Influence of high and low glycaemic index carbohydrate meals on exercise capacity and substrate metabolism in men

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INFLUENCE OF HIGH AND LOW GLYCAEMIC INDEX CARBOHYDRATE MEALS ON EXERCISE CAPACITY AND SUBSTRATE METABOLISM IN MEN

by

Ching-Lin Wu

A Doctoral Thesis

Submitted in partial fulfillment of the requirements for the award of Doctor of Philosophy of Loughborough University

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ABSTRACT

Ingestion of carbohydrate (CHO) before exercise and after exercise enhances subsequent endurance exercise capacity when compared to a fasting state. However, the optimal type of CHO meal to be consumed is still unclear. Glycaemic and insulinaemic responses to a meal during the postprandial period may play an important role in subsequent exercise metabolism and performance. This thesis examined the influence of ingesting high and low glycaemic index (GI) CHO meals 3 hour before exercise and during 4 hour recovery on substrate metabolism and exercise capacity in men.

To examine the influence of pre-exercise CHO meals with different GIs on postprandial and subsequent exercise metabolism, isocaloric high and low GI meals with the same macronutrient content were consumed by healthy and active subjects after an overnight fast. The high GI meal induced higher glycaemic and insulinaemic responses than the low GI meal during the postprandial period. During subsequent exercise, the low GI meal resulted in higher fat oxidation and maintained normal plasma glucose concentrations than the high GI meal. However, there were no differences in substrate utilisation during the postprandial periods. To further investigate the effect of these two types of meals on subsequent exercise capacity, the same meals were consumed before subject’s performed a test of endurance capacity running. Consuming the low GI meal 3 hour before exercise improved endurance capacity when compared to the high GI meal.

After a glycogen-depletion exercise, the same GI meals were consumed to investigate their influence on the short-term recovery metabolism during subsequent exercise.
Ingestion of the high GI meal during post-exercise recovery induced higher glycaemic and insulinaemic responses during the postprandial period compared to the low GI meal which was similar to the pre-exercise feeding studies. However, there were no differences in substrate utilisation during the recovery period. To further investigate the effect of ingesting these meals on recovery, they were consumed after prolonged exercise 3 hours later the subjects ran to exhaustion to assess their endurance capacity. Consuming the low GI meal promoted higher fat metabolism compared to the high GI meal. However, no differences in endurance capacity were found between two types of recovery meals.

In summary, the rate of fat oxidation during exercise is affected by postprandial insulinaemic and glycaemic responses. Therefore, consumption of 2g kg\(^{-1}\) body mass of low GI CHO meals 3 hour before exercise or during 4 hour recovery does not produce the symptoms of hypoglycaemia during the onset of subsequent exercise. Furthermore there is a higher rate of fat oxidation following the consumption of a low GI CHO meal than after a high GI meal during subsequent exercise. In addition, the ingestion of a low GI CHO meal 3 hour before exercise enhances subsequent endurance capacity during running. However, it does not result in an improvement in endurance running capacity when consumed during recovery following prolonged exercise, even though the rate of fat oxidation is greater than following the consumption of a high GI CHO meal.

Key words: glycaemic index, carbohydrate, endurance capacity, substrate utilisation, fat oxidation, carbohydrate oxidation and fatigue.
This thesis is dedicated to my parents
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Publications

The findings of some of the studies have been published as follows:

**Published paper**

**Conference communications**

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CHAPTER 1
INTRODUCTION

Carbohydrate (CHO) and fat are the main energy sources during strenuous endurance exercise. It is well known that the onset of fatigue during endurance exercise is associated with muscle glycogen depletion. High CHO diets are known to increase muscle and liver glycogen stores leading to improve endurance performance (Bergstrom et al. 1967; Karlsson and Saltin, 1971). Therefore, nutritional interventions to enhance CHO stores have received wide attention. Nevertheless, ingestion of CHO induces substantial insulin secretion which depresses fat oxidation (Coyle et al. 1997; Horowitz et al. 1997) and increases muscle glycogen utilisation during subsequent exercise (Coyle et al. 1985). Therefore, to find a nutritional strategy to optimise glycogen stores without severely depressing fat oxidation, is one of the current challenges of exercise nutrition.

Carbohydrates can be described as either simple or complex according to their chemical structure. However, there is a more informative way of classifying CHO which is based the changes in blood glucose concentration following the ingestion of a reference amount of this macronutrient i.e. glycaemic responses. Jenkins and colleagues established the glycaemic index (GI) concept to classify CHO-rich foods in the early 1980s (Jenkins et al. 1981). The glycaemic index classifies CHO-rich foods according to the 2-hour postprandial blood glucose response which is expressed as a percentage of a reference food. Thomas et al. (1991) first reported that ingestion of a low GI food 1 hour before submaximal cycling exercise at a fixed load improved endurance capacity (Thomas et al. 1991). They suggested that ingestion of low GI CHO induced less postprandial hyperglycaemia and hyperinsulinaemia, and
maintained plasma glucose and free fatty acid (FFA) at higher values during critical periods of exercise than after the consumption of high GI CHO. Several subsequent studies examined the effects of ingesting high and low GI CHO foods within 1 hour before exercise, on exercise performance, although performance results from these studies are inconsistent (Thomas et al. 1994; Febbraio and Stewart, 1996; Kirwan et al. 1998; Sparks et al. 1998; DeMarco et al. 1999; Kirwan et al. 2001a). Most of these performance studies required their subjects to complete a set amount of work as fast as possible, whereas the original study of Thomas et al. (1991) required their subjects to exercise for as long as possible. There are two main limitations to these earlier studies. The first was the timing of the food intake. The food was consumed only an hour before exercise and so there was probably too little time for complete digestion and absorption. The second was the studies were mainly performance trials that were not long enough to severely challenge the carbohydrate stores of the participants. For these reasons, Wee et al. (1999) investigated the influences of ingesting high and low GI CHO foods 3 hours before a constant pace treadmill run to exhaustion. They reported an increase in fat oxidation during the postprandial period and during exercise following the ingestion of the low GI CHO compared with the response to the high GI CHO. However, there were no differences in endurance capacity between the high GI CHO and low GI CHO trials (Wee et al. 1999). It is important to note that most of these GI studies used single foods rather than meals made up of a mixture of foods. People normally eat meals rather than single foods or simple drinks 2-3 hours before training or competition. Therefore, useful as these early studies are to science, they are not readily applicable to the real world where meals rather than single foods make up the diets of most people. With these questions in mind two studies were undertaken to examine the influences of high and low GI CHO meals consumed 3 hours before exercise on fat metabolism and endurance capacity during constant pace
treadmill running. The results of these two studies are reported in Chapters 4 and 5 of this Thesis.

One of the other challenges facing exercise nutrition is how to restock the body's carbohydrate stores as quickly as possible in order to enhance the recovery process. Muscle glycogen can be fully restored in 24 hours after exercise when subjects ingest sufficient amounts of CHO foods. However, athletes may train or compete more than once a day. Therefore, nutritional strategies are required to meet their needs. Ivy and colleagues examined the effects of ingesting CHO immediately after exercise on muscle glycogen resynthesis (Ivy et al. 1988a; Ivy et al. 1988b). They showed that glycogen resynthesis is more rapid when CHO is consumed sooner rather than later during the early part of recovery. Later studies showed that ingestion of CHO-electrolyte solutions during short-term recovery improved subsequent exercise capacity when compared with a placebo solution (Fallowfield et al. 1995; Wong et al. 1998; Wong et al. 2000). Nevertheless, in these studies the subjects ingested CHO solutions during their 4 hour recovery when they could have ingested real meals. However, the effects of ingesting high CHO mixed meals, during short-term recovery on metabolism and performance during subsequent exercise has not been reported in the literature.

Therefore, with these questions in mind the aim of this thesis was to examine the influence of carbohydrate meals with high and low glycaemic indices on metabolism and running endurance capacity when consumed before and after exercise. Thirty-three recreational active men participated in four studies presented in this Thesis.
This Thesis contains eight chapters. Chapter 2 provides a summary of the relevant literature on the interrelationship between CHO and fat during exercise. It also covers the effects of different types of CHO ingestion on CHO and fat metabolism, post-exercise glycogen restoration, and subsequent exercise performance. Chapter 3 describes the general methodology employed in the four studies of the thesis. Chapter 4 discusses the influence of ingesting two different GI meals comprising of 2g CHO·kg⁻¹ body mass or fasting on postprandial and subsequent exercise substrate utilisation. Chapter 5 examines the effects of two different GI breakfasts containing 2g CHO·kg⁻¹ body mass on substrate utilisation and endurance capacity during running at 70% VO₂max. Chapter 6 examines the influence of high GI and low GI CHO recovery meals on metabolism during subsequent exercise. Chapter 7 compares the effects of two high CHO meals with different GI s on substrate utilisation and endurance capacity after a 4 hour recovery. The experimental chapters, from Chapter 4 to Chapter 7, are structured according to journal paper format with the exception of the methods sections. Therefore, the introduction and discussion in these chapters may overlap. Finally, Chapter 8 summarises the findings of the four studies in this Thesis and discusses the implication.
CHAPTER 2
REVIEW OF LITERATURE

2.1 Introduction
This purpose of this chapter is to present a review of the relevant literature on the influence of CHO ingestion on the postprandial and exercise metabolism, and endurance performance. The review of literature consists of four main sections:
(i) Carbohydrate and fat metabolism during endurance exercise (Section 2.2)
(ii) The glycaemic index (Section 2.3)
(iii) Pre-exercise carbohydrate intake- the effects on metabolism and endurance performance (Section 2.4)
(iv) Post-exercise short-term recovery carbohydrate intake- the effects on metabolism and subsequent endurance performance (Section 2.5)
2.2 Carbohydrate and fat metabolism during endurance exercise

Carbohydrate and fat are the main fuels of ATP production during submaximal exercise (Fig 2.1). Metabolism of these fuels must be increased in order to meet the increased energy demand during exercise. At the onset of exercise, the skeletal muscle cell activates key metabolic steps to increase availability and metabolism of CHO and fat which includes complex intracellular and extracellular signals and transport. Whilst both substrates are important during exercise, there are many factors influencing substrate utilisation during submaximal exercise such as substrate availability, activity of rate-limiting enzymes, level of plasma hormones, exercise intensity, etc. Increasing the availability of endogenous or exogenous CHO increases the rate of CHO oxidation and similarly increasing exogenous free fatty acid (FFA) availability can increase the rate of fat oxidation during exercise (Hickson et al. 1977; Costill et al. 1977; Hargreaves et al. 1987; Horowitz et al. 1997).

Carbohydrate contributes to aerobic and anaerobic exercise energy production. However, CHO stores in the body can be depleted following 1-2 h of intensive exercise (Hermansen et al. 1967). It is known that increased rate of fat oxidation and decreased CHO use (i.e. sparing the limited muscle and liver glycogen stores) is advantageous during prolonged strenuous exercise. Therefore “glycogen sparing” can be achieved by increasing fat oxidation and this is beneficial for prolonged endurance exercise because it helps to delay the onset of fatigue.

In this section, the review consists of two parts: (1) increased fat availability on the interaction between fat and CHO oxidation during exercise, and (2) increased CHO availability on the interaction between fat and CHO oxidation during exercise.
Fig 2.1 Schematic overview of energy production in skeletal muscle.

