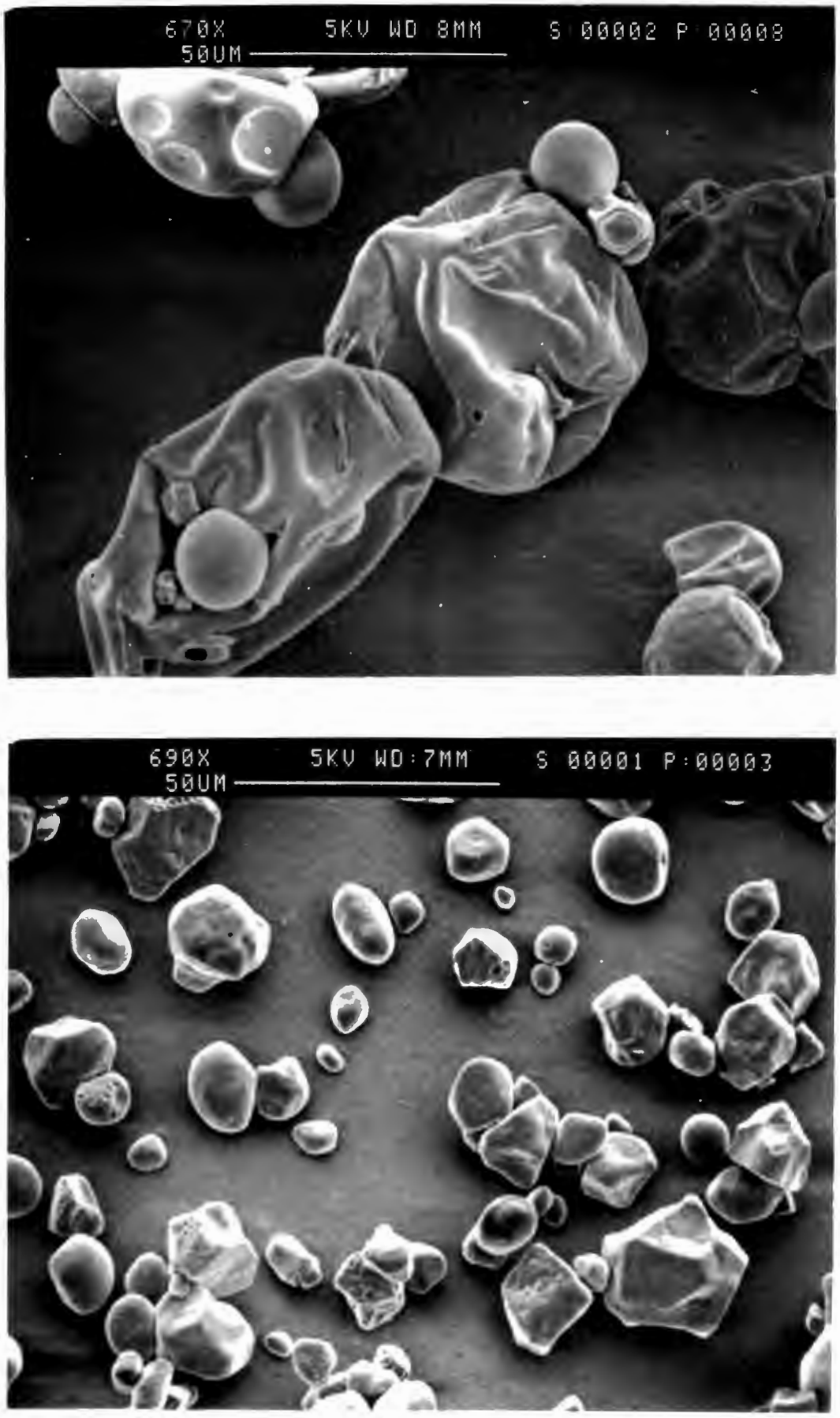

Figure 5.3. Scanning electron micrograph showing **(A)** typical distribution of amylopectin particles (Magnification 670 x) and **(B)** typical distribution of the particles from amylose-amylopectin maize-starch (Magnification 690 x).

5.3 Discussion

The first finding of this study was that differences in the physical structure and composition of the label and ingested starch led to the U-^{14}C `soluble' starch tracer failing to track the bulk of the ingested (unlabelled) starch.

A retrospective comparison of the commercially available U-^{14}C starch label with that of the unlabelled ingested starch revealed that whereas the `soluble' starch label was 100% amylopectin, the ingested starch preparation contained 24% amylose and only 76% amylopectin. Previous studies have shown that starches with a high amylopectin content are digested and absorbed more rapidly than those with a high amylose content (Behall et al. 1988; Berry 1986; Goddard et al. 1984). The glucose chains of amylose starch are bound more tightly by hydrogen bonds making them less available for amylitic attack than the many branched chains of glucose in amylopectin (Leach 1965). Thus, it is almost certain that the starch label used in the current investigation would have been digested and absorbed far more rapidly than the (unlabelled) ingested starch. A failure of the U-^{14}C label to track the tracee resulted in erroneously elevated plasma glucose specific activities and a subsequent overestimation of the rates of ingested starch oxidation.

More direct evidence that the rates of oxidation of ingested `soluble' starch would have been overestimated comes from recent experiments conducted in our laboratory (JA Hawley, SC Dennis, KD Derman, TD Noakes, unpublished observations). Results from these studies reveal that in trained subjects who ingested a 24% amylose-76% amylopectin starch preparation during 120 min of cycling at 63% of PPO, plasma glucose (plus lactate) oxidation peaked at 0.65 g/min after 60-70 min of exercise, and thereafter remained relatively constant for the remainder of the exercise bout. This finding, along with the more rapid appearance of the U-^{14}C label in the plasma after the ingestion of starch, compared to glucose polymer (Figure 5.1), confirm that the rates of ingested `soluble' starch oxidation were, indeed, spurious.

In this regard, Saris et al. (1993) recently compared the rates of ingested CHO oxidation of two naturally enriched ^{13}C starch preparations of different solubility ingested repetitively by eight subjects during 150 min of moderate-intensity cycling (i.e. 60% of PPO). The starch preparations were a soluble partially hydrolysed corn starch and an insoluble corn starch, both ingested at concentrations of 15 g/100 ml. Both the mean (0.84 ±0.21 versus 0.50 ±0.15 g/min) and peak (1.10 ±0.18 versus 0.81 ±0.25 g/min) rates of ingested CHO oxidation were significantly higher ($P < 0.05$) after ingestion of the soluble, compared to the insoluble starch. More to the point, they found that adding an extrinsic tracer ($^{13}C_6$-glucose) to the insoluble starch resulted in a significant overestimation (i.e. 1.23 ±0.41 g/min) of the rates of ingested CHO oxidation. Saris et al. (1993) concluded that "*soluble CHO was oxidised at a higher rate during exercise than an isocaloric insoluble CHO*" and that "*the addition of a soluble ($^{13}C_6$-glucose) tracer to an insoluble starch leads to overestimation of the exogenous* (ingested) *CHO oxidation rates.*"

With respect to the ingestion of glucose polymers during exercise, it has been proposed that, because of their lower osmolalities, these solutions would be preferable to isocaloric glucose solutions as a source of ingested CHO (Murray 1987). Indeed, several studies have shown that the rates of gastric emptying of glucose polymer solutions are faster than those of isocaloric glucose solutions (Foster et al. 1980; Owen et al. 1986; Sole and Noakes 1989).

However, the second finding of the current investigation was that, despite differences in osmolality, the rates of gastric emptying for glucose polymer and `soluble' starch drinks were not significantly different. Hunt (1960) also observed that isocaloric solutions of starch and glucose left the stomach at the same rate, despite marked differences in their osmotic pressures. Possibly the osmolalities of the solutions tested in the present study were too low for any effect of osmolality to be apparent. Alternatively, the retarding effect of osmolality on gastric emptying may have been overridden in this trial by the effects of a large intragastric volume produced by the initial bolus feeding and repetitive

ingestion (Noakes et al. 1991a). The similar rates of gastric emptying for glucose polymer and starch preparations in the present study, along with the comparable rates found for isocaloric solutions of glucose and maltose (Chapter Four) again suggest that the drinking pattern of an athlete during exercise is a more important determinant of gastric emptying than is solute osmolality.

The peak rate of ingested CHO oxidation found in the current study from the 15 g/100 ml, 22 chain-length glucose polymer solution (0.9 g/min) is very similar to the peak rates reported by others for maltodextrins with varying chain-lengths (Massicotte et al. 1989; Moodley et al. 1992; Rehrer et al. 1992). It also matches the peak rate of ingested CHO oxidation found for isocaloric solutions of maltose and glucose ingested under identical experimental conditions (Chapter Four). As maltodextrins are rapidly hydrolised in the small intestine before subsequent glucose absorption (Kenny and Maroux 1982), it is, perhaps, not surprising that the metabolic response to the ingestion of a short-chain (i.e. 22 glucose units) glucose polymer is similar to that found after the ingestion of equally concentrated glucose or maltose solutions. Although Jones et al. (1987) report more rapid glucose absorption from short-chain (i.e. three to 11 glucose units) oligomers than from free glucose, the results of this and the previous investigation (Chapter Four) provide additional evidence that intestinal glucose digestion and absorption may not ultimately limit the rate at which ingested CHO is utilised by the working muscles, at least for glucose oligomers with a chain length less than 22 glucose units.

Rather, it would appear that provided that the CHO is ingested sufficiently frequently in appropriate volumes, there will not be any physiologically important differences in the rates of ingested CHO oxidation from glucose, maltose and glucose polymers ingested during exercise. During prolonged exercise all these ingested CHO's are ultimately oxidised at a rate of approximately 1 g/min.

In summary, the results of the current study provide evidence that neither gastric emptying nor the rate of ingested CHO oxidation is influenced by increasing the glucose polymer

chain length to 22 glucose units, at least for concentrations up to 15 g/100 ml. Rather, it would appear that the rate of ingested CHO oxidation must be either limited by a regulated transport of glucose into the systemic blood supply regardless of the type of CHO ingested, or by the rate of working muscle glucose uptake and oxidation.

CHAPTER SIX

EFFECTS OF GLUCOSE INGESTION AND GLUCOSE INFUSION ON FUEL SUBSTRATE KINETICS DURING PROLONGED, MODERATE-INTENSITY EXERCISE

6.1 Introduction

Results from the experiments described in Chapters Four and Five have shown that the peak rates of ingested CHO oxidation are limited to ~1 g/min at the end of 90 min moderate-intensity exercise. These findings agree with the results from other studies utilising both arteriovenous balance methods and isotopic tracer techniques which have shown that the peak rates of plasma glucose oxidation also appear to be limited to around 1 g/min (Bosch et al. 1993a; Broberg and Sahlin 1989; Coggan et al. 1991; Stein et al. 1989). Whether the rate of exogenous glucose oxidation is limited by the rate of digestion, absorption and subsequent transport of ingested glucose into the systemic blood supply, *or* by the rate of glucose uptake and oxidation by the exercising muscles has yet to be determined. Therefore, the first aim of the current study was to determine if intravenous glucose infusion, which by-passes both intestinal absorption and hepatic glucose uptake, might lead to rates of muscle glucose oxidation greater than the peak rates of ~1 g/min measured after CHO ingestion.

As hepatic glucose appearance is known to be greater after administration of an oral glucose load than with peripheral intravenous glucose infusion, despite the maintenance of similar insulin and glucagon concentrations (Abumrad et al. 1982; Barrett et al. 1985; Bergman et al. 1982; DeFronzo et al. 1978; Ishida et al. 1983), an additional purpose of the current study was to evaluate the effects of euglycaemic (i.e. 5 mmol/L) concentrations of glucose, maintained by either CHO ingestion or intravenous glucose infusion, on the rates of liver glucose turnover during exercise.

6.2 Results

6.2.1 Subjects characteristics

Ten experienced endurance-trained cyclists participated in this investigation. In order to minimise the subject's exposure to radioactivity in these investigations, they were divided into two groups (n = five/group) and any one subject only performed one experimental trial. The characteristics of the subjects are displayed in Table 6.1. There were no significant differences for any of the variables measured.

Table 6.1 Subject characteristics

	CHO ingestion	Glucose infusion
Age (yr)	25.8 0.7	23.8 0.9
Mass (kg)	80.4 2.2	78.9 1.9
FFM (kg)	67.7 1.9	67.4 0.7
PPO (W)	411.6 26.6	388.9 15.2
VO_2max (L/min)	4.98 0.21	4.84 0.15
Workrate at 63% PPO (W)	260.4 14.4	246.2 8.0

CHO ingestion, subjects ingested carbohydrate (a 15 g/100 ml U^{14}-C glucose solution) throughout exercise; Glucose infusion, subjects received a variable-rate intravenous glucose infusion throughout exercise; FFM, fat free mass; PPO, peak sustained power output attained during the maximal test; VO_2max, maximal oxygen consumption. All values are mean ±SEM of 5 subjects/group.