PM, plasma membrane; OM, IM, outer and inner mitochondrial membranes; FFA, free fatty acid; FABPc, cytoplasmic fatty acid binding protein; TG, triacylglycerol; CoA, coenzyme A; CPT I, II, carnitine palmitoyltransferase I and II; CAT, carnitine-acylcarnitine translocase; NAD, NADH, oxidized and reduced nicotinamide adenine dinucleotide; G-6-P, G-1-P, glucose 6- and 1-phosphate; TCA, tricarboxylic acid; ETC, electron transport chain (Modified from Spriet 2002)
2.2.1 Effects of increase fat availability on the interaction between fat and CHO oxidation during exercise.

Randle and his colleagues proposed the glucose-fatty acid cycle (Fig 2.2) to explain the reciprocal relationship between fat and CHO oxidation (Randle et al. 1963; Randle, 1998). They found that increasing the availability of free fatty acid (FFA) to heart and diaphragm muscles increased fat oxidation and reduced CHO oxidation in vitro. They also observed an increase in muscle acetyl-CoA, citrate and glucose 6-phosphate (G-6-P). Acetyl-CoA inhibits the activity of pyruvate dehydrogenase (PDH) by activating PDH kinase, which is the enzyme that phosphorylates PDH to the inactive form. Citrate is also an inhibitor of phosphofructokinase (PFK) in the cytoplasm which leads to a decrease in glycolysis. In addition, increasing G-6-P concentration has been shown to inhibit hexokinase (HK). Increasing FFA availability increased muscle acetyl-CoA and citrate leading to down regulation of PDH and PFK activities. The reduced flux through the glycolytic pathway causes accumulation of G-6-P, which inhibits HK activity and finally decreased glucose uptake.

The evidence to support this proposed mechanism in human skeletal muscle is generally lacking. Odland and co-workers (1998a) using FFA infusion increased FFA availability in the blood stream, and found fat oxidation increased, CHO oxidation decreased, and muscle glycogen degradation was reduced (Odland et al. 1998a). However the subsequent study showed that there were no effects of increased fat availability on acetyl-CoA and G-6-P concentrations but a small increase in citrate and a lower PDH activity (Odland et al. 2000). The entire mechanism by which an increased fat oxidation modifies carbohydrate metabolism in human muscle during exercise has yet to be explained.
Fig 2.2 The glucose-fatty acid cycle. (Randle 1963)
G-6-P: Glucose-6-phosphate
F-6-P: Fructose-6-phosphate
F-1,6-diP: Fructose-1,6-diphosphate
HK: Hexokinase
PFK: Phosphofructokinase
PHD: Pyruvate dehydrogenase
2.2.1.1 Increased FFA availability during exercise

Romijn and colleagues investigated substrate utilisation using isotope tracer techniques (Romijn et al. 1993). Figure 2.3 shows the substrate utilisation during 30min of exercise at 25, 65 and 85% VO2 max. Lipolysis was increased from 25 to 65% VO2 max and plasma FFA concentration maintained at high levels. However, at an exercise intensity of 85% VO2 max plasma FFA concentration decreased approximately 50%. Their subsequent study used intralipid infusion to maintain plasma FFA concentration at the same level when subjects performed at 85% VO2 max (Romijn et al. 1995). The plasma FFA uptake and oxidation was higher but did not returned to the rate observed at 65% VO2 max in the previous study (Romijn et al, 1993). These findings indicate that fat oxidation is normally impaired during exercise at 85% VO2 max because of the failure of FFA mobilization to increase above resting levels. However, this explains only part of the decline in fat oxidation when exercise intensity is increased from 65 to 85% VO2 max.

A recent study by van Loon et al. (2001) using stable isotope methodology in combination with muscle biopsy sampling to assess substrate utilisation, investigated the regulation of muscle fuel selection during exercise. Eight cyclists were studied at rest and during three consecutive 30 min stages of exercise at intensities of 40, 55 and 75 % maximal workload (W max). Figure 2.4 shows the substrate utilization during the four different workloads. They found muscle glycogen and plasma glucose oxidation rates increased with every increment in exercise intensity. Whole-body fat oxidation increased at 55 % W max, but declined at 75 % W max. This decline involved a decrease in the oxidation rate of both plasma FFA and triacylglycerol fat sources. It was accompanied by increases in muscle pyruvate dehydrogenase complex activation and acetylation of the carnitine pool, resulting in a decline in muscle free carnitine.
Fig 2.3 Maximal contribution to energy expenditure derived from glucose and FFA taken up from blood and minimal contribution of muscle triglyceride and glycogen stores after 30 min of exercise, expressed as function of exercise intensity. Total amount of energy (Cal) available from plasma does not change in relation to exercise intensity. (Reproduced from Romijn et al. 1993)
Fig 2.4 Energy expenditure and fuel selection during variety of exercise intensity. Values are means. (Reproduced from van Loon et al. 2001)
concentration. They conclude that the most likely mechanism for the reduction in fat oxidation during high intensity exercise is a down-regulation of CPT I by this marked decline in free carnitine availability.

Early studies showed that increased plasma FFA concentrations might have a muscle glycogen sparing effect (Hickson et al. 1977; Costill et al. 1977). Several studies examined exercise at 80% $\dot{V}O_2_{max}$ when increased FFA availability decreased muscle glycogen utilisation and increased fat oxidation (Dyck et al. 1993; Romijn et al. 1995; Dyck et al. 1996). Dyck et al. (1996) examined muscle glycogenolysis and the regulation of glycogen phosphorylase (PHOS) activity during 15 min of cycling at 85% $\dot{V}O_2_{max}$ in control and high FFA (by intralipid technique) conditions. They found seven subjects used less muscle glycogen during the intralipid trial, and four did not respond. In this study, those subjects who spared glycogen, glycogen PHOS transformation into the active (alpha) form was unaffected by high FFA. Total AMP and IMP contents were not significantly different during exercise between trials, but total ADP was significantly lower with intralipid only at 15 min. The authors pooled the data with the results from the same research group (Dyck et al., 1993), and found the calculated free ADP, AMP, and Pi contents were lower with intralipid but not significantly different. The findings suggest that the elevation of plasma FFA during intense cycling spares muscle glycogen by post-transformational regulation of PHOS. This may be due to blunted increases in the concentrations of AMP, an allosteric activator of PHOS alpha, and Pi, a substrate for PHOS. However, another study reported no effect of high fat provision on muscle glycogen use but reduced glucose uptake during knee extension exercise (Hargreaves et al., 1991).

Odland et al. (2000) examined the effects of elevated FFA provision on the regulation
of pyruvate dehydrogenase (PDH) activity and malonyl-CoA content in human skeletal muscle during moderate-intensity exercise. They demonstrated that elevated FFA provision led to decrease RER values during exercise and to decrease human skeletal muscle malonyl-CoA content at rest and after 20 min of exercise. The lower malonyl-CoA may have contributed to the increased fat oxidation by reducing CPT I inhibition and increasing mitochondrial FFA transport (Odland et al. 2000).

Kiens et al. (1999) measured the content of long-chain fatty acids (LCFA) in biopsies obtained from the vastus lateralis muscle in humans at rest and after different exercise intensities. They concluded that in human skeletal muscle, oxidation rather than cellular availability of LCFA governs the rate of LCFA utilization at rest and during exercise. Consequently reduction in muscle LCFA oxidation during high-intensity exercise (e.g., 90% \( \dot{V}O_2_{max} \)) is due primarily to a decrease in mitochondrial LCFA oxidation rate rather than an insufficient cellular availability of LCFA.

2.2.1.2 Increase intramuscular triglyceride availability

Intramuscular triglyceride (IMTG) is one of the main fuels during submaximal exercise (Fig 2.3) (Romijn et al. 1993). Dietary manipulation is the most common process that changes IMTG concentrations. It appears that IMTG concentration increases following both long- (Kiens et al. 1987) and short-term (Jansson and Kaijser, 1982; Starling et al. 1997) consumption of high fat diets, whereas reduced fat intake for 1 week reduces IMTG (Coyle et al. 2001).

Coyle et al. (2001) reported a low-fat diet reduces IMTG concentration, whole body lipolysis, total fat oxidation, and calculated non-plasma fatty acid oxidation during exercise. During the 1st week of this study, subjects were fed a diet containing fat that
provided 32% of energy. During the 2nd and 3rd week, subjects were fed a diet of either 2 or 22% of energy from fat (2%FAI or 22%FAT). The results showed that 2%FAI lowered IMIG concentration and raised muscle glycogen concentration at rest. When subjects performed 1 hour of exercise at 67% \( \text{VO}_{2\text{max}} \) the 2%FAI trial resulted in a 27% reduction in total fat oxidation compared to the 22%FAI trial without altering the stable isotopically determined rates of plasma free fatty acid or glucose disappearance. In the 2%FAI trial the calculated non-plasma fatty acid oxidation was reduced by 40% in association with a 19% reduction in whole body lipolysis, while the calculated muscle glycogen oxidation was elevated by 19% compared with the 22%FAI trial. Therefore, an extremely low fat and high-carbohydrate diet lowers whole body lipolysis, total fat oxidation, and non-plasma fatty acid oxidation during exercise in the fasted state in association with a reduced concentration of IMIG.

Studies on the long-term consumption of high fat diets have shown reduced reliance on CHO as a fuel during moderate intensity exercise (Burke et al. 2000; Helge et al. 2001). In contrast, when IMIG use decreases then CHO oxidation and muscle glycogen utilisation is higher. However, there appeared to be no differences in glucose uptake either with increases or decreases in IMIG concentrations.

2.2.2 Effects of increase CHO availability on the interaction between fat and CHO oxidation during exercise.

Several studies have demonstrated that increasing the availability of CHO before or during exercise increases CHO oxidation and depresses fat oxidation (Coyle et al. 1997; Horowitz et al. 1997). Indeed, ingestion of CHO increased glycolytic flux and CHO oxidation and reduced both plasma FFA and IMTG oxidation (Coyle et al.
2.2.2.1 Increase glucose availability

Ingestion of glucose before exercise may increase glucose availability. Several studies have investigated the influence of glucose ingestion on subsequent exercise performance and metabolism (See section 2.4). Studies demonstrated that ingesting glucose prior to exercise induces increased insulin secretion during the postprandial period. Insulin plays an important role in CHO and fat metabolism (Kiens and Richter, 1996; Richter et al. 2001). It is commonly observed during the postprandial period and subsequent exercise, the rate of CHO oxidation is increased and the rate of fat oxidation is decreased when subjects consume glucose (Table 2.1).

Sidossis and Wolfe (1996) proposed a “reversed glucose-fatty acid cycle which indicated the importance of circulating glucose rather than FFA in controlling the reciprocal interplay between CHO and fat metabolism. They used hyperinsulinaemic and hyperglycaemic clamps to increase glucose availability. They concluded that glucose and/or insulin directly inhibits fatty acid oxidation. Subsequent studies also suggest that CHO might be the dominated influence on the oxidation rates of fat and CHO (Coyle et al. 1997; Horowitz et al. 1997).

Indeed, Coyle and colleagues demonstrated that pre-exercise CHO ingestion increases CHO oxidation and reduces both plasma fatty acid concentrations and IMTG oxidation (Coyle et al. 1997). The regulatory mechanism may involve FFA transport into muscle, which includes fatty acid transport protein and fatty acid translocase (FAT/CD36) (Turcotte, 2000), and also transport into mitochondria, which involve carnitine palmitoyl transferase (CPT I and CPT II) for long chain fatty acid (Coyle et al. 1997)(Fig 2.1). Carnitine palmitoyl transferase I is located on the outer
mitochondrial membrane which is believed to be the rate-limiting enzyme that is inhibited by malonyl-CoA (M-CoA) (McGarry et al. 1978, 1983, Berthon et al. 1998). Malonyl-CoA is produced in the cytoplasm by acetyl-CoA caboxylase. It was observed that when CHO availability increased, malonyl-CoA concentration was also increased which lead to the suggestion that it depresses fat oxidation by inhibiting of CPT I. However, the observation that during high intensity exercise fat oxidation is decreased cannot be attributed to an increase in muscle malonyl-CoA concentration (Winder & Hardie, 1996; Odland et al. 1998b; Winder, 1998; Dean et al. 2000).