6.2.2 Rates of glucose infusion and ingestion

The rate of intravenous glucose infusion required to maintain plasma glucose at 5 mmol/L increased progressively during exercise (Figure 6.1). For the first 25 min of the ride, the rate of glucose infusion averaged 18.0 ±4.9 umol/min/kg FFM and increased to 60.8 ±8.7 umol/min/kg FFM during the 105-125 min period of exercise ($P < 0.01$). The total amount of glucose infused in order to maintain euglycaemia during the 125 min ride was 3.92 ±0.92 mmol/kg FFM (47.8 ±11.6 g). In contrast, far more glucose was consumed during the CHO ingestion trial (19.7 umol/min/kg FFM, or 240 g; Figure 6.1).

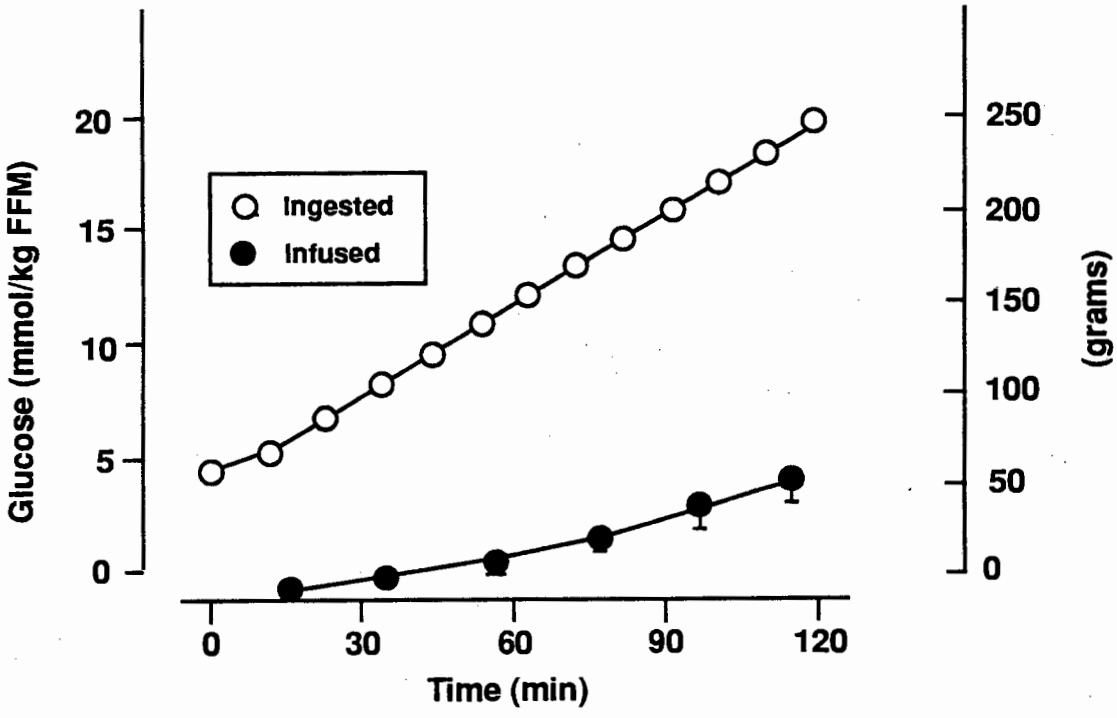

Figure 6.1. The cumulative amount of carbohydrate ingested and the rate of intravenous glucose infusion required to maintain euglycaemia (a plasma glucose concentation of 5 mmol/L) during 125 minutes of exercise. Values are mean ±SEM.

6.2.3 Plasma glucose and plasma insulin concentrations

Figure 6.2 displays the plasma glucose and plasma insulin concentrations measured during the two experimental rides. Glucose ingestion and glucose infusion both maintained euglycaemia during the 125 min rides. With CHO ingestion, plasma glucose concentration averaged 5.3 ±0.13 mmol/L (range 4.6 ±0.60 to 5.7 ±0.26 mmol/L), and with glucose infusion it averaged 5.0 ±0.09 mmol/L (range 4.6 ±0.34 to 5.36 ±0.29 mmol/L). However, despite the similar plasma glucose concentrations in the two experimental trials, CHO ingestion resulted in a significantly higher average plasma insulin concentration throughout exercise than did intravenous glucose infusion (12.9 ±1.0 versus 4.74 ±0.5 mU/ml respectively, $P < 0.001$). During the CHO ingestion trial, plasma insulin concentration increased significantly from 7.1 ±0.95 mU/ml at rest to 12.2 ±2.3 mU/ml after only 5 min of exercise ($P < 0.05$). Thereafter plasma insulin concentration continued to climb to 17.6 ±4.2 mU/ml after 25 min ($P < 0.05$ compared to rest) and then declined to 9.7 ±3.3 mU/ml at the end of exercise. In contrast, plasma insulin concentration was significantly lower after 25 min of exercise compared to resting values with intravenous glucose infusion (5.3 ±1.6 versus 8.3 ±2.3 mU/ml respectively, $P < 0.05$) and eventually declined to 3.9 ±0.9 mU/ml at the end of the ride.

6.2.4 Glucose turnover

During the CHO ingestion trial, total (endogenous plus ingested) glucose Ra and Rd (not shown) increased significantly throughout exercise to 81 ±21 umol/min/kg FFM during the last 20 min of the ride ($P < 0.05$ compared to first 20 min; Figure 6.3, left panel). At that stage, the contribution of ingested glucose to the total Ra was 65 ±13 umol/min/kg FFM or 81 ±7%.

In the glucose infusion trial, total glucose (endogenous plus infused) Ra increased to 64 ±68 umol/min/kg FFM during the last 20 min of the ride ($P < 0.05$ compared to first 20 min; Figure 6.3, right panel). Here the contribution from infused glucose to total Ra was 61 ±9 umol/min/kg FFM, or 93 ±5%. Although glucose Ra values tended to be lower

with intravenous glucose infusion than with CHO ingestion, these differences were not statistically significant.

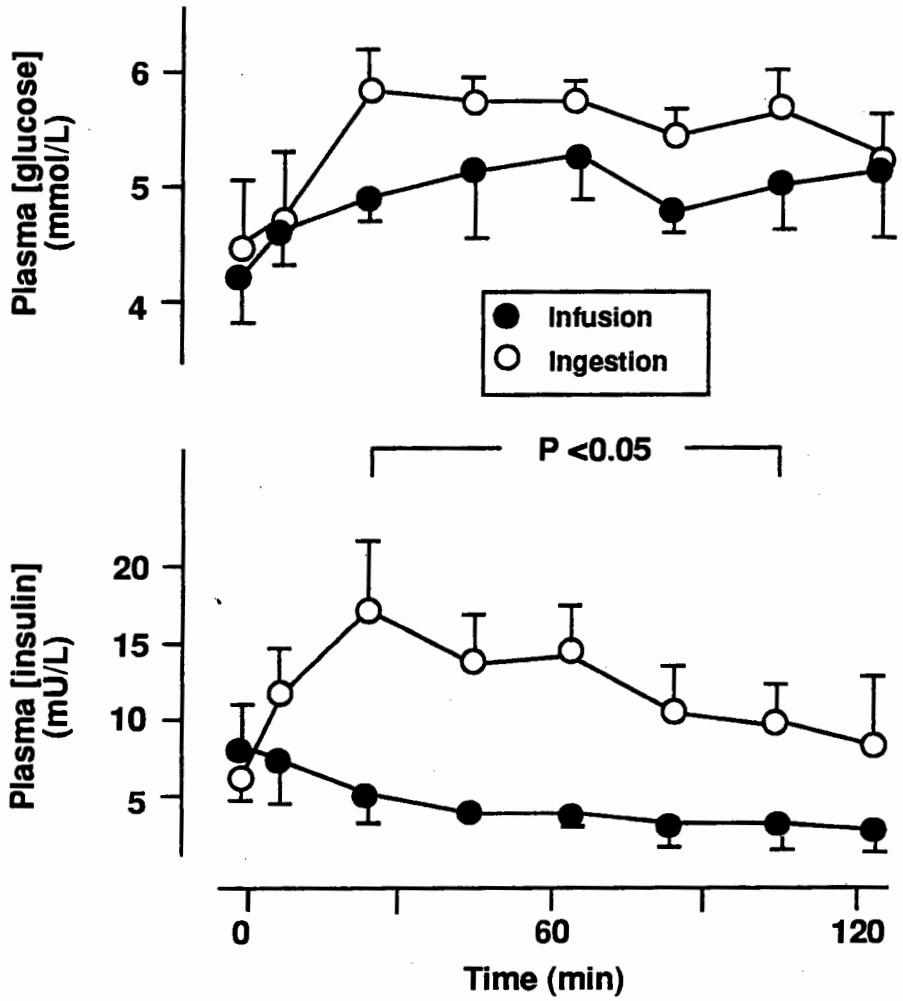

Figure 6.2. Plasma glucose and plasma insulin concentrations during 125 minutes of cycling when subjects either ingested carbohydrate or received a variable-rate intravenous glucose infusion. Plasma insulin concentrations during carbohydrate ingestion trial significantly greater than during glucose infusion trial between 25 and 125 min, $P < 0.05$. Values are mean ±SEM.

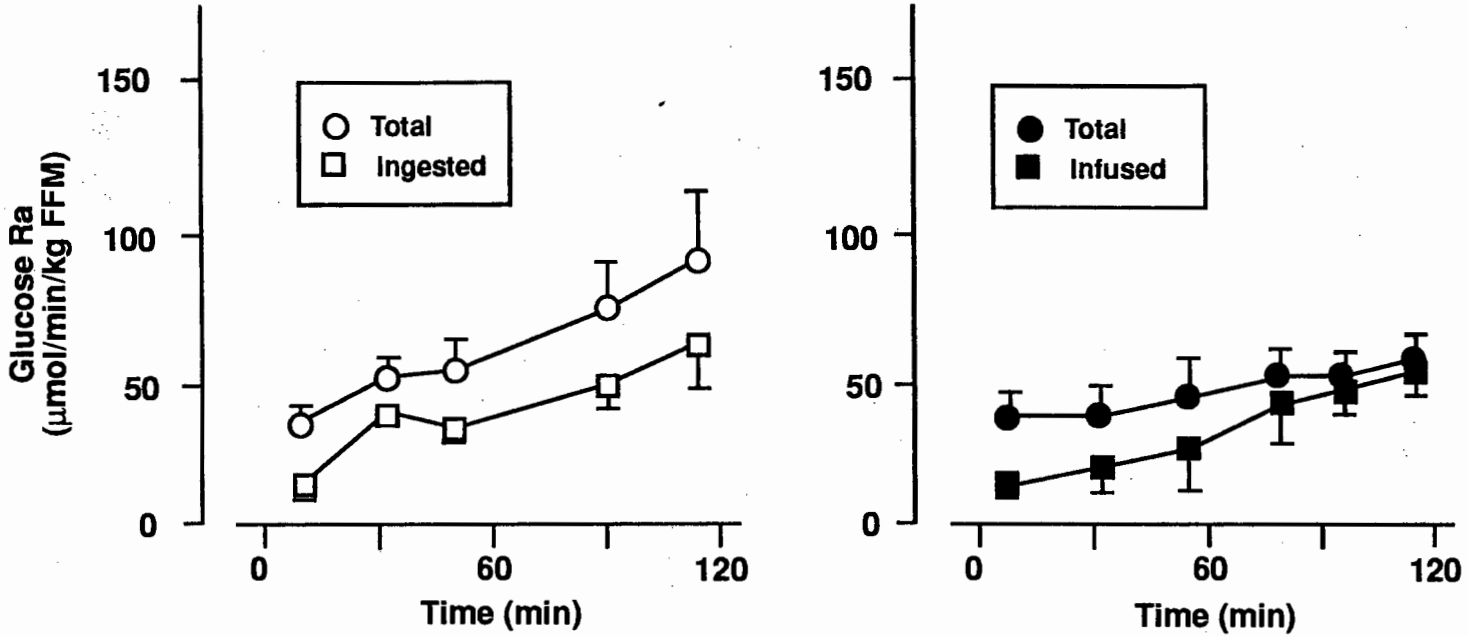

Figure 6.3. The rates of whole-body glucose appearance (Ra) during 125 minutes of cycling when subjects either ingested carbohydrate or received a variable-rate glucose infusion. Values are mean ±SEM.

6.2.5 Rates of plasma glucose oxidation

Evidence to suggest that the higher plasma insulin concentrations in the CHO ingestion trial *may* have increased glucose turnover and oxidation comes from the measurements of the rates of plasma glucose oxidation (Rox) in the two experimental rides (Figure 6.4).

Whereas during the CHO ingestion trial, Rox increased to a final value of 93 ±8 umol/min/kg FFM within 90 min, the final Rox value with intravenous glucose infusion was 85 ±5 umol/min/kg FFM and still climbing. Hence, CHO ingestion significantly increased Rox values from 25 to 105 min ($P < 0.05$; Figure 6.4).

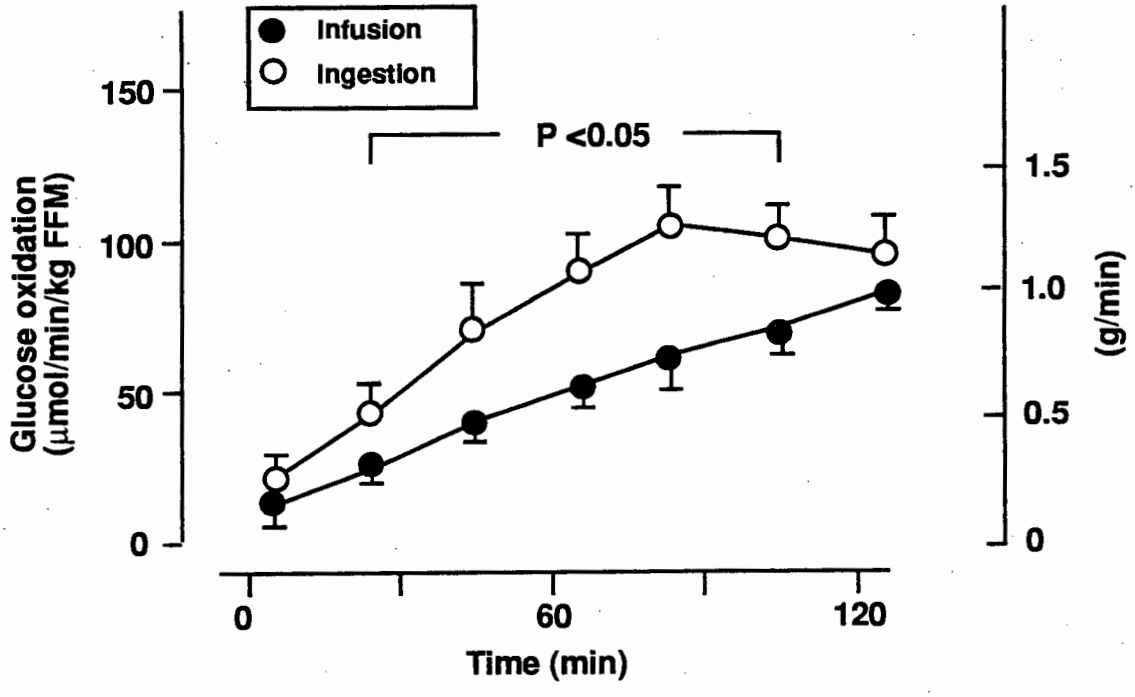

Figure 6.4. The rate of plasma glucose oxidation (Rox) during 125 minutes of cycling when subjects either ingested carbohydrate or received a variable-rate intravenous glucose infusion. Values are mean ±SEM.

6.2.6 Total carbohydrate and fat oxidation

Table 6.2 shows the steady-state gas exchange data (VO_2, VCO_2, RER) during the two experimental rides, while the rates of CHO and fat oxidation expressed relative to FFM are shown in Table 6.3.

During the glucose ingestion trial the rate of CHO oxidation declined from 306.3 ±19.1 umol/min/kg FFM after 5 min of exercise to 282.2 ±21.6 umol/min/kg FFM at the end of the ride (Table 6.3). This non significant decline in CHO oxidation was associated with a concomitant rise in the rate of fat oxidation from 10.6 ±2.7 umol/min/kg FFM to 20.5 ±4.6 umol/min/kg FFM, such that the contribution from fat oxidation to the total energy demands of exercise increased from 10.9 ±4.7% after 5 min of exercise to 18.2 ±4.5% at the end of the 125 min ride (Figure 6.5).

In contrast, despite a higher absolute work rate, RER values (Table 6.2) and calculated rates of CHO oxidation (Table 6.3) were significantly lower throughout the glucose infusion trial than when subjects ingested CHO. With intravenous glucose infusion, CHO oxidation declined from 223 ±32 umol/min/kg FFM after 5 min of exercise to 178 ±35 umol/min/kg FFM at the end of the ride (Table 6.3). Accordingly, calculated rates of fat oxidation were significantly higher throughout the glucose infusion trial ($P < 0.05$; Table 6.3), such that the contribution from fat oxidation to the total energy demands of exercise reached 51 ±10% at the end of the ride (Figure 6.5).

Table 6.2. Steady-state gas exchange data during 125 minutes of cycling when subjects either ingested carbohydrate or received a varable-rate intravenous glucose infusion.

Exercise duration (min)	5	25	45	65	85	105	125
VO_2 (L/min)							
Ingestion	3.14 0.04	3.13 0.05	3.19 0.08	3.16 0.10	3.22 0.10	3.23 0.13	3.23 0.13
Infusion	3.51* 0.15	3.53* 0.16	3.50* 0.14	3.50 0.16	3.54 0.16	3.63* 0.15	3.72* 0.17
VCO_2 (L/min)							
Ingestion	3.03 0.07	3.04 0.08	3.06 0.10	3.00 0.12	3.09 0.14	3.05 0.14	3.04 0.15
Infusion	3.06 0.04	3.05 0.04	3.00 0.04	2.98 0.07	3.01 0.04	3.05 0.06	3.10 0.07
RER							
Ingestion	0.97# 0.02	0.97# 0.02	0.96# 0.01	0.95# 0.02	0.95# 0.02	0.94# 0.01	0.94# 0.01
Infusion	0.88 0.03	0.87 0.04	0.86 0.03	0.85 0.03	0.85 0.03	0.84 0.03	0.84 0.03

Ingestion, subjects ingested carbohydrate (a 15 g/100 ml U14-C glucose solution) throughout exercise; Infusion, subject received a variable-rate intravenous glucose infusion throughout exercise; VO2, oxygen consumption; VCO2, carbon dioxide production; RER, respiratory exchange ratio. *VO2 measured during the glucose infusion trial significantly greater than the carbohydrate ingestion trial, $P < 0.05$; #RER measured during the carbohydrate ingestion trial significantly greater than the glucose infusion trial. All values are mean ±SEM.

Table 6.3 Rates of carbohydrate and fat oxidation during 125 minutes of cycling when subjects either ingested carbohydrate or received a variable-rate intravenous glucose infusion.

Exercise duration (min)	5	25	45	65	85	105	125
CHO oxidation (g/min)							
Ingestion	3.73#	3.78#	3.69#	3.52#	3.74#	3.49#	3.45#
	0.04	0.29	0.23	0.25	0.37	0.26	0.30
Infusion	2.69	2.56	2.40	2.34	2.31	2.21	2.15
	0.37	0.21	0.40	0.29	0.43	0.40	0.40
CHO oxidation (μmol/min/kg FFM)							
Ingestion	306.2#	310.3#	303.0#	288.3#	306.6#	286.8#	282.2#
	19.9	21.9	16.7	17.5	28.4	18.1	21.6
Infusion	222.3	212.6	207.6	193.4	191.7	183.5	177.8
	32.3	38.6	39.9	25.6	36.9	34.6	34.7
Fat oxidation (g/min)							
Ingestion	0.17	0.15	0.21	0.26	0.21	0.31	0.32
	0.09	0.10	0.07	0.06	0.11	0.06	0.06
Infusion	0.74*	0.79*	0.84*	0.87*	0.90*	0.98*	1.04*
	0.21	0.24	0.21	0.18	0.23	0.22	0.22
Fat oxidation (μmol/min/kg FFM)							
Ingestion	10.6	13.5	13.7	16.6	17.7	19.4	20.5
	2.7	4.6	4.2	4.1	4.8	3.9	4.6
Infusion	45.7*	45.7*	52.4*	53.9*	55.8*	60.9*	64.9*
	12.6	16.6	12.8	10.8	13.3	13.2	13.6

Ingestion, subjects ingested carbohydrate (a 15 g/100 ml U^{14}-C glucose solution) throughout exercise; Infusion, subjects received a variable-rate intravenous glucose infusion throughout exercise; # Rates in carbohydrate ingestion trial significantly greater than in glucose infusion trial, $P < 0.05$; * Rates in glucose infusion trial significantly greater than in carbohydrate ingestion trial, $P < 0.05$. All values are mean ±SEM.

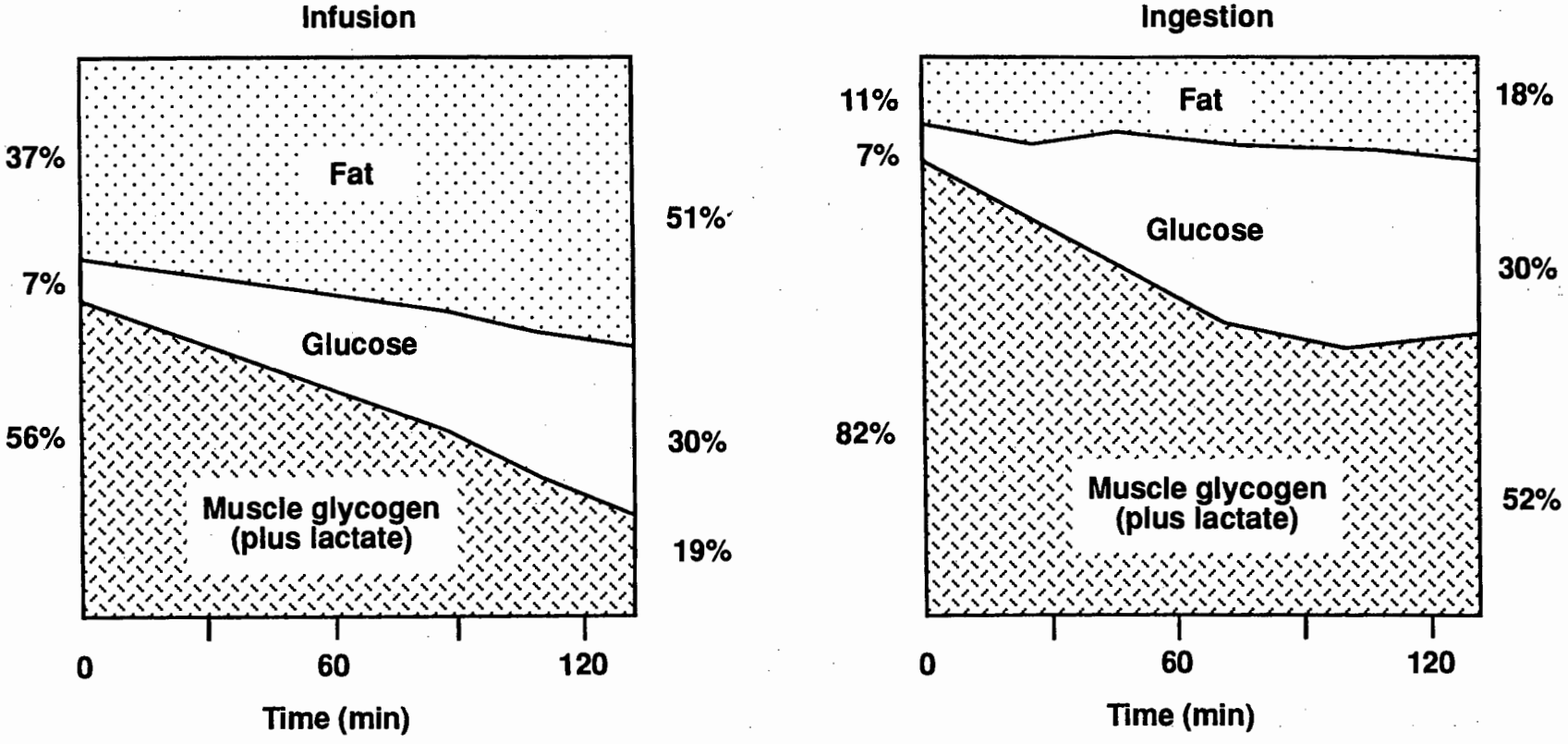

Figure 6.5. The relative contribution of fuel substrates to total energy production during 125 minutes of cycling when subjects either ingested carbohydrate or received a variable-rate intravenous glucose infusion. The contribution from muscle glycogen (plus lactate) was estimated from the difference between the total carbohydrate oxidation and total plasma glucose oxidation.

Ingested CHO oxidation increased significantly from 0.32 ±0.13 g/min after 25 min of exercise to 0.98 ±0.13 g/min at the end of the 125 min ride ($P < 0.01$) and accounted for 82.8 ±11.3 g (17.5 ±1.4%) of total CHO oxidation during the 125 min ride. The contribution of ingested CHO oxidation to total CHO oxidation also increased significantly from 8.7 ±3.8% after 25 min of exercise to 27.6 ±1.9% at the end of the ride ($P < 0.01$).

Areas under the total CHO oxidation and plasma glucose oxidation versus time curves during the two rides are shown in Figure 6.6. Both total CHO oxidation (37.2 ±2.8 mmol/125 min/kg FFM versus 24.1 ±3.9 mmol/125 min/kg FFM; $P < 0.05$) and total plasma glucose oxidation (9.5 ±1.2 umol/125 min/kg FFM versus 6.2 ±0.74 umol/125 min/kg FFM; $P < 0.05$) were significantly higher with CHO ingestion compared to glucose infusion (Figure 6.6). The contribution from muscle glycogen (plus lactate) utilisation (as estimated from the difference between the total CHO oxidation and total plasma glucose oxidation) was also greater with CHO ingestion versus intravenous glucose infusion (ingestion 27.6 ±4.6 mmol/125 min/kg FFM versus 17.8 ±4.6 mmol/125 min/kg FFM), but these differences only became statistically significant after 125 min ($P < 0.05$).