2.2.2.2 Increase muscle glycogen availability

Blomstrand and Saltin reported that exercise with a leg that is low in glycogen results in an increase release of glycerol into the circulation. This circumstantial evidence suggests that muscle uses IMTG during exercise when muscle glycogen is low (Blomstrand and Saltin, 1999). This is consistent with the observations that the ingestion of high fat diets reduces muscle glycogen and exercise performance but promotes fat oxidation when compared to high CHO diet (Starling et al. 1997). Coyle et al. (2001) demonstrated that after a higher CHO diet, muscle glycogen concentration was higher than after a high fat diet. In this study, the high muscle glycogen trial elicited higher CHO oxidation during the 1 hour submaximal exercise in the fasted state (Coyle et al. 2001). The similar phenomenon was observed in a similar study by Burke et al. (2000). Therefore, muscle glycogen concentration may influence the regulatory enzymes controlling fat metabolism. However, further evidence is needed.

Glucose ingestion before exercise results in increases in plasma glucose and insulin concentrations, PDH activity and reduced plasma FFA concentration (Marmy-Conus
et al. 1996; Watt et al. 2002). Pre-exercise CHO ingestion may lead to muscle and liver glycogen synthesis. Ingestion of high GI CHO promoted an increase in insulin secretion which may enhance glucose uptake and reduces liver glucose output (Marmy-Conus et al. 1996) Increased insulin concentration is associated with increased CHO availability and may decrease IMTG hydrolysis (Enoksson et al. 1998; Jacob et al. 1999). Therefore, the choice of CHO that does not result in a highly insulinaemic response yet supplies glucose to muscle and liver and suppress fat oxidation less, may help improve endurance exercise performance.

In summary, CHO and fat are the primary metabolic substrates during submaximal exercise. The interrelation between CHO and fat metabolism during exercise is that increases in CHO availability can increase CHO oxidation and that increases in fat availability can increase fat oxidation (Coyle et al. 1997; Horowitz et al. 1997). The evidence indicated that the classic glucose-fatty acid cycle is unlikely to explain this interaction. The increase exogenous CHO availability can decrease fat oxidation probably through increase in plasma insulin and decreased FFA availability and also by decreasing the rate of fat transport into the muscle and/or mitochondria. However, further studies examining the mechanism on the CPT I activity, fat transport and the effect of IMTG along with muscle glycogen concentration on the interaction between CHO and fat metabolism awaited for further investigation.
2.3 The Glycaemic Index

The glycaemic index (GI) was initially proposed in 1981 (Jenkins et al. 1981). The GI of a food is defined as the incremental area under the blood glucose response curve following the intake of CHO-rich food containing 50g available CHO, expressed as a percent of the incremental area under the curve (IAUC) of blood glucose response following the intake of 50g CHO from the standard food (glucose or white bread, GI=100) as applied to the same individual.

\[
\text{GI} = \frac{\text{Blood glucose IAUC of Test food}}{\text{Blood glucose IAUC of standard food}} \times 100
\]

The calculation of an IAUC was proposed by Wolever and Jenkins in 1986, using trapezoid rules, which ignored the area below the fasting value during a 2 hour postprandial period.

The Food and Agricultural Organisation (FAO) and World Health Organisation (WHO) Expert Consultation has proposed a standardised protocol to determine the GI of a food. The tests have to be repeated in six or more subjects and the resulting GI values averaged. Subjects are studied on separate days, in the morning following a 10-12 hour overnight fast. A standard drink of water, tea or coffee, should be given with each test meal. Blood glucose is determined from capillary whole blood samples. Plasma glucose may also be used to determine the GI values because it gives similar values to those obtain from capillary blood. However capillary blood is preferred because it yields result that are similar to those from arterial blood. The proportion of food tested should contain 50g of available CHO. Either white bread or glucose can
be used as the standard food. Each GI value provided should also mention which food was used as the control.

The GI of a mixed meal can be calculated from the contribution of single food to the whole meal. The mixed meal GI value calculation method was proposed by Wolever and Jenkins (1986) as follows,

\[
\text{TC: Total carbohydrate content of meal (in gram)} \\
\text{Gl}_a, \text{Gl}_b, \text{Gl}_c, \ldots: \text{GI value of individual food} \\
\text{Wa, Wb, Wc, \ldots: Carbohydrate content of each food (in gram)} \\
\text{Total GI of the meal} = \frac{\text{Wa}}{\text{TC}} \cdot \text{Gl}_a + \frac{\text{Wb}}{\text{TC}} \cdot \text{Gl}_b + \frac{\text{Wc}}{\text{TC}} \cdot \text{Gl}_c + \ldots
\]

In a report published in 1998, the FAO and WHO recommended the use of the GI as a method of categorising CHO foods because the GI provides information on the likely physiological effects of ingested CHO. The GI has been widely used for clinical nutrition, especially for diabetics, and more recently in health promotion, because of the concerns over obesity, cardiovascular disease and specific cancers.

The database on GI of different foods consumed by both healthy and diabetic subjects that has been published providing international GI tables with more than 500 foods (Foster-Powell and Miller, 1995; Foster-Powell et al. 2002). The GI range has been classified into the high (GI>70), moderate (55<GI<70) and low (GI<55) CHO foods (glucose was used as the standard food).

2.3.1 Factors which modulate the GI

The GI of foods is affected by several variables included individual factors (within
and between subjects' effects), food factors, gut fermentation, and time of day effect. The following section will describe in detail the above factors which influence glycaemic indices of foods.

2.3.1.1 Individual factors

The individual factors which influence glycaemic response included insulin sensitivity, pancreatic $\beta$ cell function, gastrointestinal motility, physical activity, metabolism of previous meals, and day-to-day variation in metabolic parameters. Blood glucose responses show considerable day-to-day variation within subjects (Wolever et al. 1999). Therefore, it is recommended by the FAO/WHO that the standard food test be repeated at least three times in each subject to avoid day to day variation in blood glucose within subjects (FAO/WHO, 1998). Although glycaemic responses in healthy versus diseased subjects are different, it has been established that GI values for the same foods are highly correlated regardless of the subjects' glucose tolerance status.

2.3.1.2 Food factors influencing glycaemic responses

A) Choice of standard food

The choice of standard food affects the outcome of the GI. According to the FAO/WHO protocol, either white bread or glucose can be used as the standard food. In the same food, using white bread as the standard food can obtain 1.4 times GI values when comparing to glucose as the standard food. In this thesis, all the GI values were taken from the table that used glucose as the standard food i.e. they were adopted from Foster-Powell and Brand Miller's 1995 GI table (Foster-Powell and Brand-Miller, 1995).

B) Nature of the starch

Amylose and amylopectin are different chemical bond linkages in starch. Amylose is
a linear type of starch, consisting of glucose residues in $\alpha 1,4$ linkage. Amylopectin is the branched form of starch which has about one $\alpha 1,6$ linkage per thirty $\alpha 1,4$ linkages (Stryer, 1988). Amylopectin has been described as a branched chain starch, and is more readily hydrolysed than amylose. It has been reported that starch with a high amylopectin content could induce higher glycaemic responses compared to amylose (Kabir et al. 1998; Larsen et al. 1996). Thus, the high ratio of amylose/amylopectin in starch may attenuate glycaemic responses. Some observations suggest that the amylose content of the starch used in the acute meal needs to be greater than 50% in order to significantly reduce the postprandial plasma glucose and insulin responses. In addition, resistant starch, which is an indigestible form of starch, might result in slower digestion and absorption of CHO, and therefore, decreases glycaemic responses (Raben et al. 1994).

C) Cooking and food processing

During cooking and food processing of CHO foods the degree of gelatinisation (Vaaler et al. 1984; Miller et al. 1992), food particle size (Holt and Miller, 1994), and physical form of food affect glycaemic responses (Collings et al. 1981). The starch granules are compacted in raw starchy foods. During cooking, water and heat expand the starch granules which become swollen and burst in a process known as gelatinisation (Cummings and Englyst, 1995). A fully gelatinised starch is easy to digest as the swollen granules have a greater surface to contact the hydrolytic enzymes in the intestine. It is therefore, the level of gelatinisation that will affect the glycaemic index. A gelatinised starch will retrograde after cooling down. A retrogradation starch is more resistant to hydrolysis (Cummings and Englyst, 1995). Modern food processing methods, such as grinding, flaking and popping, and increasing gelatinisation have been known to increase glycaemic responses. Starch and protein mixture can also become unavailable for digestion and absorption in the
browning reaction (Maillard reaction). These processes either reduce the food particle size or increase the enzymatic hydrolysis surface which may highly affect the outcome of glycaemic indices (Wolever et al. 1986).

D) Fat and Protein

Ingestion of fat and protein in addition to CHO is known to affect the glycaemic response to foods. Fat ingestion may decrease the rate of gastric emptying by increasing the viscosity of the chyme and also interfere with the action of digestive enzymes on CHO. High fat food will therefore tend to have lower GI values. Protein may induce insulin secretion (Rabinowitz et al. 1966). Addition of protein to potato and spaghetti (Gulliford et al. 1989) and liquid glucose polymer drinks (Spiller et al. 1987) greatly increase the insulin response of these foods. Several studies reported that adding protein to CHO ingestion post-exercise induces higher glucose responses when compared to ingestion of CHO alone (van Hall et al. 2000b; Ivy et al. 2002).

E) Dietary fibre

Viscous fibre, i.e. water-soluble fibre, increases the viscosity of food which leads to a reduced transit rate in the small intestine (Leeds, 1979; Blackburn et al. 1984; Leeds, 1987). Viscous fibre also reduces the interaction between the starch enzymes for starch hydrolysis, and lowers the rate of diffusion of glucose in the lumen of the small intestine (Jenkins et al. 1987). It therefore may lower glycaemic responses. However, insoluble fibre has no such effect as there are no differences in glycaemic responses between white and wholemeal bread, white and brown spaghetti, and white and brown rice (Jenkins et al. 1983).

F) Organic acids and anti-nutrients

The organic acids and anti-nutrients also incur blood glucose lowering effects. Several studies indicated that ingestion of vinegar or lemon juice with mixed meals significantly lowers glycaemic responses (Brand-Miller et al. 1997). Several reports
also indicated that CHO which contains phytic acid or phytate lowers the glycaemic responses (Thompson et al. 1984; Thompson et al. 1987). It is believed that the lowered glycaemic effects might be induced by interfering with amylase activities and decreased rate of gastric emptying (Yoon et al. 1983; Liljeberg and Bjorck, 1996; Liljeberg and Bjorck, 1998). Moreover, some anti-nutrients such as lectins and amylase inhibitors may reduce amylase activity, thus decreasing postprandial blood glucose responses (Wolever, 1990).

2.3.1.3 Time of day effects

The time of the day when the test is conducted has an important influence on the GI (Wolever et al. 1988). However, the GI of a food remains the same even when measured fasted at different times of the day. The FAO/WHO protocol recommends that testing should take place in the morning, after an overnight fast (FAO/WHO, 1998).

2.3.1.4 Gut fermentation

There is a considerable amount of CHO left undigested in the small intestine which may be available for microbial fermentation in the colon. Colonic fermentation of undigested CHO yields short chain fatty acids. These short chain fatty acids may impact upon gastric and small intestinal processes and thereby affect glycaemic response (Jenkins and Jenkins, 1985; Luo et al. 1996).