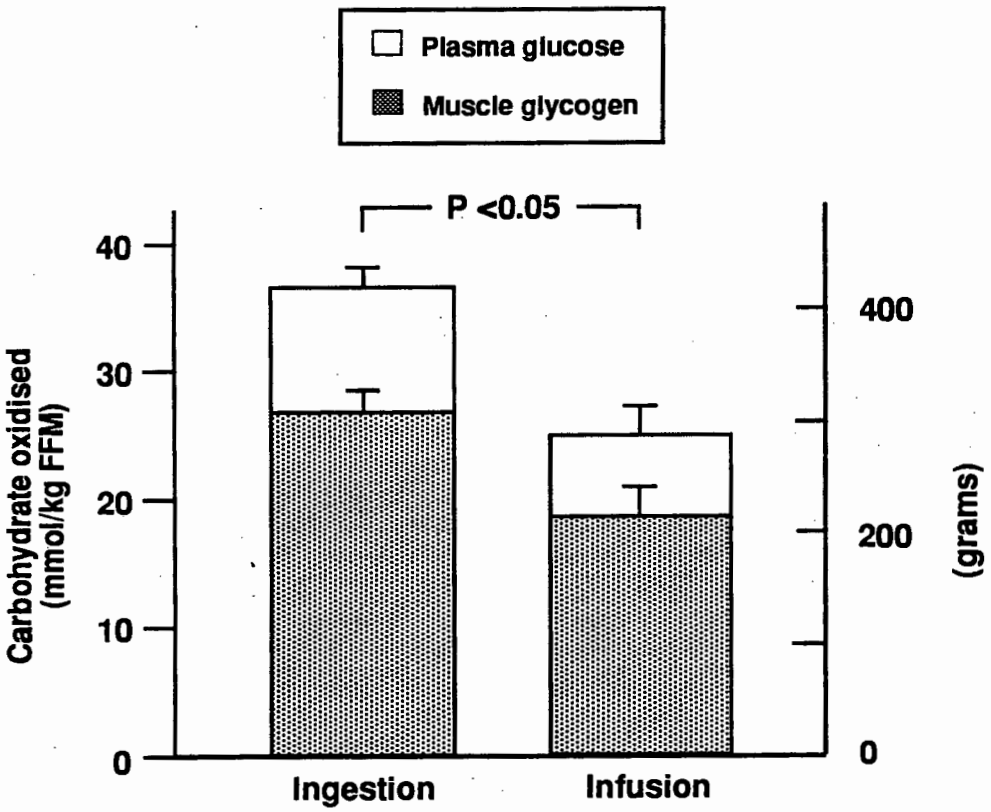

Figure 6.6. Carbohydrate oxidation during 125 minutes of cycling when subjects either ingested carbohydrate or received a variable-rate intravenous glucose infusion. Both total carbohydrate oxidation and plasma glucose oxidation were significantly greater with carbohydrate ingestion than with glucose infusion, $P < 0.05$. The contribution from muscle glycogen (plus lactate) was estimated from the difference between the total carbohydrate oxidation and total plasma glucose oxidation. Values are mean ±SEM.

6.3 Discussion

The first finding of the present study was that when CHO was ingested to maintain plasma glucose concentration at 5 mmol/L, plasma insulin concentrations were significantly greater throughout most of the 125 min ride than when euglycaemia was maintained via intravenous glucose infusion (Figures 6.1 and 6.2). Higher plasma insulin concentrations with CHO ingestion could probably result from the large differences in the amounts of glucose administered (Figure 6.1). Whereas 240 g of glucose was ingested during the 125 min of exercise, only 48 ±12 g was required to maintain euglycaemia during the intravenous glucose infusion.

Alternatively, the differences in plasma insulin concentrations may have been due to the routes of glucose administration. Glucose ingestion is known to cause a greater insulin secretion than intravenous glucose infusion (Abumrad et al. 1982; Barrett et al. 1985; Bergman et al. 1982; DeFronzo et al. 1978; Ishida et al. 1983).

Despite the differences in plasma insulin concentrations (Figure 6.2), there were no measurable differences in the rates of glucose appearance (Ra, Figure 6.3). With glucose ingestion, glucose Ra tended to be higher towards the end of exercise but the differences were neither significant nor, during the early (< 60 min) stages of exercise, that reliable. Comparisons between the final glucose Ra values in Figure 6.3, and the final rates of glucose oxidation (Rox) in Figure 6.4 indicated that glucose Ra determinations probably underestimated actual rates of glucose appearance during both experimental trials by up to 15% with CHO ingestion and 25% with glucose infusion.

Although the isotope dilution technique has been used extensively to quantify glucose kinetics and turnover, it is based on a number of assumptions. These assumptions are that; i) the metabolism of radioactive and non radioactive glucose is identical in all experimental conditions (McMahon et al. 1989); 2) the pool fraction being sampled (i.e. blood) is equilibrated with the metabolically active pool (McMahon et al. 1989); 3) the radioactive tracer is not recycled in the reactions of the glycolytic and gluconeogenic pathways

(Altszuler et al. 1975; Bell et al. 1986; Bier et al. 1977; Dunn et al. 1976; Finegood and Vranic 1985; Finegood et al. 1988; Hue and Hers 1974; Kalhan et al. 1980; Katz and Rognstad 1976; Katz et al 1992; Weber et al. 1990; 4) the measurement of plasma specific activity and the rate of isotope infusion is accurate (Argoud et al. 1987; Best et al. 1982); 5) steady-state conditions are met and 6) that under such conditions the model chosen to calculate glucose turnover (generally the one compartment model originally proposed by Steel in 1959) is appropriate (Allsop et al. 1978; Altszuler et al. 1968; Argoud et al. 1987; Cherrington and Vranic 1971; Cherrington et al. 1974; Cowan et al. 1969; Finegood et al. 1987, 1988; Ninomiya et al. 1965; Vranic and Wrenshall 1969).

These assumptions and limitations do not invalidate the isotopic tracer method, but suggest that the contribution of plasma glucose oxidation to overall substrate metabolism during prolonged exercise may be under estimated in investigations which have used the isotopic tracer approach (Coggan 1991).

In the present investigation (and the study subsequently described in Chapter Seven), a 2-^{3}H glucose label was used for the determination of glucose turnover because there is no recycling of this label (Bosch et al. 1993a) and little incorporation of the label into glycogen during the pre-exercise infusion period (Sonne and Galbo 1985). As the presence of $^{3}H_2O$ from the metabolism of 2-^{3}H glucose in the glycolytic pathway was less than 2%, it is most unlikely that underestimates of glucose disposal could have been due to methodological processing of the tritiated glucose samples. Further, although there were perturbations in the dpm/mmol plasma 2-^{3}H activity during the early (i.e. 30-40 min) stages of infusion, true steady-state conditions were attained during the final 75 min of exercise when changes in plasma 2-^{3}H were small (i.e. < 5%). Thus, the most likely explanation for the current underestimation of tracer-derived glucose disposal rates is inappropriate application of the one-compartment, fixed pool volume model of glucose kinetics, as originally described by Steele (1959).

Although it has been proposed that a variable volume approach might yield more accurate estimates of glucose turnover than the single compartment model (Allsop et al. 1978; Cobelli et al. 1987, 1989; Cowan and Hetenyi 1971; Finegood et al. 1987; Issekutz et al. 1984; Wolfe 1984), it is worth noting that the equations of Steele (1959) have been validated and found to yield similar results when either single or multiple glucose pools are employed (Radziuk et al. 1978b). Nevertheless, the glucose Ra data in the current investigation should probably be regarded as more directional than absolute. They show that, towards the end of exercise, most of the glucose Ra was from ingested or infused glucose.

The more reliable measurements of plasma glucose oxidation, which do not rely on assumed distribution volumes, showed that between 25 and 105 min of exercise, rates of glucose oxidation and plasma insulin concentrations rose together in the CHO ingestion trials (Figure 4; $P < 0.05$). Whereas with CHO ingestion, Rox increased to 93 ±8 umol/min/kg FFM within 90 min of exercise, the final Rox value after 125 min of intravenous glucose infusion was 85 ±5 umol/min/kg FFM and still rising, despite the falling plasma insulin concentrations. Wolfe et al. (1986) also showed that, despite markedly different plasma insulin (19.8 versus 9.2 uU/ml) and plasma FFA (0.18 versus 0.70 umol/ml) concentrations, glucose oxidation rose to a similar extent after 60 min of light (40% of VO_{2max}) exercise. The rates of plasma glucose oxidation found in the current study are also in accord with the 92 umol/min/kg FFM rates of glucose disposal *estimated* by Coggan and Coyle (1987) at the end of 215 min of exhaustive cycling in subjects who received an intravenous glucose infusion in the latter stages of exercise to maintain plasma glucose concentration at ~5.0 mmol/L.

The peak (~1 g/min) rates of plasma glucose oxidation found in the current study are also similar to the values reported by Bosch et al. (1993a) in moderately-trained subjects who ingested 170 ml of a 10 g/100 ml glucose beverage every 20 min during 3 hr of cycling at 70% of VO_{2max}. Although such rates of plasma glucose uptake and oxidation are considerably higher than some previous reports (Ahlborg and Felig 1976, 1982; Wahren

1977), they are in close agreement with findings from recent studies using either arteriovenous sampling or isotopic tracers during dynamic exercise (Broberg and Sahlin 1989; Coggan et al. 1991; Katz et al. 1986; Stein et al. 1989).

As rates of glucose oxidation by muscle eventually increased to similar values with both intravenous glucose infusion and CHO ingestion (Figure 6.4), these findings suggest that when sufficient CHO is ingested, it is not the rate of appearance of ingested glucose in the systemic blood supply that limits the rate of glucose oxidation by muscle during exercise. Rather, it seems that the prevailing blood glucose concentration regulates the rate of hepatic glucose release which, in turn, governs the rate of glucose oxidation by working muscles. Thus, the data in Figure 6.4 and the results from other studies cited previously suggest that when plasma glucose concentration is ~5 mmol/L and plasma insulin concentrations are not greatly increased, the rate of muscle glucose oxidation is probably limited to ~1 g/min. Certainly, this would explain why the ingestion of a variety of mono-, di-, and oligosaccharides, all of which elicit similar (i.e. 5 mmol/L) plasma glucose concentrations are limited to ~1 g/min (Massicotte et al. 1986, 1989; Moodley et al. 1992; Pallikarakis et al. 1986; Rehrer et al. 1992; Saris et al. 1993).

In this regard, the estimated 2 g/min rates of plasma CHO oxidation found by Coyle et al. (1986) from gas exchange measurements at the end of continuous, exhaustive moderate-intensity cycling when muscle glycogen was depleted and plasma glucose concentrations were 4-5 mmol/L are of interest. Since rates of muscle glucose oxidation appear to be limited to 1 g/min, Coyle et al's (1986) data suggest that the final rates of plasma CHO oxidation during exhaustive exercise may have been due to a combination of glucose and lactate oxidation. A breakdown of glycogen in non-working muscles to provide lactate to glycogen depleted muscles for oxidation has been proposed as an important mechanism for redistributing CHO during exercise (Brooks 1986; Walsh and Bannister 1988).

A second finding of the current study was that the reduced plasma insulin concentrations with intravenous glucose infusion were associated with lower RER values (Table 2) and

hence higher calculated rates of fat oxidation (Table 3). Whereas the contribution to energy production from fat oxidation increased from 11 ±4% at the beginning of exercise to only 18 ±4% after 125 min with CHO ingestion, it rose from 37 ±10% to 51 ±10% with glucose infusion (Figure 5). These high percentage contributions to energy production from fat oxidation with intravenous glucose infusion are similar to those found in subjects ingesting water during exercise of similar intensity and duration (Coyle et al 1986, 1991). Costill et al. (1977) have reported that oxidation of fat is significantly greater throughout 30 min of treadmill running at 68% of VO_{2max} when plasma FFA concentration are elevated above those found when either water or 75 g of glucose is ingested. An elevated rate of fat oxidation with hypoinsulinaemia probably arises from a decreased re-esterification of the FFAs released from adipocyte triglyceride lipolysis, and supports the concept that the utilisation of plasma FFA during moderate to high intensity exercise may be limited by the release into the bloodstream of FFA from adipocyte triglyceride breakdown (Romijn et al. 1992b). Elevated plasma insulin concentrations are known to reduce circulating plasma free fatty acid concentrations (Wolfe et al. 1986), to facilitate a high rate of glucose disposal and to inhibit fat oxidation (Costill et al. 1977; Rennie and Holloszy 1977).

Conversely, artificially raised plasma FFA concentrations have been shown to increase the rates of FFA oxidation and inhibit glucose utilisation in some (Balasse and Neef 1974; Ferrannini et al. 1983), but not all (Hargreaves et al. 1991; Wolfe et al. 1988) investigations. Studies in animals (Rennie and Holloszy 1976) and humans (Costill et al. 1977) have shown that an elevation of plasma FFA reduces the utilisation of working muscle glycogen by up to 45% during submaximal exercise, although more recent experiments have reported that CHO oxidation (Ravussin et al. 1986) and glycogen metabolism (Hargreaves et al. 1991) are unaffected by physiological elevations in plasma FFA. In the current study we found that the higher rates of fat oxidation in the glucose infusion trial, than with CHO ingestion, were associated with a significant decrease in muscle glycogen (plus lactate) utilisation (estimated from the difference between the rate of total CHO oxidation minus the plasma glucose oxidation) after 125 min of exercise, and

a reduced plasma glucose oxidation and overall CHO oxidation throughout exercise (Figure 6.6).

In conclusion, these results show that, compared to repetitive CHO ingestion, intravenous glucose infusion and the associated hypoinsulinaemia result in reduced initial rates of muscle glucose oxidation, decreased rates of CHO oxidation, and increased rates of fat oxidation during 2 hr of moderate-intensity exercise. However, despite considerably lower plasma insulin concentrations with glucose infusion, the rates of muscle glucose oxidation were the same at the end of 125 min of exercise as with CHO ingestion, which elicited significantly higher plasma insulin concentrations. These findings indicate that conditions which elevate plasma insulin concentrations during the first 90-120 min of prolonged (i.e. 3-4 hr) exhaustive exercise *may* be detrimental to endurance performance due to an inhibition of fat metabolism with an accelerated rate of CHO metabolism, the opposite effect of that which is believed to aid performance during prolonged exercise (Coggan and Coyle 1991). In this regard, Gisolfi and Duchman (1992) have recently recommended that during the initial stages of athletic events lasting between one and three hr "*fat metabolism should be promoted*" and "*the inclusion of carbohydrate in beverages (early in these events) should be avoided*" as it would "*turn on carbohydrate metabolism and promote glycogen utilization ..... which could lead to premature fatigue.*" Further studies will be needed to test this hypothesis.

CHAPTER SEVEN

THE EFFECTS OF EUGLYCAEMIA AND HYPERGLYCAEMIA ON FUEL SUBSTRATE KINETICS DURING PROLONGED, MODERATE-INTENSITY EXERCISE

7.1 Introduction

The results of the previous studies have demonstrated that the rates of oxidation of euglycaemic (i.e. 5 mmol/L) concentrations of plasma glucose are limited to ~1 g/min in trained subjects after 90-125 min of moderate-intensity cycling. Even by-passing both intestinal absorption and hepatic glucose uptake via an intravenous glucose infusion (Chapter Six) failed to increase rates of glucose oxidation by muscle above the rates of ingested CHO found when subjects ingest a variety of CHO solutions during exercise (Chapters Four and Chapter Five). Thus, when sufficient quantities of glucose are ingested (or infused), the appearance of glucose into the systemic circulation does not limit the rate of exogenous glucose oxidation by the working muscles, at least when plasma glucose concentrations are 5 mmol/L and plasma insulin concentrations are not greatly increased.

However, the recent results of Coyle et al. (1991) suggest that trained cyclists may be able to oxidise plasma glucose at rates considerably greater than the peak rates of 1 g/min found during euglycaemic conditions. These workers infused glucose into well-trained subjects during two hr of intense cycling and found that the rate of glucose infusion needed to maintain plasma glucose concentration at 10 mmol/L increased from 1.6 g/min early in exercise to over 2.6 g/min during the last 20 min of the ride (Coyle et al. 1991). As rates of glucose infusion provide an indirect estimate of plasma glucose disposal (DeFronzo et al. 1978, 1979), the results of Coyle et al. (1991) show that trained cyclists have a remarkable capacity to dispose of exogenous glucose. Without the use of isotopic tracers, however, Coyle et al. (1991) were unable to determine the proportion of the infused glucose that was oxidised.

Therefore, the purpose of this study was to quantify fuel substrate kinetics during prolonged, steady-state exercise in highly-trained subjects when plasma glucose concentration was either maintained at 5 mmol/L (euglycaemic trial [ET]) or raised to 10 mmol/L (hyperglycaemic trial [HT]) by continuous variable-rate intravenous glucose infusions. Specifically this study aimed to determine whether the rate of muscle glucose

oxidation can be increased when plasma glucose is doubled from 5 mmol/L to 10 mmol/L, via an intravenous glucose infusion.

7.2 Results

7.2.1 Subject characteristic

A separate set of twelve male endurance-trained cyclists, randomly divided into two groups (n = six/group), participated in this investigation. To minimise their exposure to radioactivity, each subject performed only one experimental trial. Subject characteristics are displayed in Table 7.1. There were no significant differences in any of the variables measured in the two groups.

Table 7.1 Subject characteristics

	ET	HT
Age (yr)	23.5 (1.0)	26.0 (1.1)
Mass (kg)	79.8 (1.8)	72.8 (4.9)
FFM (kg)	68.6 (1.4)	64.6 (4.7)
PPO (W)	450.0 (21.5)	388.5 (13.4)
VO_2max (L/min)	4.86 (0.17)	4.81 (0.14)
Workrate at 63% PPO (W)	255.4 (13.5)	244.8 (8.4)

ET, euglycaemic clamp (5 mmol/L); HT, hyperglycaemic clamp (10 mmol/L); FFM, fat free mass; PPO, peak sustained power output attained during the maximal test; VO_2max, maximal oxygen consumption. All values are mean ±SEM of 6 subjects/group.

7.2.2 Rates of glucose infusion

The rates of glucose infusion required to maintain plasma glucose at euglycaemic concentrations of either 5 mmol/L (ET) or hyperglycaemic concentrations of 10 mmol/L (HT) increased progressively during exercise (Figure 7.1).

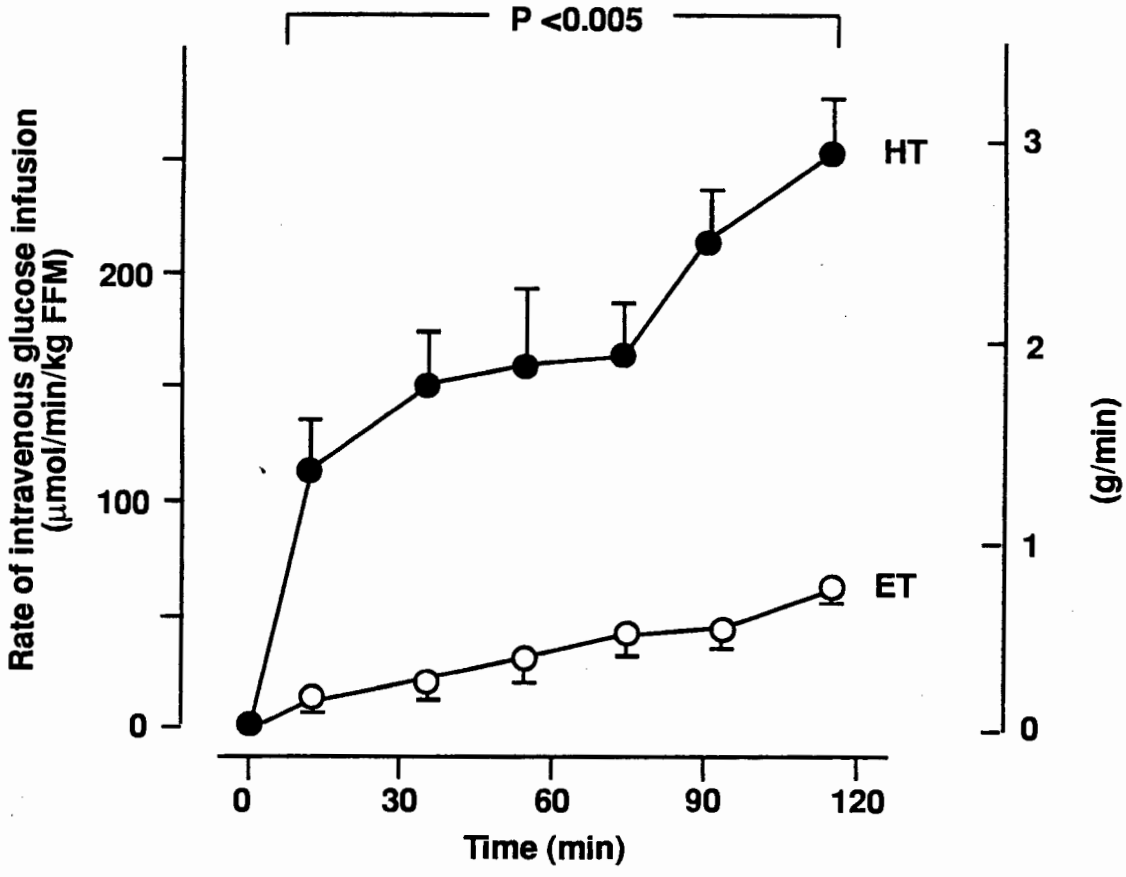

Figure 7.1. The rate of intravenous glucose infusion required to maintain plasma glucose concentration at 5 mmol/L (Euglycaemic trial; ET) and 10 mmol/L (Hyperglycaemic trial; HT) during 125 minutes of exercise. The rate of glucose infusion required to maintain hyperglycaemia was significantly higher than that required to maintian euglycaemia throughout exercise, P <0.005. Values are mean ±SEM.

For the first 25 min of the ET, the rate of glucose infusion averaged 15.8 ±5.3 umol/min/kg FFM, and increased to 63.7 ±7.6 umol/min/kg FFM during the 105-125 min period of exercise ($P < 0.0005$). In the HT the corresponding glucose infusion rates increased from 114.1 ±23.9 umol/min/kg FFM in the first 25 min of exercise to 251.5 ±29.1 umol/min/kg FFM in the last 20 min of exercise ($P < 0.005$). As would be expected, a significantly greater amount of glucose was infused over 125 min in order to maintain plasma glucose at a concentration of 10 mmol/L than at a concentration of 5 mmol/L (21.9 ±2.9 mmol/kg FFM versus 4.4 ±0.8 mmol/kg FFM; $P < 0.005$).

7.2.3 Plasma glucose concentration

As a result of the increasing rate of glucose infusion (Figure 7.1), the plasma glucose concentration during the ET was maintained at a mean value of 4.8 ±0.1 mmol/L and ranged between 4.4 and 5.3 mmol/L during the 125 min ride (Figure 7.2).

During the HT, plasma glucose concentration increased significantly from 5.0 ±0.3 mmol/L at rest to 8.7 ±0.6 mmol/L after five min of exercise ($P < 0.05$), and thereafter remained at a relatively constant concentration of 9.7 ±0.3 mmol/L for the duration of the ride. As intended, differences in glucose concentration between the ET and HT were highly significant ($P < 0.0001$).

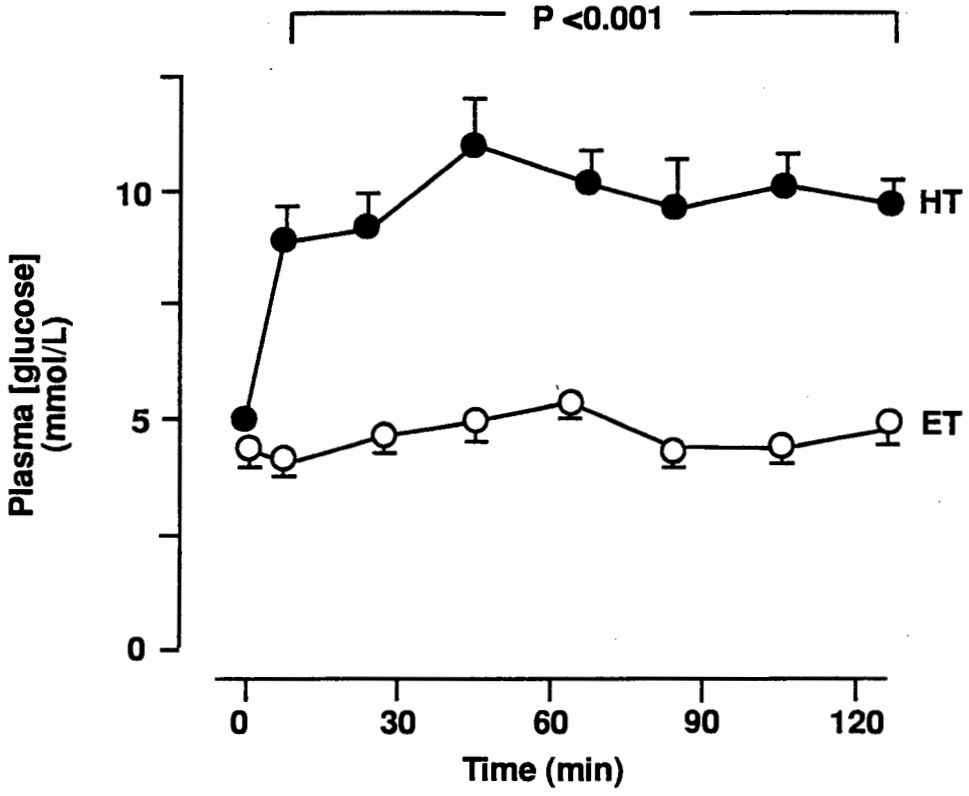

Figure 7.2. Plasma glucose concentration during 125 minutes of cycling under euglycaemic (5 mmol/L; ET) and hyperglycaemic (10 mmol/L; HT) conditions. Plasma glucose concentration was significantly higher in the hyperglycaemic trial than in the euglycaemic trial throughout exercise, P <0.001. Values are mean ±SEM.