2.3.1.5 Gorging and nibbling

It has been suggested that the glycaemic response could be improved when CHO is digested slowly rather than through gorging (Jenkins et al. 1989; Jenkins et al. 1990; Jenkins et al. 1992). Jenkins et al. (1990) fed subjects with 50 g glucose in 700 ml
water on two occasions: over 5-10 min (bolus) and at a constant rate over 3.5 h (sipping). Despite similar 4-h blood glucose areas, large reductions were seen in serum insulin (54±10%) areas after sipping. There was also prolonged suppression of plasma glucagon, growth hormone, and free-fatty acid (FFA) levels after sipping, whereas these levels rose 3-4 h after the glucose bolus. An intravenous glucose tolerance test at 4 h demonstrated a 48±10% more rapid decline in blood glucose after sipping than after the bolus. Furthermore, FFA and total branched-chain amino acid concentrations as additional markers of insulin action, were lower over this period despite similar absolute levels of insulin and C-peptide. These findings indicate that prolonging the rate of glucose absorption enhances insulin economy and glucose disposal.

In the study by Jenkins et al. (1992), their subjects consumed 13 snacks (the nibbling diet) on one day and on another day the same diet was taken as three meals and one snack (the three-meal diet). The nibbling diet reduced mean blood glucose, serum insulin, and C peptide concentrations over the 9.5 h of observation. Twenty-four hour low urinary C peptide concentrations indicated that prolonging the rate of glucose absorption enhances insulin economy and glucose disposal (Jenkins et al. 1992).

2.3.2 Glycaemic index – Implication in health and diseases
The GI has been well used as a tool in planning diets for diabetic patients. Recently, the GI has been used as one of the key factors in prevention of obesity, Type II diabetes, hyperlipidaemia, cardiovascular diseases and some specific cancers in the general population (Brand-Miller et al. 2002; Pi-Sunyer, 2002; Leeds, 2002; Jenkins et al. 2002; Liu and Willett, 2002; Willett et al. 2002). It has been suggested that high GI foods are detrimental to health due to postprandial hyperglycaemia and
hyperinsulinaemia which can lead to insulin resistance (Brand-Miller 2003).

Epidemiological studies have shown that a high GI diet increases the risk of coronary heart disease (CHD) (Ford and Liu, 2001; Katan, 1999; Liu and Willett, 2002; Frost et al. 1999; Liu et al. 2000). In addition to epidemiological studies, nutritional intervention studies indicate that a low GI diet might be beneficial in maintaining normal blood lipid profiles in healthy subjects, hyperlipidaemic subjects, diabetes mellitus patients, and patients with CHD (Jenkins et al. 1987; Wolever et al. 1992a; Frost et al. 1996; Frost et al. 1998; Jarvi et al. 1999).

Whether the long-term intake of high GI carbohydrates increases the risk of Type II diabetes has been a long-standing debate. In recent years, epidemiological and nutritional intervention data suggest that hyperglycaemia leads to loss of pancreatic $\beta$ cell function that can result in glucose intolerance and ultimately an irreversible state of diabetes (Willett et al. 2002). Although the mechanism of $\beta$ cell exhaustion is still unclear, high glycaemic index foods and a high glycaemic diet has been considered as one of the main risk factors.

Ingestion of low GI foods may be beneficial for weight control in two ways: by promoting satiety and by promoting fat oxidation at the expense of CHO oxidation (Holt et al. 1992; Holt et al. 1996; Wee et al. 1999; Ludwig et al. 1999; Brand-Miller et al. 2002; Ludwig, 2000; Febbraio et al. 2000). Some long-term studies in human and animal models have shown the benefit of low GI foods for weight control in obese populations (Wolever et al. 1992b; Slabber et al. 1994; Kabir et al. 1998; Pawlak et al. 2001; Spieth et al. 2000).
2.3.3 Glycaemic index and physical performance

Thomas and co-workers first reported that pre-exercise low GI CHO improved exercise performance (Thomas et al. 1991). Thereafter, a number of studies investigated the effect of the GI of CHO on exercise performance and post-exercise recovery. However, the effects of the GI of CHO on exercise performance are still inconclusive. The influence of the GI of CHO on exercise performance will be discussed in the later section, both in the pre-exercise feeding (section 2.4) and post-exercise recovery feeding sections (section 2.5).
2.4 Pre-exercise carbohydrate intakes - the effects on metabolism and endurance performance

The link between pre-exercise glycogen concentrations and human endurance exercise capacity has been well established since the 1960s (Bergstrom and Hultman, 1967; Bergstrom et al. 1967; Karlsson and Saltin, 1971). Since then a numbers of studies have tried to find the optimal pre-exercise nutritional strategy to increase muscle glycogen stores before endurance exercise or competition. Carbohydrate intake is known to increase muscle and liver glycogen stores. In addition to increasing muscle glycogen concentrations, reducing the rate of muscle glycogen use by promoting fat oxidation during exercise has also received attention (Hawley et al. 1998; Jeukendrup, 1999; Horowitz and Klein, 2000).

Pre-exercise CHO ingestion introduces a variety of effects on subsequent exercise metabolism and performance. The different feeding and exercise protocols between studies have produced different physiological responses and exercise performance results. The protocols differ in time of ingestion, types and amounts of CHO, and exercise intensity and duration. These factors influence the magnitude of the insulin response which is the dominant factor affecting substrate utilisation during subsequent exercise.

The following section considers the influence of pre-exercise CHO feeding on metabolism and exercise performance. It is divided into three subsections: (1) ingestion of CHO within 1 hour before exercise; (2) ingestion of CHO 2-4 hours before exercise; (3) the effect of pre-exercise GI CHO on metabolism and exercise performance.
2.4.1 Effects of carbohydrate ingestion within the hour before exercise

It has been reported that ingesting CHO in the hour prior to exercise may have a positive (Gleeson et al. 1986; Thomas et al. 1991; Sherman et al. 1991; Goodpaster et al. 1996; Kirwan et al. 1998; Kirwan et al. 2001), negative (Foster et al. 1979) or neutral effect (Hargreaves et al. 1987; Chryssanthopoulos et al. 1994; Jentjens et al. 2003) on subsequent exercise performance. The range of studies on this question and their principal findings are summarised in Table 2.1. The inconsistent results may be due to the timing of ingestion, the amount and type of CHO, and the different exercise protocols employed.

Studies examining ingestion of CHO within an hour before exercise mainly used simple carbohydrate i.e. glucose (high GI), fructose (low GI), sucrose (medium GI), or other simple CHO (Table 2.1). Foster et al (1979) reported that ingesting 75 g of glucose 30 min before exercise resulted in a reduction in cycling endurance capacity by 19% compared to plain water or milk. They hypothesized that the rapidly increase in blood glucose after ingesting the concentrated glucose solution caused insulin over-secretion. Insulin secretion inhibits hepatic glucose release for maintenance of blood glucose concentration, and also increases blood glucose uptake into muscles and the liver. This triggered rebound hypoglycaemia during early stage of exercise. At the same time, insulin also inhibited mobilisation and use of fat for energy production, i.e. suppression of lipolysis. The authors suggested that a greater muscle glycogen degradation caused early glycogen depletion and fatigue (Foster et al. 1979). Therefore athletes are sometimes advised to avoid CHO intake within the hour before exercise since this may have a negative effect on performance. However, the later studies have not shown negative effects on exercise performance when subjects...
ingested glucose within the hour before exercise (Gleeson et al. 1986; Hargreaves et al. 1987; Sherman et al. 1991; Chryssanthopoulos et al. 1994; Goodpaster et al. 1996; Jentjens and Jeukendrup 2003). Nevertheless, a consistent observation in all the studies on this topic was a significantly decrease in blood glucose concentration during the first 15-30 min during subsequent exercise. It could be argued that because a decrease in blood glucose concentrations during prolonged endurance exercise has been associated with onset fatigue that this early fall in blood glucose might also contribute to a early onset of fatigue (Coyle et al. 1986; Wright et al. 1991). However, a rebound hypoglycaemia in the early stages of exercise seems to be of little functional significance as this does not affect subsequent exercise performance in most of studies (Alberici et al. 1993; Chryssanthopoulos et al. 1994; Jentjens et al. 2003; Jentjens and Jeukendrup 2003).

Several studies examined the effect of different amount of glucose feedings within the hour before exercise (Sherman et al. 1991; Seifert et al. 1994; Short et al. 1997; Jentjens et al. 2003). Despite the different amounts of glucose solutions ingested (22.5 g to 200 g), blood glucose concentrations at the onset of exercise and 10-20 min into exercise were similar between all studies (Sherman et al. 1991; Seifert et al. 1994; Short et al. 1997; Jentjens et al. 2003). A comparatively hypoglycaemic response was observed after subjects ingested 25, 75 or 200 g of glucose solutions compared with a placebo solution (Jentjens et al. 2003). Short et al. (1997) and Jentjens et al. (2003) reported that although insulin responses were greater with a larger glucose intake, blood glucose concentrations were similar throughout subsequent exercise.

Ingestion of fructose, unlike glucose, induces lower insulinaemic and glycaemic responses during the postprandial period. Ingesting fructose within the hour before
exercise resulted in less perturbations in blood glucose concentrations during subsequent exercise (Levine et al. 1983; Decombaz et al. 1985; Koivisto et al. 1985; Hargreaves et al. 1987; Fielding et al. 1987). This lower insulinaemic may also have a less inhibitory influence lipolysis. Therefore, these responses may offer a rationale for ingesting fructose prior to exercise as it may be of benefit to exercise performance. However, fructose is mainly taken up in the liver for maintaining hepatic glucose production. The plasma free fatty acid concentrations after fructose ingestion appear to be similar to those after glucose (Koivisto et al. 1985) or placebo ingestion (Okano et al. 1988). Levine et al (1983) observed muscle glycogen utilisation appeared to be lower during exercise after ingestion of fructose when compared with ingestion of glucose or placebo solutions. However, the glycogen sparing effect was not observed in other studies (Koivisto et al. 1985; Hargreaves et al. 1987; Fielding et al. 1987). The studies that have reported muscle glycogen sparing (Levine et al. 1983) and improvement in endurance capacity (Okano et al. 1988) fed their subjects before exercise. This is different from other studies in which subjects were overnight fasted before exercise (Koivisto et al. 1985; Hargreaves et al. 1987; Fielding et al. 1987). It seems that the benefit of ingesting fructose within the hour before exercise on exercise performance is still inconclusive. In practice, ingesting high fructose solutions is often accompanied by gastrointestinal distress that includes diarrhoea and vomiting (Craig, 1993). Therefore, these responses would not make fructose the first CHO of choice for pre-exercise feeding.

Other limitation of consuming CHO within the hour before exercise is the timing of ingestion. Ingesting CHO only 30-60 min before exercise may not allow enough time for complete digestion and absorption. Several studies reported a number of cases of gastrointestinal discomfort during exercise when subjects ingested CHO foods within
an hour before performing exercise (Hargreaves et al. 1987; Thomas et al. 1991; Costill and Hargreaves, 1992). The discomfort might be due to the food still in the gut. The gastrointestinal stress may lead to impair the performance during subsequent exercise.

In summary, ingestion of CHO within the hour before exercise may have the following limitations:

1. Insufficient time to digest and to absorb the CHO.
2. High GI CHO may result in hyperinsulinaemia in subjects leading them to develop hypoglycaemia in the early stages of exercise.
3. Promote CHO oxidation and depress fat oxidation.