7.2.4 Plasma insulin concentrations

With the high rates of glucose infusion (Figure 7.1) and the resultant elevation of plasma glucose concentration in the HT (Figure 7.2), plasma insulin concentrations in the HT were also increased (Figure 7.3; P <0.0001).

Whereas in the ET plasma insulin concentrations declined from 7.5 ±1.4 to 4.8 ±1.3 uU/ml at the end of exercise, in the HT plasma insulin concentrations rose from 5.9 ±1.7 uU/ml to 26.5 ±3.3 uU/ml after five minutes and remained elevated thereafter (P <0.0001).

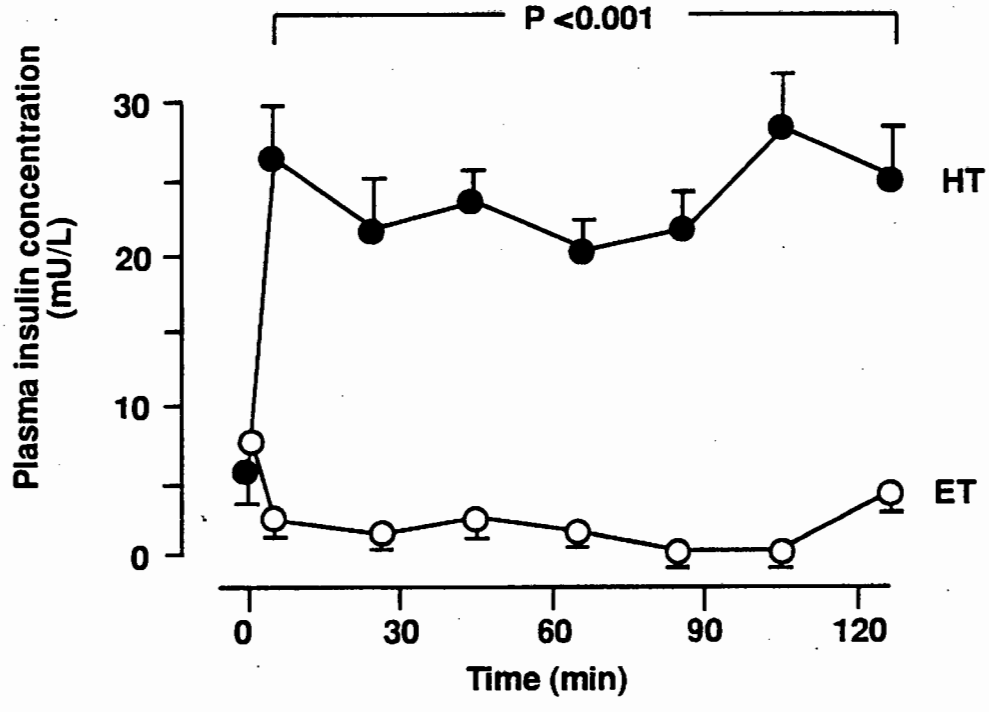

Figure 7.3. Plasma insulin concentration during 125 minutes of cycling under euglycaemic (5 mmol/L; ET) and hyperglycaemic (10 mmol/L; HT) conditions. Plasma insulin concentration was significantly higher in the hyperglycaemic trial than in the euglycaemic trial throughout exercise, P <0.001. Values are mean ±SEM.

7.2.6 Rates of plasma glucose oxidation

Figure 7.5 displays the Rox for the two experimental rides. During the ET, Rox increased from 16.5 ±6.1 umol/min/kg FFM after five min of exercise to 92.3 ±8.6 umol/min/kg FFM at the end of the ride (P <0.0001). During the HT, the corresponding values were 30.2 ±4.4 umol/min/kg FFM and 151.8 ±20.8 umol/min/kg FFM (P <0.005).

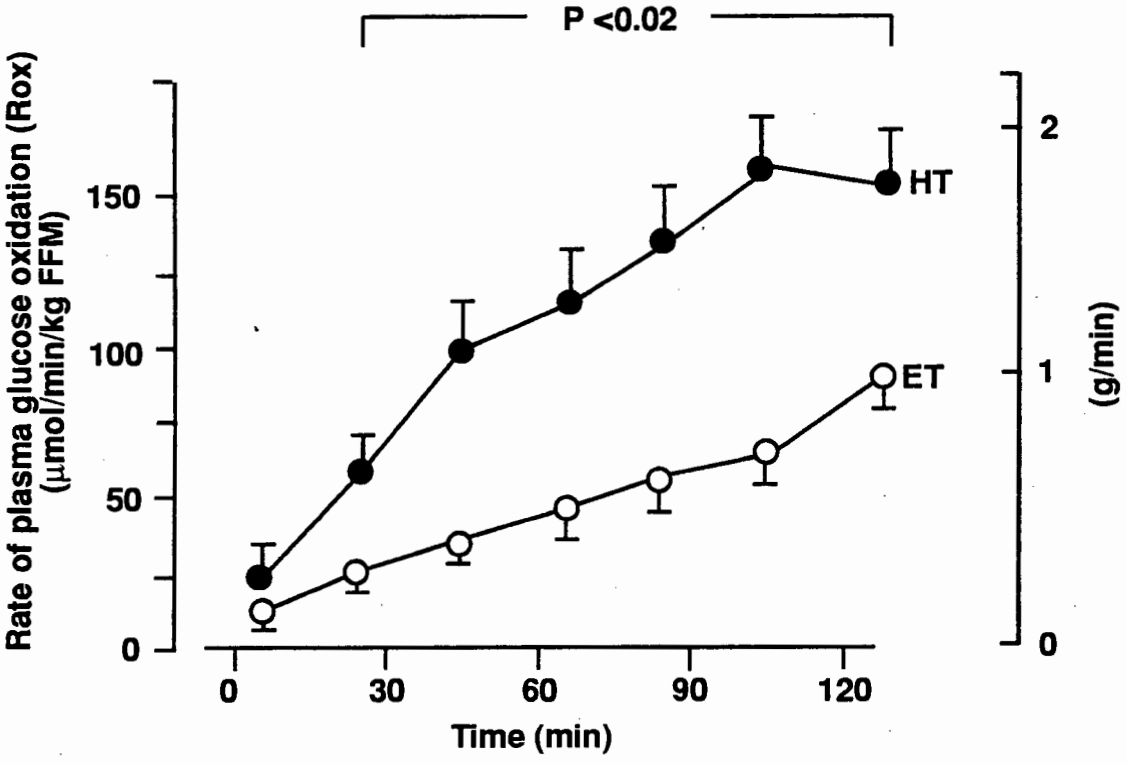

Figure 7.5. The rate of plasma glucose oxidation (Rox) during 125 minutes of cycling under euglycaemic (5 mmol/L; ET) and hyperglycaemic (10 mmol/L; HT) conditions. Rox was significantly higher in the hyperglycaemic trial than in the euglycaemic trial throughout exercise between 25-125 minutes of exercise, P <0.02. Values are mean ±SEM.

7.2.7 Total carbohydrate and fat oxidation

Tables 7.2 and 7.3 show the steady-state gas exchange data and the total calculated CHO and fat oxidation rates during the two experimental rides. As the absolute metabolic demand of the slightly heavier subjects in the ET was greater than that of the subjects in the HT (average VO_2 3.55 ±0.03 L/min versus 3.20 ±0.02 L/min; P <0.05), the CHO and fat oxidation rates have been expressed relative to FFM (Table 7.3).

During the ET, the rate of CHO oxidation declined from 228.3 ±27.4 umol/min/kg FFM at the start of exercise to 178.8 ±28.3 umol/min/kg FFM at the end of the ride (Table 7.3). Associated with the fall in CHO oxidation in the ET was a rise in the rate of fat oxidation from 45.1 ±12.9 umol/min/kg to 62.1 ±11.4 umol/min/kg FFM. In contrast, the rate of CHO oxidation in the HT was maintained at an average of 310.1 ±3.1 umol/min/kg FFM and rates of fat oxidation only rose from 9.0 ±2.9 umol/min/kg FFM to 19.8 ±1.7 umol/min/kg FFM (Table 7.3).

The higher rates of CHO oxidation in the HT than the ET (Table 7.3) reduced the percentage contribution to total energy production from fat oxidation from between 35-55% in the ET to between 12-18% in the HT (Figure 7.6). Instead, more energy was derived from plasma glucose oxidation in the HT. With hyperglycaemia, the percentage contribution to total energy production from plasma glucose oxidation increased from 8.2 ±1.6% at the beginning of exercise to 40.8 ±12.2% at the end of the ride (P <0.001; Figure 7.6). Corresponding values for the ET were 4.7 ±1.7% versus 26.3 ±3.1%; P <0.001; Figure 7.6).

Table 7.2. Steady-state gas exchange data and total carbohydrate and fat oxidation during 125 minutes of cycling under euclycaemic and hyperglycaemic conditions.

Exercise duration (min)	5	25	45	65	85	105	125
VO_2 (L/min)							
ET	3.50 0.12	3.52 0.13	3.51 0.11	3.51 0.13	3.53 0.13	3.61 0.13	3.70 0.14
HT	3.11 0.16	3.20 0.13	3.17 0.13	3.19 0.10	3.21 0.10	3.24 0.12	3.31 0.14
VCO_2 (L/min)							
ET	3.09 0.04	3.08 0.04	3.06 0.07	3.03 0.07	3.02 0.03	3.05 0.05	3.09 0.06
HT	3.00 0.16	3.04 0.13	3.02 0.15	3.01 0.11	3.02 0.12	3.05 0.14	3.13 0.14
RER							
ET	0.89 0.03	0.88 0.03	0.87 0.03	0.87 0.02	0.86 0.03	0.85 0.03	0.84 0.03
HT	0.97* 0.01	0.95* 0.01	0.95* 0.01	0.94* 0.01	0.94* 0.01	0.94* 0.01	0.94* 0.01

ET, euglycaemic clamp (5 mmol/L); HT, hyperglycaemic clamp (10 mmol/L); VO2, oxygen consumption; VCO2, carbon dioxide production; RER, respiratory exchange ratio; * HT significantly greater than ET, $P < 0.05$. All values are mean ±SEM.

Table 7.3 Carbohydrate and fat energy during 125 minutes of cycling under euglycaemic and hyperglycaemic conditions.

Exercise duration (min)	5	25	45	65	85	105	125
CHO oxidation (g/min)							
ET	2.82 0.33	2.72 0.40	2.62 0.39	2.52 0.30	2.40 0.36	2.28 0.34	2.20 0.33
HT	3.69 0.28	3.59 0.21	3.57 0.28	3.46 0.22	3.42 0.23	3.48 0.28	3.60 0.23
CHO oxidation (μmol/min/kg FFM)							
ET	228.3 27.4	220.1 32.2	212.2 31.5	204.2 23.3	195.0 30.7	185.3 28.0	178.8 28.3
HT	321.6* 24.3	314.2* 20.7	313.7* 27.1	303.2* 21.8	299.6* 22.2	302.9* 23.1	315.8* 23.2
Fat oxidation (g/min)							
ET	0.68# 0.18	0.73 0.21	0.77# 0.19	0.80# 0.16	0.85# 0.19	0.94# 0.18	1.01# 0.18
HT	0.18 0.07	0.25 0.06	0.25 0.06	0.30 0.06	0.32 0.05	0.32 0.07	0.30 0.03
Fat oxidation (μmmol/min/kg FFM)							
ET	45.1# 12.9	44.7 12.6	47.4# 11.8	49.4# 10.4	52.7# 12.3	57.9# 11.6	62.1# 11.4
HT	9.0 2.9	16.6 4.0	14.3 4.3	19.8 4.1	21.6 3.9	21.3 5.3	19.8 1.7

ET, euglycaemic clamp (5 mmol/L); HT, hyperglycaemic clamp (10 mmol/L); carbohydrate and fat oxidation calculated from gas exchange data. * HT significantly greater than ET, $P < 0.05$; # ET significantly greater than HT, $P < 0.05$. All values are mean ±SEM.