In addition to the above effects, ingesting a bolus of a CHO solution or eating single foods within the hour before exercise seems less applicable to the real world of competition or trainings.
Table 2.1 Summary of studies examining the effects of CHO ingestion within the hour before exercise on metabolism and performance during subsequent exercise.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Type of CHO</th>
<th>Amount of CHO</th>
<th>Time before exercise</th>
<th>Exercise protocol</th>
<th>Blood glucose during exercise</th>
<th>CHO oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koivisto et al 1981</td>
<td>Glucose (G) Fructose (F) Placebo (P)</td>
<td>75g</td>
<td>45 min</td>
<td>75% VO₂ max cycling for 45 min</td>
<td>P&gt;F&gt;G</td>
<td></td>
<td>G, F&lt;P</td>
<td></td>
</tr>
<tr>
<td>Hargreaves et al 1987</td>
<td>Glucose (G) Fructose (F) Placebo (P)</td>
<td>75g</td>
<td>45 min</td>
<td>75% VO₂ max cycling till exhaustion</td>
<td>G&lt;F, P at 15min</td>
<td></td>
<td></td>
<td>No difference</td>
</tr>
<tr>
<td>Decombeaz et al 1985</td>
<td>Glucose (G) Fructose (F)</td>
<td>1 g/kg bw</td>
<td>1 h</td>
<td>61% VO₂ max cycling for 45 min + 15 min maximal output</td>
<td>G&lt;P</td>
<td></td>
<td>No difference</td>
<td>No difference</td>
</tr>
<tr>
<td>Fielding et al 1987</td>
<td>Glucose (G) Fructose (F) Placebo (P)</td>
<td>75g</td>
<td>30 min</td>
<td>70% VO₂ max running for 30 min</td>
<td>G&lt;F, P at 15 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sherman et al 1991</td>
<td>Low (L) High (H) Placebo (P)</td>
<td>1.1g/kg bw</td>
<td>1 h</td>
<td>70% VO₂ max cycling for 90 min + performance trial</td>
<td>L, H&lt;P</td>
<td>L, H&gt;P</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: H > P indicates a significant difference favoring the high CHO group, F > G indicates a significant difference favoring the CHO group, and L, H > P indicates a significant difference favoring the high CHO group in the performance trial.
### Table 2.1 Summary of Studies examining the effects of CHO ingestion within the hour before exercise on metabolism and performance during subsequent exercise

<table>
<thead>
<tr>
<th>Studies</th>
<th>Type of CHO</th>
<th>Amount of CHO</th>
<th>Time before exercise</th>
<th>Exercise protocol</th>
<th>Blood glucose during exercise</th>
<th>CHO oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foster et al 1979</td>
<td>Water (W) Glucose (G) Liquid Meal (M)</td>
<td>0g 75g 15g CHO + 10g protein + 12.5g fat</td>
<td>30min</td>
<td>80% VO₂ max cycling to exhaustion</td>
<td>G&lt;W, M</td>
<td>G&gt;W, M</td>
<td>G&lt;W, M</td>
<td>G&lt;W, M</td>
</tr>
<tr>
<td>Guezennec et al 1989</td>
<td>Glucose (G) Fructose (F) Corn starch (Cs)</td>
<td>100g</td>
<td>1 h</td>
<td>60% VO₂ max cycling for 120min</td>
<td>G, CS&lt;F during 1st h</td>
<td>G, CS&gt;F</td>
<td>G, CS&lt;F during 1st h</td>
<td>-</td>
</tr>
<tr>
<td>Gleeson et al 1986</td>
<td>Glucose (G) Fast (F)</td>
<td>70 g</td>
<td>45min</td>
<td>70% VO₂ max cycling till exhaustion</td>
<td>-</td>
<td>-</td>
<td>G&gt;F</td>
<td></td>
</tr>
<tr>
<td>Goodpaster et al 1996</td>
<td>Waxy starch (W), Resistant starch (RS), Glucose (G), Placebo (P)</td>
<td>1g/kg</td>
<td>30min</td>
<td>90min cycling at 66% VO₂ max + 30min performance ride</td>
<td>No difference</td>
<td>W, RS, G&gt;P</td>
<td>Plasma glycerol P&gt;W,RS, G</td>
<td>W, RS, G&gt;P</td>
</tr>
</tbody>
</table>
Table 2.1 Summary of Studies examining the effects of CHO ingestion within the hour before exercise on metabolism and performance during subsequent exercise

<table>
<thead>
<tr>
<th>Studies</th>
<th>Type of CHO</th>
<th>Amount of CHO</th>
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<th>Blood glucose during exercise</th>
<th>CHO oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guezennec et al 1993</td>
<td>Glucose (G)</td>
<td>836KJ</td>
<td>1h</td>
<td>120min cycling at 60% VO$_2$ max</td>
<td>G, P &lt; R, S at 30min</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Potato (P)</td>
<td>286KJ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bread (B)</td>
<td>178KJ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rice (R)</td>
<td>492KJ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spaghetti (S)</td>
<td>264KJ</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Jentjens et al 2003</td>
<td>Placebo</td>
<td>0g (P)</td>
<td>45min</td>
<td>20min at 65% max power output (SS) + time trial (TT)</td>
<td>P &gt; L, M, H</td>
<td>RER values</td>
<td></td>
<td>No difference</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>75g</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>25g (L)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>75g (M)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>200g (H)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jentjens &amp; Jeukendrup 2003</td>
<td>Glucose (GLU)</td>
<td>75g</td>
<td>45min</td>
<td>20min at 65% max power output (SS) + time trial (TT)</td>
<td>GLU &lt; GAL, TRE</td>
<td>No difference</td>
<td></td>
<td>No difference</td>
</tr>
<tr>
<td></td>
<td>Galactose (GAL)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Trehalose (TRE)</td>
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</tr>
</tbody>
</table>
2.4.2 Effects of carbohydrate ingestion 2-4 hour before exercise

Consuming CHO within the hour before exercise is insufficient time for digestion and absorption, and may cause gastrointestinal stress and hyperinsulinaemia. Therefore, feeding so soon before exercise is not applicable to the real world of training and competition. Some studies investigated ingestion of CHO 2-4 hours before exercise in an attempt to prevent hyperinsulinaemia leading to hypoglycaemia during the early stages of exercise (Table 2.2). In addition to preventing hypoglycaemia, feeding 2-4 hours before exercise means that a larger amount of CHO can be consumed and there is a longer postprandial period during which some liver and muscle glycogen synthesis may take place (Taylor et al. 1993).

Coyle et al (1985) reported that subjects who consumed a meal containing 2g·kg\(^{-1}\) CHO after an over-night fast 4 hour before exercise increased muscle glycogen concentrations when compared to the fasting state. Later studies showed that ingestion of either CHO-electrolyte solutions or CHO-rich meals 2-4 hour before exercise improve subsequent exercise performance when compared with exercise after fasting (Neufer et al. 1987; Wright et al. 1991; Chryssanthopoulos and Williams, 1997; Schabert et al. 1999; Chryssanthopoulos et al. 2002). However, this improvement did not occur in all studies (Whitley et al. 1998).

The studies mentioned above used different exercise protocols to assess subsequent exercise with either endurance capacity (Wright et al. 1991; Chryssanthopoulos and Williams, 1997; Schabert et al. 1999; Chryssanthopoulos et al. 2002) or endurance performance (Neufer et al. 1987; Whitley et al. 1998) being the assessed outcome. Endurance capacity is defined as the time to volitional fatigue during submaximal exercise at a fixed intensity between 65-85% \(\text{VO}_2\max\) (Williams, 1989). Time trials
where subjects have to complete a fixed amount of work load as fast as possible or perform as much of a work load as possible during a fixed time period are example of endurance performance (Jeukendrup et al., 1996). An endurance performance test may not be as severe as endurance capacity test in challenging CHO stores i.e. muscle and liver glycogen. It seems clear that ingestion of CHO 2-4 hour before exercise improved endurance capacity (Wright et al. 1991; Chryssanthopoulos and Williams, 1997; Schabert et al. 1999; Chryssanthopoulos et al. 2002) but not all endurance performance trials were improved (Neufer et al. 1987; Whitley et al. 1998) when compared to the fasting state.

When subjects ingested either CHO solutions or CHO meals an increase in plasma insulin and glucose concentrations during the postprandial period in these endurance studies was consistently observed. Some studies showed the concentrations of insulin and glucose retuned to fasting values before exercise (Coyle et al. 1985; Neufer et al. 1987; Sherman et al. 1989), but not others (Wright et al. 1991; Chryssanthopoulos and Williams, 1997; Whitley et al. 1998; Chryssanthopoulos et al. 2002). Even though the plasma insulin concentrations had returned to fasting values, the persistent effect of insulin still caused a transient decline in blood glucose concentrations during the early stage of exercise. However, the phenomenon did not impair subsequent exercise as the studies showed an improvement in endurance capacity and (Wright et al. 1991; Chryssanthopoulos and Williams, 1997; Schabert et al. 1999; Chryssanthopoulos et al. 2002) performance (Neufer et al. 1987). Furthermore, the persistent insulin effect leads to a greater CHO oxidation and a suppression of plasma glycerol and free fatty acid concentrations during subsequent exercise (Coyle et al. 1985). Coyle et al. (1985) reported glycogen utilisation was higher in the fed trials compared to the fasting trials during
subsequent exercise. The phenomenon might be the result of using the test foods that were mainly high GI CHO. Therefore, the choice of CHO may need further consideration when planning a pre-exercise meal.

In summary, ingestion of CHO 2-4 hours before exercise seems to cause less gastrointestinal stress and is more applicable to daily training and competition for athletes. The current relevant literature seems to be clear that ingestion of CHO 2-4 hour prior to exercise improves endurance capacity compared to fasting before exercise. These studies also showed a greater rate of CHO oxidation during subsequent exercise and a lower fat oxidation.
Table 2.2 Summary of Studies examining the effects of CHO ingestion 2-4 hour before exercise on metabolism and performance during subsequent exercise

<table>
<thead>
<tr>
<th>Studies</th>
<th>Type of CHO</th>
<th>Amount of CHO</th>
<th>Time before exercise</th>
<th>Exercise protocol</th>
<th>Blood glucose during exercise</th>
<th>CHO oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coyle et al 1985</td>
<td>CHO meal (C)</td>
<td>2.0 g/kg +</td>
<td>4 h</td>
<td>70% ( \text{VO}_2 ) max cycling for 105 min</td>
<td>F&lt;C during the 1st h</td>
<td>C&gt;F (45% greater in the 1st h)</td>
<td></td>
<td>C&lt;F</td>
</tr>
<tr>
<td></td>
<td>Fast (F)</td>
<td>0.3 g/kg Protein</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Neufer et al 1987</td>
<td>(I) Liquid CHO (L),</td>
<td>(I) 45 g</td>
<td>(I) 15 min</td>
<td>77% ( \text{VO}_2 ) max cycling for 45 min + 15 min performance test</td>
<td>L,S&gt;P at 10, 20 min</td>
<td>MS&gt;S, L&gt;P during 15 min performance</td>
<td>Glycerol</td>
<td>MS&gt;L, S&gt;P</td>
</tr>
<tr>
<td></td>
<td>Solid CHO (S),</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Placebo (P)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(II) meal + solid CHO (MS)</td>
<td>(II) 200 g + 45 g</td>
<td>(II) 4 h + 15 min meal</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Chryssanthopoulos and Williams</td>
<td>CHO + CHO drink</td>
<td>2.5 g (+CHO and placebo drink during exercise)</td>
<td>3 h</td>
<td>70% ( \text{VO}_2 ) max running till exhaustion</td>
<td>MC&gt;PC, PP during 1st 60 min</td>
<td></td>
<td></td>
<td>MC&gt;PC&gt;PP</td>
</tr>
<tr>
<td>1997</td>
<td>(MC)</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>Placebo + CHO drink</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(PC)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo + Placebo drink (PP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schabot et al 1999</td>
<td>CHO (C)</td>
<td>100 g CHO breakfast</td>
<td>3 h</td>
<td>70% ( \text{VO}_2 ) max cycling till exhaustion</td>
<td>No difference</td>
<td></td>
<td></td>
<td>C&gt;F</td>
</tr>
<tr>
<td></td>
<td>Fast (F)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Table 2.2 Summary of Studies examining the effects of CHO ingestion 2-4 hour before exercise on metabolism and performance during subsequent exercise

<table>
<thead>
<tr>
<th>Studies</th>
<th>Type of CHO</th>
<th>Amount of CHO</th>
<th>Time before exercise</th>
<th>Exercise protocol</th>
<th>Blood glucose during exercise</th>
<th>CHO oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
<th>Exercise capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright et al 1991</td>
<td>25% glucose polymer</td>
<td>5g/kg</td>
<td>3h</td>
<td>70% VO2 max cycling with high intensity interval every 45 min till exhaustion</td>
<td>CC, CP &lt; fasting</td>
<td></td>
<td></td>
<td>CC, CP, PC &gt; PP</td>
</tr>
<tr>
<td></td>
<td>CHO+CHO drink (CC)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>CHO+Placebo drink (CP)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Placebo +CHO drink (PC)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Placebo+Placebo drink (PP)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sherman et al 1989</td>
<td>Low (L)</td>
<td>45g</td>
<td>4h</td>
<td>70% VO2 max 95min + time trial</td>
<td>H&lt;M, L, P at 15 min</td>
<td></td>
<td></td>
<td>H&gt;M, L, P</td>
</tr>
<tr>
<td></td>
<td>Medium (M)</td>
<td>156g</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>High (H)</td>
<td>312g</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Placebo (P)</td>
<td>0g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chryssanthopoulos et al 2002</td>
<td>CHO+CHO drink (MC)</td>
<td>2.5g (+CHO and water drink during exercise)</td>
<td>3h</td>
<td>70% VO2 max running till exhaustion</td>
<td></td>
<td>MC,MW&gt;PW during 1st h</td>
<td>MC,MW&lt;PW during 1st h</td>
<td>MC&gt;MW&gt;PW</td>
</tr>
<tr>
<td></td>
<td>CHO+Water drink (MW)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Placebo+Water drink (PW)</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Whitley et al 1998</td>
<td>CHO meal</td>
<td>215g/70kg</td>
<td>4h</td>
<td>70% VO2 max cycling for 90min and 10km time trial</td>
<td></td>
<td>CHO&gt;Fast</td>
<td></td>
<td>No difference</td>
</tr>
<tr>
<td></td>
<td>Fast</td>
<td></td>
<td></td>
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</tbody>
</table>
2.4.3 Effect of different GI carbohydrate ingestion before exercise

Early studies on pre-exercise CHO feeding resulted in hyperinsulinaemia leading to hypoglycaemia in the early stage of exercise. To protect against hypoglycaemic symptoms, ingestion of CHO without inducing hyperinsulinaemia during the postprandial period has received attention. Thomas et al. (1991) first considered the effect of the GI of foods on endurance exercise performance. They reported that ingestion of lentils, a low GI CHO, one hour before exercise improved endurance capacity when compared to ingestion of potatoes, a high GI CHO (Thomas et al. 1991). However, subsequent studies on the effect of the GI of CHO on endurance performance are controversial (Table 2.3). Some studies report that exercise performance in trials where pre-exercise feeding used high GI food was worse than with medium GI or low GI foods (Thomas et al. 1991; Kirwan et al. 1998; DeMarco et al. 1999; Kirwan et al. 2001a; Wong et al. 2003). Another study claimed that medium GI foods improved exercise performance more than that of placebo trials (Kirwan et al. 2001b). However, some studies reported similar performances after ingestion of either high GI or low GI CHO foods (Febbraio and Stewart, 1996; Burke et al. 1998; Sparks et al. 1998; Wee et al. 1999). The range of studies on this question and their principal findings are summarised in Table 2.3. The effect of ingesting different GI CHO pre-exercise on subsequent exercise performance seems to be inconclusive.

When comparing the influence of the GI of CHO on subsequent exercise performance, different protocols have been employed (Table 2.3). Some studies included control or fasting trials (Thomas et al. 1991; Febbraio and Stewart, 1996; Burke et al. 1998; Kirwan et al. 1998; Sparks et al. 1998; DeMarco et al. 1999; Kirwan et al. 2001b), which provided no CHO, but not all (Thomas et al. 1994; Wee et al. 1999; Wong et al. 2003).
2003). Regarding performance test protocols, some studies measured endurance capacity, in terms of time to exhaustion at a constant workload on a cycle ergometer or at a constant pace on a treadmill (Thomas et al. 1991; Thomas et al. 1994; Kirwan et al. 1998; Wee et al. 1999; DeMarco et al. 1999; Kirwan et al. 2001a; Kirwan et al. 2001b). Other studies measured endurance performance, in terms of work output during a fixed time (Febbraio and Stewart, 1996; Sparks et al. 1998), or time to complete a fixed distance or workload (Burke et al. 1998; Wong et al. 2003) after a bout of prolonged submaximal exercise. Endurance performance tests are known to challenge the energy systems less than endurance capacity tests. Those studies that claimed exercise performance was improved used mainly endurance capacity as the performance test protocol (Thomas et al. 1991; Kirwan et al. 1998; DeMarco et al. 1999; Kirwan et al. 2001a). However, ingestion of low GI CHO within the hour before exercise did not affect endurance performance (Febbraio and Stewart, 1996; Sparks et al. 1998).

The underlying mechanism behind improved endurance capacity of ingesting low GI food has been suggested to be related to lower glycaemia and insulinaemia during postprandial period. In most studies, the low GI pre-exercise foods maintained a better blood glucose concentration during exercise when compared with high GI food (Table 2.3). Several studies observed higher plasma FFA concentrations during exercise in the low GI trials than in the high GI trials (Thomas et al. 1991; Thamoas et al. 1994; DeMarco et al. 1999; Wee et al. 1999). Furthermore, the low insulinaemic effect of ingesting low GI foods might lead to a lower rate of CHO oxidation and/or a higher rate of fat oxidation (Guezennec et al. 1993; Thomas et al. 1994), which might spare muscle glycogen (Thomas et al. 1991; Guezennec, 1995). It has been reported that when FFA concentrations are increased, muscle glycogen utilization is reduced.
Although these studies claimed the test foods or meals used supplied the same amount of CHO with different GIs, it is worth noting that the macronutrient contents were different (Hargreaves et al. 1987; Thomas et al. 1991; Costill and Hargreaves, 1992; Thomas et al. 1994; Febbraio and Stewart, 1996; Burke et al. 1998; Kirwan et al. 1998; Kirwan et al. 2001a). As a consequence, test meals were neither isocaloric nor provided the same amount of macronutrients that might cause subsequent exercise performance to differ. Studies using well designed isocaloric test meals with the same macronutrient content but with different GI values are much in need.

The studies that examined the GI CHO effect on exercise performance have used mainly cycle ergometer exercise and the test foods or meals were ingested within the hour before exercise (Table 2.3). Ingestion of different GI foods a few hours before exercise is more practical in the real world but has received less attention. Wee and colleagues first reported the influences of ingesting different GI foods 3 hours before a treadmill run to exhaustion (endurance capacity) (Wee et al. 1999). Even though a higher rate of fat oxidation was observed in the low GI trial compared with the high GI trial, there was no difference in endurance running capacity. When the ratio of glycaemic response between the low GI trial and the high GI trial was examined, it was found to be inconsistent with the values given in the international GI Table (Foster-Powell and Miller, 1995). Wee et al. (1999) reported the ratio of the high GI trial and low GI trial glycaemic responses as 5.8:1.0 which were much higher than the estimated values for the GI ratios of 2.8:1.0 (high GI: 80; low GI: 29). It is reasonable to suggest that the low GI test food, lentils, produced lower glycaemic responses than the calculated values. This might signify that the low GI test food was indigestible, i.e.
the CHO could not be readily absorbed. As a consequence, the low GI CHO did not provide sufficient glucose and was not taken up by muscle which might explain the non-improvement in endurance capacity.

In summary, ingestion of low GI CHO results lower insulinaemia and glycaemia during the postprandial period. As a result, it maintains better blood glucose concentrations, decreases in CHO oxidation and increase in fat oxidation during subsequent exercise. However, the effect of ingesting different GI CHO on subsequent exercise endurance capacity is still inconclusive, especially when ingestion is 2-4 hour before exercise. Furthermore, most of the current published studies focused on the effects of single foods rather than a meal. Therefore further studies are much needed.
Table 2.3 Summary of Studies examining the effects of ingesting CHO of different glycaemic indices before exercise on metabolism and performance during subsequent exercise

<table>
<thead>
<tr>
<th>Studies</th>
<th>Type of CHO</th>
<th>Amount of CHO</th>
<th>Time before exercise</th>
<th>Exercise protocol</th>
<th>Blood glucose during exercise</th>
<th>CHO oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thomas et al 1991</td>
<td>Potato (P), Glucose (G), Water (W)</td>
<td>1g/kg bw</td>
<td>1 h</td>
<td>65-70% $\text{VO}_2\max$ cycling to exhaustion</td>
<td>P, L&lt;G, W</td>
<td>W&lt;L&lt;G&lt;P</td>
<td>W&gt;L&gt;G&gt;P</td>
<td>L&gt;P</td>
</tr>
<tr>
<td>Horowitz and Coyle, 1993</td>
<td>Potatoes (P), rice (R), sucrose (S), potatoes+margarine (PM), rice+margarine (RM), confectionery bar (B), Control (C)</td>
<td>0.7kg/kg bw</td>
<td>30min</td>
<td>1 h cycling: 30min at 60% $\text{VO}_2\max$, 15min at 50% $\text{VO}_2\max$, 15min at 70% $\text{VO}_2\max$</td>
<td>Decline in plasma glucose RM,PM&lt;P, S and B at 20min</td>
<td>P,S &gt; C during 1st 30min, P,S,B,PM&gt;C at 30-45min P&gt;C at 45-60min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomas et al, 1994</td>
<td>Potato (P), Rice (R), Lentil (L), Bran (B)</td>
<td>1.0 g/kg bw</td>
<td>1 h</td>
<td>65-70% $\text{VO}_2\max$ cycling to exhaustion</td>
<td>Correlate inversely with GI after 90 min of exercise</td>
<td>P&gt;R, L, B</td>
<td></td>
<td>No difference</td>
</tr>
<tr>
<td>Febbraio and Stewart 1996</td>
<td>Lentil (LGI), Potato (HGI), Placebo (P)</td>
<td>1.0g/kg bw</td>
<td>45min</td>
<td>70% $\text{VO}_2\max$ cycling for 120min and 15min performance test</td>
<td>No difference</td>
<td>No difference</td>
<td></td>
<td>LGI, P&gt;HGI</td>
</tr>
</tbody>
</table>
Table 2.3 Summary of Studies examining the effects of ingesting CHO of different glycaemic indices before exercise on metabolism and performance during subsequent exercise