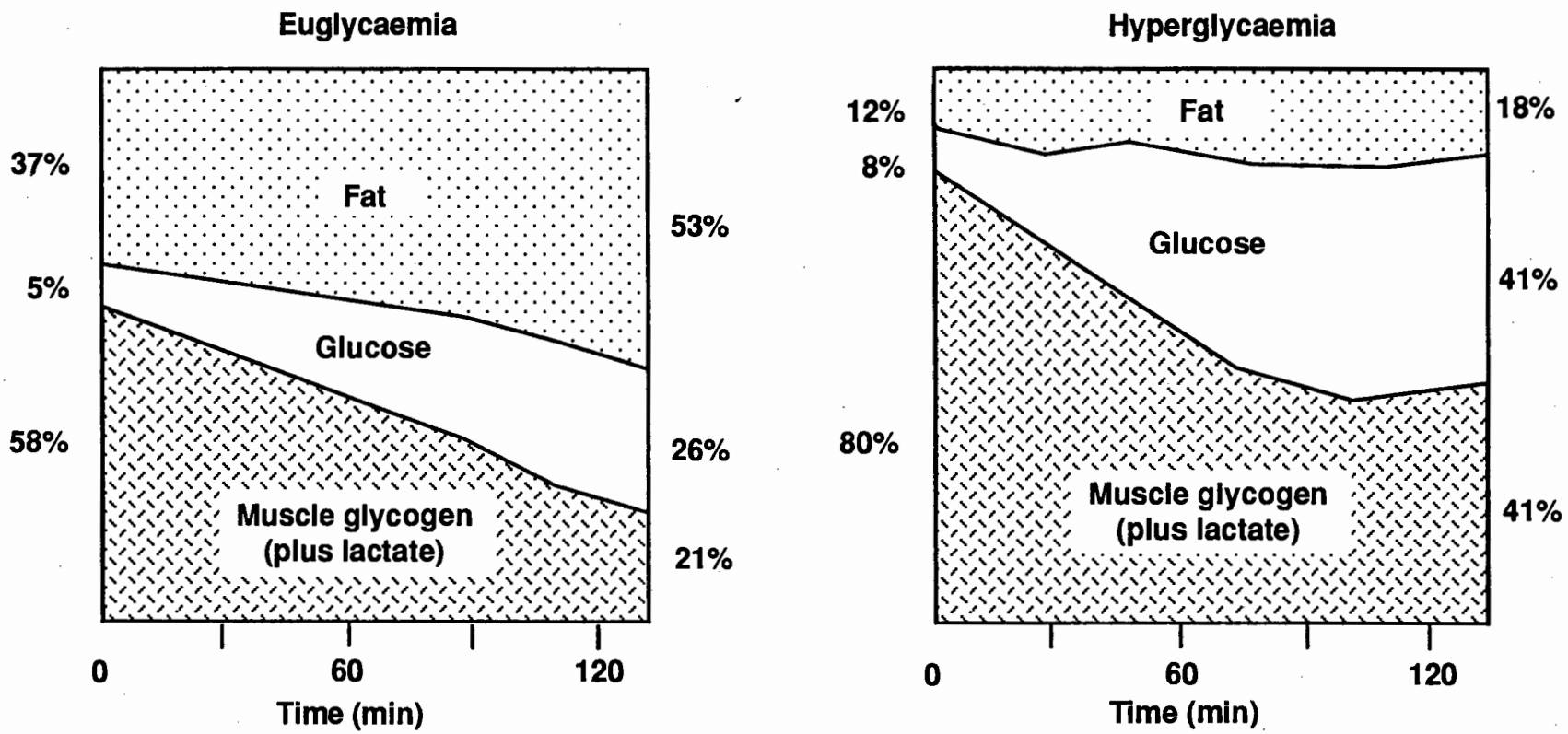

Figure 7.6. The relative contribution of fuel substrates to total energy production during 125 minutes of cycling under euglycaemic (5 mmol/L; ET) and hyperglycaemic (10 mmol/L; HT) conditions.

Areas under the total CHO oxidation and plasma glucose oxidation versus time curves during the HT and ET are shown in Figure 7.7. The total CHO oxidation in the HT was significantly higher than in the ET (37.9 ±2.3 mmol/kg FFM versus 25.5 ±3.5 mmol/kg FFM; P <0.05). Total plasma glucose oxidation was also significantly higher in the HT than in the ET (13.3 ±1.5 mmol/kg FFM versus 5.8 ±0.6 mmol/kg FFM (P <0.005). On the other hand, muscle glycogen (plus lactate) utilisation (estimated from the difference between the total CHO oxidation minus the total plasma glucose oxidation) was not significantly different between the HT and ET (24.64 ±2.1 mmol/kg FFM versus 19.7 ±4.0 mmol/kg FFM, respectively; Figure 7.7).

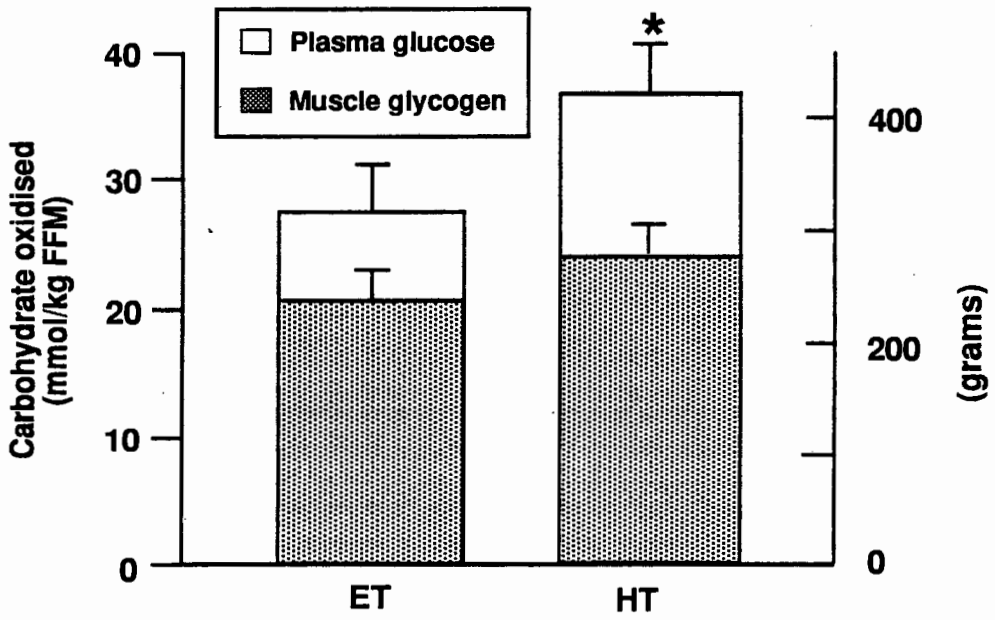

Figure 7.7. Carbohydrate oxidation during 125 minutes of cycling under euglycaemic (5 mmol/L; ET) and hyperglycaemic (10 mmol/L; HT) conditions. Muscle glycogen (plus lactate) oxidation has been estimated from the difference between total carbohydrate oxidation and plasma glucose oxidation. *Total carbohydrate oxidation significantly greater during HT than ET, P <0.05. Values are mean ±SEM.

7.2.8 Glucose oxidation versus glucose infusion

Figure 7.8 compares the amount of glucose that was infused to the amount of glucose that was oxidised during the two experimental rides. Whereas in the ET the amount of glucose infused was similar to the total amount of plasma glucose oxidised (4.4 ±0.8 mmol/kg FFM versus 5.8 ±0.6 mmol/kg FFM), during the HT, the amount of glucose infused (21.9 ±2.9 mmol/kg FFM) was significantly greater than the amount oxidised (13.3 ±1.4 mmol/kg FFM; P <0.05).

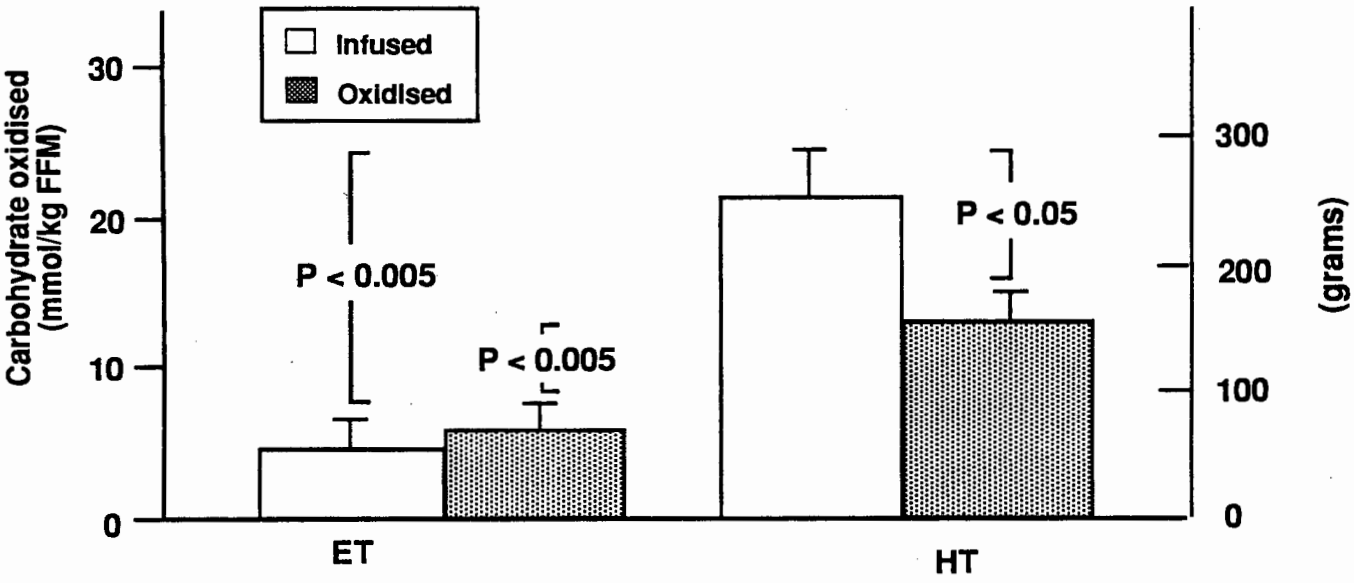

Figure 7.8. The amount of glucose infused and oxidised during 125 minutes of cycling under euglycaemic (5 mmol/L; ET) and hyperglycaemic (10 mmol/L; HT) conditions. Both the amount of glucose infused and the total plasma glucose oxidised are significantly greater during HT than ET, P <0.005. The amount of glucose infused is significantly greater than the amount of plasma glucose oxidised for the HT, P <0.05. Values are mean ±SEM.

7.3 Discussion

The first finding of the present study was that when glucose was infused (Figure 7.1) to elevate plasma glucose concentration to 10 mmol/L in the hyperglycaemia trial (HT; Figure 7.2), the rate of glucose infusion required to maintain plasma glucose concentration rose from 1.6 ±0.2 g/min during the first 60 min of exercise to 2.9 ±0.3 g/min during the last 20 min of the ride. These rates are almost identical to 1.6 to 2.6 g/min infusion rates reported by Coyle et al. (1991) in their studies of hyperglycaemia in highly trained cyclists during two hours of intense (i.e. 73 ±2% of VO_{2max}) exercise. Such high rates of infusion confirm the exceptional ability of well-trained subjects to dispose of high concentrations of infused glucose (Coyle et al. 1991).

Associated with the elevation of plasma glucose concentration in the HT (Figure 7.2), there was modest (24.5 ± 0.9 uU/ml) hyperinsulinaemia (Figure 7.3) comparable to the 16-24 uU/ml plasma insulin concentrations reported by Coyle et al. (1991). This combination of hyperglycaemia (Figure 7.2) and hyperinsulinaemia (Figure 7.3) totally suppressed endogenous liver glucose Ra throughout the ride, and increased the rates of glucose disappearance (Figure 7.4) and plasma glucose oxidation in the final 30 min of exercise to ~1.8 g/min (Figure 7.5).

In skeletal muscle the physiologically most important stimuli for an increase in the rate of glucose uptake are contractile activity and the prevailing plasma glucose and insulin concentration (Wallberg-Henriksson 1987). In the presence of basal circulating insulin concentrations skeletal muscle is responsible for only 20% of Rd. Baron et al. (1988) showed that during hyperinsulinemia, insulin-mediated glucose uptake into skeletal muscle represented 75% of Rd at euglycaemia (i.e. 5 mmol/L) and 95% of Rd during hyperglycaemia, making muscle the predominant glucose-uptake site in response to elevations in circulating insulin concentration. Yet despite the high glucose uptake that occurs in human muscle during hyperglycaemia, the intracellular concentration of glucose does not change (Katz et al. 1988). This indicates that glucose is rapidly metabolised by muscle and that glucose transport across the cell membrane is probably *the* rate-limiting

step in glucose utilisation (Berger et al. 1975; Crofford and Reynolds 1965; Klip and Paquet 1990; Ozand et al. 1962; Richter et al. 1982). Glucose transport and glucose transporters and their metabolic control have been reviewed in detail elsewhere (Klip and Paquet 1990).