<table>
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<tr>
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<th>Blood glucose during exercise</th>
<th>CHO oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
<th>Exercise Capacity for Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirwan et al 2001</td>
<td>Rolled oat (MGI),</td>
<td>75g</td>
<td>45min</td>
<td>60% VO\textsubscript{2} max cycling to exhaustion</td>
<td>MGI&gt;HGI, C at 60 and 90min</td>
<td>MGI&gt;C</td>
<td>MGI,HGI&lt;C at 30, 60 and 120min</td>
<td>MGI&gt;C</td>
</tr>
<tr>
<td>Kirwan et al 1998</td>
<td>Puffed rice (HGI),</td>
<td>75g</td>
<td>45min</td>
<td>60% VO\textsubscript{2} max cycling to exhaustion</td>
<td>No difference</td>
<td>RO, OF&gt;C for first 60min and 90 min</td>
<td>RO&gt;C</td>
<td></td>
</tr>
<tr>
<td>Kirwan et al 2001</td>
<td>Whole-oat flour (OF),</td>
<td>75g</td>
<td>45min</td>
<td>60% VO\textsubscript{2} max cycling to exhaustion</td>
<td>No difference</td>
<td>RO&gt;C</td>
<td>RO&lt;C for first 120min</td>
<td>No difference (5% longer in RO)</td>
</tr>
<tr>
<td>DeMarco et al 1999</td>
<td>HGI</td>
<td>1.5g</td>
<td>30min</td>
<td>70% VO\textsubscript{2} max cycling for 2h + 100% VO\textsubscript{2} max to exhaustion</td>
<td>HGI,LGI&lt;C at 20min; LGI&gt;HGI at 120min</td>
<td>HGI&gt;LGI, C</td>
<td>LGI&gt;HGI, C</td>
<td>LGI&gt;HGI, C</td>
</tr>
<tr>
<td>Sparks et al 1998</td>
<td>HGI</td>
<td>1.0g/kg bw</td>
<td>45 min</td>
<td>50% VO\textsubscript{2} max cycling for 50min + 15min performance</td>
<td>HGI&lt;LGI, C till 30min</td>
<td>HGI&gt;LGI, C</td>
<td>LGI&gt;HGI</td>
<td>No difference</td>
</tr>
</tbody>
</table>
### Table 2.3 Summary of Studies examining the effects of ingesting CHO of different glycaemic indices before exercise on metabolism and performance during subsequent exercise

<table>
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<tr>
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<th>CHO oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burke et al 1998</td>
<td>HGI, LGI and control (+CHO ingestion during exercise)</td>
<td>2g/kg bw</td>
<td>2 h</td>
<td>70% ( \dot{V}O_2 \text{ max} ) cycling for 2h + time trial</td>
<td>No difference</td>
<td></td>
<td></td>
<td>No difference</td>
</tr>
<tr>
<td>Wee et al 1999</td>
<td>HGI, LGI</td>
<td>2.0g/kg bw</td>
<td>3 h</td>
<td>70% ( \dot{V}O_2 \text{ max} ) running till exhaustion</td>
<td>LGI &gt; HGI at 15min</td>
<td>HGI &gt; LGI</td>
<td>LGI &gt; HGI</td>
<td>No difference</td>
</tr>
</tbody>
</table>
2.5 Post-exercise short-term recovery carbohydrate intakes - the effects on metabolism and subsequent endurance performance

It is well known that the onset of fatigue during prolonged strenuous exercise is associated with muscle glycogen depletion. A nutritional intervention strategy to increase the rate of recovery from prolonged glycogen depleting exercise is much needed. Many athletes train or compete more than once a day. Therefore to replenish muscle glycogen stores to meet the substrate requirement of the second training session is important. Although during short-term recovery muscle glycogen is unlikely to be fully restored, it would be of benefit to maximise the rate of glycogen resynthesis in the few hours between training sessions or competitions. In this section of the review, a summary of relevant literature on the effects of post-exercise CHO ingestion during short-term recovery on metabolic changes and subsequent endurance performance will be presented.

In the post glycogen-depleting exercise recovery period, CHO intake plays an important role for glycogen resynthesis. The restoration of muscle glycogen stores following glycogen depleting exercise is probably the most important factor in determining the time to recover. It appears that muscle glycogen resynthesis has metabolic priority during post-exercise recovery. Several studies have shown that muscle glycogen can be fully restored within 24 hours (Bergstrom et al, 1967; Kochan et al 1979; Keizer et al. 1987; Casey et al. 1995). Furthermore, it has been shown that if a high CHO diet is consumed for 3 days after exhaustive exercise, muscle glycogen can increase two to three fold above normal concentrations, as a “glycogen super compensation” effect occurs (Bergstrom et al. 1967). Several studies also indicated the importance of muscle glycogen resynthesis during the first few hours of
post-exercise recovery. Therefore, the short-term recovery nutrition strategy has received much attention.

The following section considers the effects of CHO ingestion during short-term recovery on muscle glycogen resynthesis and subsequent exercise performance and metabolism. It is subdivided into four sections: (1) the amount of CHO required during short-term recovery. (2) the timing of ingestion. (3) the type of CHO. (4) CHO ingestion during recovery on subsequent exercise metabolism and performance.

2.5.1 The amount of CHO required during short-term recovery

When no CHO is consumed after glycogen depletion exercise, very little glycogen resynthesis occurs (Ivy et al. 1988b). Blom and colleagues (1987) demonstrated that ingesting CHO early during post-exercise recovery increases muscle glycogen resynthesis. They reported that when the amount of CHO is increased from 0.35 g·kg⁻¹·h⁻¹ to 0.7 g·kg⁻¹·h⁻¹, this resulted in the same muscle glycogen restoration (Blom et al. 1987). Ivy et al (1988b) confirmed that the ingestion of either 0.75 or 1.5 g·kg⁻¹·2h⁻¹ of CHO during post-exercise recovery produced no differences in muscle glycogen resynthesis (19.6 vs 22.0 mmol·kg⁻¹·dW·h⁻¹, respectively). However, later studies by van Loon and colleagues showed a higher rate of muscle glycogen resynthesis (van Loon et al. 2000) when CHO ingestion was increased from 0.8 g to 1.2 g·kg⁻¹·h⁻¹ (16.6 vs 35.4 mmol·kg⁻¹·dW·h⁻¹, respectively). The earlier studies (Blom et al. 1987; Ivy et al. 1988b) fed subjects at 2 hour intervals, while van Loon et al. (2000b) provided CHO at 30 min intervals. This feeding pattern might have a large lasting effect in providing glucose for muscle glycogen resynthesis. Although some studies reported very high rates of glycogen resynthesis (Doyle et al. 1993; Casey et al. 1995; Piehl Aulin et al. 2000; Jentjens et al. 2001), which supported van Loon et al.
it could be influenced by other factors, for example, the degree of muscle glycogen depletion, type of \( \text{CHO} \) ingested and subjects' training status.

The ACSM Joint position statement – on 'nutrition and athletic performance' (2000), recommended athletes to consume the equivalent of 1.5g\( \text{kg}^{-1} \) body weight \( \text{CHO} \) for the first 30min and every 2 hours for 4-6 hours after glycogen depletion exercise. This recommendation is equivalent to 3g\( \text{kg}^{-1} \) body mass per 4 hours. However, this suggestion seems to be somewhat high, especially if sustained over 24 hours. Fallowfield and Williams (1997) reported that when subjects’ ingested 2g\( \text{kg}^{-1} \) or 6g\( \text{kg}^{-1} \) glucose polymer solutions during a 4 hour recovery period there were no differences in endurance capacity during subsequent exercise (Fallowfield and Williams, 1997). In addition, there is another important nutritional consideration which is whether or not ingesting large amounts of \( \text{CHO} \) is tolerable during a short-term recovery. Therefore, 2g \( \text{CHO} \)\( \text{kg}^{-1} \) body weight was chosen for the studies reported in this Thesis, during recovery studies (Chapter 6 and 7) based on the experience of published studies and on the fact that it is closer to the amount of \( \text{CHO} \) consumed by athletes.

2.5.2 The timing of \( \text{CHO} \) ingestion

There are two phases in muscle glycogen resynthesis following its depletion by exercise: non-insulin dependent and insulin dependent (Price et al. 1994). The first phase is non-insulin dependent, which has been found to last for 30-60 min. The second phase is insulin dependent because it is directly related to the insulin response to \( \text{CHO} \) consumed. The non-insulin dependent phase may be due to the increase in rate of glucose transporter-4 (GLUT-4) translocation at the plasma membrane and in glycogen synthase activity (Goodyear et al. 1990). Glycogen synthase activity appears
to be inversely related to the glycogen concentration in skeletal muscle whereas the increase in GLUT 4 activity appear to result from the contractile activity per se.

Ivy et al. (1988a) fed subjects with liquid CHO solution (70% maltodextrin, 15% glucose and 15% sucrose) of 1 g kg\(^{-1}\) h\(^{-1}\) either immediately after exercise or at 2 hour post-exercise. They observed that when CHO feeding is delayed by 2 hours after exercise that the muscle glycogen resynthesis rate was significantly lower than when CHO was ingested immediately after exercise (Ivy et al. 1988a). Nevertheless, Parkin et al. (1997) reported that delaying feeding 2 hours post-exercise did not affect muscle glycogen resynthesis. However, this study examined 8 hour and 24 hour post-exercise glycogen concentration which was different from the study by Ivy et al. with 4 hour recovery. It is worth noting that in the study by Parkin et al. (1997) where feeding was immediately after exercise, subjects were fed only for the first 4 hours of recovery. There was no CHO supplement thereafter. Therefore, it has been suggested that athletes should ingest CHO immediately after prolonged strenuous exercise to increase the rate of muscle glycogen resynthesis.

Doyle et al. (1993) studied the rate of muscle glycogen resynthesis when subjects consumed 0.4g CHO kg\(^{-1}\) body weight every 15 min for 4 hour. They reported that the muscle glycogen resynthesis rate was approximately 10 mmol kg\(^{-1}\) h\(^{-1}\). This rate was higher than following feeding schedules of 0, 2 and/or 4 hour post-exercise recovery which was 5-7 mmol kg\(^{-1}\) h\(^{-1}\) (Blom et al. 1987; Ivy et al. 1988a; Ivy et al. 1988b). More frequent CHO feeding schedule might maintain more stable elevation in blood glucose and insulin concentrations after glycogen depletion exercise and so produce a greater rate of glycogen resynthesis.
2.5.3 The choice of carbohydrate

2.5.3.1 The effect of glycaemic index carbohydrate

In planning CHO repletion following post-glycogen depletion exercise, the GI of the food consumed should be considered (Hargreaves et al. 1991; Coyle 1991; Burke et al. 1993; Jentjens and Jeukendrup 2003). It has been suggested that a higher insulinaemic and glycaemic responses to ingesting CHO during post-glycogen-depleting exercise restored a greater muscle glycogen (Burke et al. 1993). Early studies have examined the effect of fructose (low GI) and glucose (high GI) on the rate of muscle glycogen synthesis during post-exercise recovery (Nilsson and Hultman, 1974; Conlee et al. 1987; Blom et al. 1987; Van Den Bergh et al. 1996). Nilson and Hultamn (1974) investigated liver glycogen synthesis rate using liver biopsy technique. They infused or fed glucose or fructose during the post-absorptive state. The rate of liver glycogen synthesis was 3.7 fold higher following fructose infusion than after of glucose infusion. However, ingestion of fructose produced a lower rate of resynthesis of muscle glycogen when compared to glucose (Conlee et al. 1987; Blom et al. 1987). Blom et al (1987) reported that the muscle glycogen resynthesis rate was only 3 mmol·kg⁻¹·h⁻¹ when fructose was ingested compared to 5-7 mmol·kg⁻¹·h⁻¹ following the ingestion of glucose or sucrose (medium GI).