In the current investigation, the high rates of plasma glucose oxidation found in the HT maintained the total rates of CHO oxidation at between 3.4 to 3.7 g/min throughout two hr of exercise (Tables 7.2 and 7.3) and limited the percentage contribution to total energy production from fat oxidation to between 12 and 18% (Figure 7.6). Since Coyle et al. (1991) found that hyperglycaemia and hyperinsulinaemia suppressed the rise in plasma FFA concentrations during two hr of exercise when compared to a euglycaemic (4-5 mmol/L) control group, the low rates of fat oxidation may have been due to an insulin-induced inhibition of adipocyte lipolysis. Low concentrations of circulating FFA, together with the high plasma glucose and insulin concentrations facilitate a high rate of glucose disposal by exercising muscle (Rennie and Holloszy 1977; Coyle et al. 1985).

Despite the high (1.8 g/min) peak rate of glucose oxidation in the HT, there was no sparing of muscle glycogen (Figure 7.7). Muscle glycogen (plus lactate) oxidation, as estimated from the difference between the total CHO oxidation and total plasma glucose oxidation, was similar in the HT and in the ET (286 ±25 g/125 min versus 243 ±50 g/125 min respectively). As had previously been found by Coyle et al. (1991), the lack of glycogen sparing, together with the higher rates of plasma glucose oxidation (Figure 7.5) elevated the total CHO oxidation by 40% from 325 ±43 g/125 min in the ET trial to 441 ±28 g/125 min in the HT trial (Figure 7.7; $P < 0.05$).

Higher rates of plasma glucose oxidation in the HT, however, did not account for all of the glucose that was infused (Figure 7.8). In agreement with investigations that have measured both the rates of gastric emptying and ingested CHO oxidation during prolonged exercise, and which have found that the amount of CHO oxidised is far less than the amount delivered to the intestine (Moodley et al. 1992; Rehrer et al. 1992), there was a

large disparity between the amount of glucose infused and the calculated amount oxidised in the HT (Figure 7.8). Whereas the amount of plasma glucose oxidised was 155 ±17 g/125 min, the amount of glucose infused was 255 ±35 g/125 min. Of the remaining ~100 g of infused glucose, about 10 g would be required to provide the 55 mmols of glucose needed to raise the plasma glucose concentration in the ~11 L glucose distribution volume (used in Steele's (1959) equations) by 5 mmol/L. The other ~90 g was presumably incorporated into non-working muscle glycogen stores, as hepatic tissue retains very little glucose directly, even in the presence of supra-physiological concentrations of glucose and insulin (De Fronzo et al. 1978). Hyperinsulinaemia accelerates glucose transport and hexokinase activity and elevated intracellular glucose-6-P concentrations promote glycogen synthesis.

In contrast, under more physiological euglycaemic clamp conditions, the rates of glucose infusion required to maintain plasma glucose at a concentration of 5 mmol/L was significantly less than that needed to maintain hyperglycaemia ($P < 0.005$; Figure 7.1), such that during the last 20 min of the ET, the rate of glucose infusion (0.7 ±0.1 g/min) was still lower than during the first 25 min of the HT (1.3 ±0.3 g/min).

The lower rate of glucose infusion in the ET than in the HT (Figure 7.1) resulted in a modest hypoinsulinemia (Figure 7.3). By the end of the ride, plasma insulin concentration had declined from 7.5 ±1.4 uU/ml to 4.8 ±1.3 uU/ml. The latter insulin concentrations were almost five-fold less than the corresponding values measured during the HT (Figure 7.3). Lower plasma glucose and insulin concentrations during the ET failed to suppress completely endogenous (liver) glucose Ra. With the progressively increasing rate of glucose infusion (Figure 7.1), Ra was gradually reduced from 0.27 ±0.06 g/min to 0.08 ±0.01 g/min by the end of the 125 min ride. Exogenous glucose infusion during exercise is known to depress plasma FFA concentration (Elayan and Winder 1991), and inhibit hepatic glycogenolysis (Felig and Wahren 1979; Issekutz 1981) such that the exercise-induced Ra is usually precisely regulated to maintain euglycaemia (Jenkins et al. 1985; Vissing et al. 1988). Further, although glucose and insulin both play important roles in the

regulation of hepatic glucose balance (Bergman and Bucolo 1974; Newsholme and Start 1973), the effects of glucose *per se* are quantitatively more important, particularly during glucose infusion (Bergman and Bucolo 1974).

Despite the progressive inhibition of liver glycogen to glucose conversion, the rates of glucose disappearance (Figure 7.4) and plasma glucose oxidation (Figure 7.5) increased throughout the ET, although both rates were significantly lower than those measured in the HT. In agreement with previous arteriovenous balance and isotopic tracer studies of glucose metabolism during prolonged exercise which show that the peak rates of plasma glucose oxidation range from 0.9 to 1.3 g/min (Bosch et al. 1993a; Broberg and Sahlin 1989; Coggan et al. 1991; Stein et al. 1989), euglycaemic plasma glucose concentrations were only oxidised at rates of 1.1 ±0.1 g/min after two hr of exercise (Figure 7.5). Although the rates of plasma glucose oxidation were still rising, these values closely match the rates of plasma glucose oxidation in glycogen-depleted subjects at the end of three hr of cycling at 70% of VO_{2max} (Bosch et al. 1993a). Interestingly, the highest rates of plasma glucose oxidation in euglycaemic exercising subjects (Bosch et al. 1993a; Broberg and Sahlin 1989; Coggan et al. 1991; Stein et al. 1989) are similar to the maximum rates of ingested CHO oxidation found in individuals ingesting a variety of CHO solutions during prolonged exercise (Massicotte et al 1986; Pallikarakis et al. 1986; Rehrer et al. 1992; Saris et al. 1993).

Without hyperglycaemia and hyperinsulinaemia to maintain high rates of CHO oxidation throughout two hr of exercise, there was a progressive reduction in CHO oxidation during the ET (Tables 7.2 and 7.3). CHO oxidation declined from 2.8 ±0.3 g/min at the start of exercise to 2.2 ±0.3 g/min at the end of the ET (Tables 7.2 and 7.3). Associated with the lower rate of CHO oxidation throughout the ET (Figure 7.7) was a significantly greater contribution to energy requirements from fat metabolism compared to the HT (Figure 7.6). Fat oxidation increased from 0.7 ±0.2 g/min at the start of exercise to 1.0 ±0.2 g/min during the latter stages of the ride. Related to the higher rate of fat oxidation during the ET, there was a significant reduction in the contribution to energy production from plasma

glucose oxidation compared to the HT (14.2 ±2.8% versus 29.9 ±4.8% for ET and HT respectively; $P < 0.05$). Thus, unlike the HT, in which the amount of plasma glucose oxidised was significantly less than the amount of glucose infused, the amount of plasma glucose oxidised during the ET (72 ±8 g/125 min) was not significantly different from the amount of glucose infused (55 ±10 g/125 min; Figure 7.8).

In conclusion, the results of the current study show that in well-trained subjects, hyperglycaemia (i.e. a plasma glucose concentration of 10 mmol/L) and the associated hyperinsulinaemia (i.e. a plasma insulin concentration of 25 uU/ml), caused a complete suppression of endogenous Ra, maintained high rates of CHO oxidation, and inhibited fat oxidation throughout two hr of moderate-intensity exercise. Hyperglycaemia was also associated with an increasing glucose disposal throughout the two hour ride, such that during the latter stages of exercise, well-trained cyclists were capable of utilising plasma glucose at rates approaching Coyle et al's (1986, 1991) *predicted* values of 2 g/min. Such high rates of plasma glucose oxidation confirm the exceptional capacity of skeletal muscle to oxidise glucose when hyperglycaemic and support the hypothesis that the rate of glucose oxidation by muscle is a function of the concentration of glucose and insulin to which the muscle is exposed (Bergman and Bucolo 1974; Bourey et al. 1990) and the extent to which the glucose-transport process has been stimulated by exercise (Morgan et al. 1961; Morgan and Whitfield 1973).

The results of the current investigation and those from the previous study (Chapter Six) suggest that even when glucose is infused to by-pass any possible limitation in the rates of digestion, absorption and liver glucose output, the oxidation of euglycaemic concentrations of glucose are restricted to ~1 g/min at the end of two hr of exercise. It is hypothesised that glucose oxidation by skeletal muscle is precisely regulated by the prevailing plasma glucose concentration. This, in turn, regulates hepatic glucose uptake and release. The practical implication of this postulate is that it is the physiological concentration of plasma glucose normally present during prolonged, moderate-intensity

exercise which, ultimately, limits the rate of muscle glucose uptake and its subsequent oxidation, at least in the current experimental model.

CHAPTER EIGHT

SUMMARY AND CONCLUSIONS

1.0 Summary and Conclusions

The experiments described in this thesis have sought to examine systematically some of the factors which determine the rate at which exogenous glucose is utilised by the working muscles during prolonged, moderate-intensity exercise.

Although historically it has been assumed that the rate of gastric emptying was *the* primary factor limiting the rate of CHO delivered to the blood and the working muscles (Costill 1990; Costill and Saltin 1974), the results from the experiments described in Chapter Four and Chapter Five demonstrate that after the repetitive ingestion of 15 g/100 ml glucose, maltose, and 22 chain-length glucose polymer solutions, the amount of CHO emptied from the stomach far exceeds the amount oxidised. Thus, gastric emptying cannot limit the rate of CHO absorption and subsequent oxidation from CHO solutions ingested during the early (90 min) stages of prolonged exercise. Further, the similar rates of gastric emptying for glucose, maltose, glucose polymer and `soluble' starch beverages suggests that the drinking pattern during exercise is a more important determinant of gastric emptying than is solute osmolality (Noakes et al. 1991a).

The practical implication of these findings is that provided the CHO is ingested sufficiently frequently, in appropriate volumes, there will not be any physiologically important differences in the rates of ingested CHO oxidation from glucose, maltose and glucose polymer solutions. Indeed, even the ingestion of more complex (i.e. soluble starch) solutions have recently been found to result in similar rates of ingested CHO ingestion (Saris et al. 1993). The similar rates of ingested CHO oxidation from glucose, maltose and glucose polymer solutions indicate that the rate limiting step for ingested glucose utilisation by the active muscles exists distal to intestinal digestion, and is either the transport of glucose into the systemic circulation or the rate of working muscle glucose uptake and oxidation.

That the rate of appearance of ingested glucose into the systemic circulation does not limit the rate of muscle glucose oxidation was established by the results of Chapter Six. When

sufficient quantities of glucose under euglycaemic (i.e. 5 mmol/L) conditions were either ingested or infused (the latter to by-pass any potential limitation of intestinal absorption and/or hepatic glucose uptake and release), the peak rates of plasma glucose oxidation by muscle were still limited to ~1 g/min at the end of two hr of cycling at 63% of PPO, despite markedly different plasma insulin concentrations and rates of fat and CHO oxidation throughout exercise. This would suggest that muscle glucose oxidation is a function of the prevailing plasma glucose concentration and hence the rate of hepatic glucose appearance, rather than plasma insulin concentration, or the rate of fat and CHO oxidation. This hypothesis, if correct, would explain why the ingestion of a variety of different mono-, di-, and oligosaccharides, all of which elicit similar (i.e. 5 mmol/L) plasma glucose concentrations are all limited to ~1 g/min (Moodley et al. 1992; Pallikarakis et al. 1986; Rehrer et al. 1992; Saris et al. 1993).

The practical significance of these findings is that conditions which markedly elevate plasma insulin concentrations during the first two hours of exercise *may* be of detriment to endurance due to a significant inhibition of fat metabolism with an associated acceleration of CHO metabolism (Gisolfi and Duchman 1992), the opposite effect of that which is assumed to aid performance (Coggan and Coyle 1991). Further studies are necessary to test this hypothesis.

The results from Chapter Seven confirm that exogenous glucose oxidation by skeletal muscle is a function of the prevailing plasma glucose concentration, and thus the rate of hepatic glucose appearance. Only by doubling plasma glucose concentration from 5 mmol/L to 10 mmol/L (i.e. hyperglycaemia) did the rate of glucose oxidation by muscle increase to above 1 g/min at the end of two hr of exercise. Associated with the high (~1.8 g/min) rates of plasma glucose oxidation found with hyperglycaemia was a modest hyperinsulinaemia, an elevated rate of CHO oxidation, and a progressive reduction in the contribution from fat metabolism to the energy demands of exercise, conditions which might, normally, be expected to reduce endurance.

In conclusion, the results of this thesis suggest that the rate at which exogenous glucose is utilised by the working muscles during prolonged (i.e. > 90-125 min), moderate-intensity exercise is not limited by the rate of gastric emptying of an ingested solution, nor by the rate of digestion, absorption, and subsequent transport of glucose into the systemic circulation, at least for the repetitive ingestion of 15 g/100 ml solutions of glucose, maltose and a 22 chain-length polymer. Rather, it would appear that muscle glucose oxidation is regulated by the prevailing, normal (i.e. 5 mmol/L) plasma glucose concentration during exercise (Bonen et al. 1989), which, in turn, regulates the hepatic appearance of ingested (or infused) glucose, at least in the current experimental model.

CHAPTER NINE

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