A 24 hour recovery study by Burke et al (1993) reported that ingestion of high GI CHO meals resulted in a higher muscle glycogen resynthesis compared with that of low GI meals. In this study, they provided 10 g CHO·kg⁻¹ of body mass, evenly distributed between meals eaten 0, 4, 8, and 21 h post-exercise. They found an increase in muscle glycogen content after 24 h of recovery that was greater with the high GI diet (106±11.7 mmol·kg wet wt⁻¹) than with the low GI diet (71.5±6.5 mmol·kg wet wt⁻¹). The authors explained the differences in the resynthesis rates in
of the higher glycaemia and insulinemia induced by high GI CHO during the postprandial period. As a result of these studies athletes are recommended to choose high GI CHO during recovery from exercise in order to promote a higher rate of muscle glycogen resynthesis. Although a higher muscle glycogen synthesis rate was observed, performance during subsequent exercise was not assessed.

2.5.3.2 Additional protein to carbohydrate

Several studies have reported that ingestion of CHO in combination with protein or amino acids induces a higher insulin secretion and glucose response during post-exercise recovery when compared to consuming CHO alone (Zawadzki et al. 1992; Tarnopolsky et al. 1997; Carrithers et al. 2000; van Loon et al. 2000a; van Hall et al. 2000a; van Hall et al. 2000b; van Loon et al. 2000b; Ivy et al. 2002). Adding protein or amino acid to CHO has been reported to induce a higher rate of muscle glycogen resynthesis compared to ingestion of CHO or protein alone in some studies (Zawadzki et al. 1992; van Loon et al. 2000a; Ivy et al. 2002). Other studies however have not seen this effect (Tarnopolsky et al. 1997; Carrithers et al. 2000; van Hall et al. 2000b; Jentjens et al. 2001). The effect of combining protein with CHO ingestion on muscle glycogen resynthesis seems inconclusive. The inconsistent results may be due to the choice of protein or amino acid, the choice of CHO, and the amount of CHO and protein ingested.

A recent study by Williams et al. (2003) observed the restorative capacities of a high carbohydrate-protein (CHO-PRO) beverage containing electrolytes and a traditional 6% carbohydrate-electrolyte sports beverage (SB) were assessed after glycogen-depleting exercise. Post-exercise ingestion of the CHO-PRO beverage, in comparison with the SB, resulted in a 55% greater time to exhaustion during a
subsequent exercise bout at 85% VO\(_2\)\(_\text{max}\). They claimed greater recovery after the intake of the CHO-PRO beverage could be because of a greater rate of muscle glycogen storage. In this study CHO-PRO (355 ml; approximately 0.8 g CHO kg\(^{-1}\) body wt and approximately 0.2 g protein kg\(^{-1}\) body wt) or SB (355 ml; approximately 0.3 g CHO kg\(^{-1}\) body wt) was provided immediately and 2 hours after exercise. They found that ingestion of the CHO-PRO beverage resulted in a 17% greater plasma glucose response, a 92% greater insulin response, and a 128% greater storage of muscle glycogen (159±18 and 69±32 mmol g\(^{-1}\) dry weight for CHO-PRO and SB, respectively) compared with the SB. These findings indicate that the rate of recovery is coupled with the rate of muscle glycogen replenishment and suggests that recovery supplements should be consumed to optimise muscle glycogen synthesis as well as fluid replacement.

### 2.5.4 Effects of carbohydrate ingestion during short-term recovery on subsequent exercise metabolism and performance.

The relevant literature on the effect of CHO ingestion during short-term recovery on subsequent exercise performance is limited (Table 2.4). Followfield and Williams (1995) first reported ingestion of 2 g kg\(^{-1}\) CHO electrolyte solution during 4 hour recovery improved subsequent running endurance capacity when compared to the group ingesting a placebo (Fallowfield et al. 1995). They further investigated the effect of the amount of CHO ingestion during 4 hour recovery on subsequent endurance performance. Their results showed that ingestion of either 2 g kg\(^{-1}\) or 6 g kg\(^{-1}\) glucose polymer solutions resulted in similar endurance capacities in their subjects (Fallowfield and Williams, 1997). Later studies also showed greater endurance capacity were found when subjects ingested CHO solutions compared to placebo solutions (Wong et al. 2000; Casey et al. 2000; Bilzon et al. 2000). It seems clear that
CHO ingestion during recovery restores endurance capacity to greater extent than no CHO at all.

Wong and Williams (2000) compared ingestion at 50g of CHO electrolytes solutions immediately after glycogen depletion exercise and subsequent feeding of either a placebo drink or CHO solution during a 4 hour recovery on subsequent endurance capacity. They found ingesting approximately 167g CHO restored a similar endurance capacity to that following the ingestion of only 50g CHO (Wong and Williams, 2000). A recent study used a similar feeding protocol to examine muscle glycogen resynthesis rate during recovery and utilisation during subsequent exercise (Tsintzas et al. 2003). They found despite higher muscle glycogen resynthesis during the high CHO trial compared to the low CHO trial, there were no difference in muscle glycogen utilisation during the subsequent 15 min exercise (Tsintzas et al. 2003). Authors suggested that muscle glycogen availability may not be the only limiting factor in restoring endurance capacity after short-term recovery from exercise.

Bilzon et al. (2002) used stable isotope tracer to investigate substrate utilisation during post-exercise recovery and subsequent exercise endurance capacity in the heat (Blizon et al. 2002). Subjects were fed with 55 g enriched [U-13C]-glucose immediately after a glycogen depletion run. Thereafter, subjects consumed either 55 g of [U-13C]-glucose (C220) or placebo (C55) solution at 60, 120 and 180 min during a 4 hour recovery. The estimated muscle glycogen resynthesis rate was 5 fold higher in the C220 trial than C55 trial during recovery. However, subsequent endurance capacity did not differ between the two trials. The C220 trial showed a higher rate of CHO oxidation. The authors concluded that ingestion of C220 did not spare endogenous CHO compared to C55 which may have lead to the lack of difference in endurance capacity between
trials in the heat. It is worth noting that in this study, exercise intensity was set at 60% $\text{VO}_2\text{max}$ which might not be high enough to challenge muscle glycogen stores.

Nevertheless, these studies mainly used glucose or glucose polymer, which are classified as high GI CHO sources. Casey et al. (2000) fed subjects glucose (high GI CHO) or sucrose (medium GI CHO) solution during 4 hour of recovery. They reported that there were no differences in muscle glycogen resynthesis and in subsequent exercise capacity between the two CHO treatments. Interestingly, they found a positive correlation between replenishment of liver glycogen and subsequent endurance capacity (Casey et al. 2000). Together these studies demonstrated that restoring muscle glycogen during short-term recovery from exercise is not the only key factor affecting endurance capacity.

As with pre-exercise CHO feeding, ingestion of CHO during the recovery period induces insulin secretion, therefore promoting CHO oxidation and depressing fat oxidation both during the recovery period and during subsequent exercise. Insulin secretion is known to accelerate muscle glycogen resynthesis, whilst conversely increasing CHO oxidation i.e. using either endogenous or exogenous CHO as a source of fuel (Blizon et al. 2002). Hence, ingesting CHO without inducing significant increases in CHO oxidation and decreases in fat oxidation may be beneficial for subsequent exercise following short-term recovery. An examination of the effect of different glycaemic and insulinaemic response to different CHO foods on short-term recovery has not been thoroughly investigated. Studies examining short-term recovery used mainly high GI CHO solutions or high GI CHO and additional protein solution as a recovery energy supplement, which is not practical in the real world. The current recommendation is to consume high GI CHO during recovery. However, the effect of
different GIs CHO during short-term recovery and subsequent exercise performance has not been examined.
Table 2.4 Summary of Studies examining the effects of post-exercise CHO ingestion on muscle glycogen resynthesis and subsequent exercise metabolism and performance

<table>
<thead>
<tr>
<th>Studies</th>
<th>Type of CHO</th>
<th>Amount of CHO</th>
<th>Recovery period</th>
<th>Feeding protocol</th>
<th>Exercise protocol</th>
<th>Muscle glycogen resynthesis</th>
<th>CHO oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivy et al 1988</td>
<td>25% CHO solution</td>
<td>2g/kg bw</td>
<td>4 h</td>
<td>P-EX: immediate after exercise 2P-EX: 2h post exercise</td>
<td>Glycogen depletion: 70 min at 68% VO₂ max + 6*2 min at 88% VO₂ max cycling</td>
<td>P-EX&gt;2P-EX</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ivy et al 1988</td>
<td>50% glucose polymer</td>
<td>0 g/kg bw (P)</td>
<td>4 h</td>
<td>Immediate after exercise and 2h post-exercise</td>
<td>4 times 15 min at 62% VO₂ max + 15 min at 75% VO₂ max</td>
<td>L, H&gt;P (2h, 4h)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Zawadzki et al 1992</td>
<td>CHO</td>
<td>112g/2h</td>
<td>4 h</td>
<td>Immediate after exercise and 2h post-exercise</td>
<td>Glycogen depletion: 2h at 75% VO₂ max</td>
<td>CHO+protein &gt; CHO&gt;protein</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Parkin et al 1997</td>
<td>HGI CHO</td>
<td>8, 24 h</td>
<td>0-4 h (IT) or 2-6 h (MI at 2-h intervals</td>
<td>Glycogen depletion: 2h at 70% VO₂ max + 4*30 s sprints, cycling</td>
<td>No difference</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2.4 Summary of Studies examining the effects of post-exercise CHO ingestion on muscle glycogen resynthesis and subsequent exercise metabolism and performance

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<tr>
<th>Studies</th>
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<th>Plasma FFA during exercise</th>
<th>Exercise capacity/or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamopolsky et al 1997</td>
<td>CHO+Pro+Fat (CPF)</td>
<td>0.75 kg+0.1g/kg+0.02g/kg</td>
<td>4 h</td>
<td>immediately and 1h post-exercise</td>
<td>Glycogen depletion: 90min at 65% VO$_2$ max</td>
<td>CPF, C&gt;P</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CHO (C)</td>
<td>1g/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo (P)</td>
<td>0g/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casey et al 2000</td>
<td>Glucose (G)</td>
<td>1g/kg bw</td>
<td>4 h</td>
<td>immediately after exercise</td>
<td>Glycogen depletion: ~90min at 70% VO$_2$ max</td>
<td>No difference in Muscle glycogen</td>
<td>G, S &gt; C</td>
<td>G, S &gt; P</td>
</tr>
<tr>
<td></td>
<td>Sucrose (S)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Performance correlated to liver glycogen</td>
</tr>
<tr>
<td></td>
<td>Placebo (C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reed et al 1989</td>
<td>Liquid (glucose polymer) (L)</td>
<td>3g/kg bw</td>
<td>4 h</td>
<td>immediately and 2h post-exercise</td>
<td>Glycogen depletion: 15min intervals at 60-65% VO$_2$ max and 70-75% VO$_2$ max cycling</td>
<td>No differences</td>
<td>No differences</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Solid (banana cake) (S)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Intravenous (I)</td>
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<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Loon et al 2000</td>
<td>CHO+PRO (CP)</td>
<td>0.8g/kg/h CHO + 0.4g/kg PRO + 0.8g/kg/h CHO + 1.2g/kg/h CHO</td>
<td>5h</td>
<td>Every 30min interval</td>
<td>2min interval at 90% VO_{2max} and 50% VO_{2max} till exhaustion</td>
<td>CP, CC&gt;C</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHO (C)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>CHO+CHO (CC)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ivy et al 2002</td>
<td>CHO+PRO (CP)</td>
<td>80g CHO+28g PRO+6g fat</td>
<td>4h</td>
<td>Immediately after exercise and 2h post-exercise</td>
<td>2h cycling at 65-75% VO_{2max} + series 1min maximal sprint till plasma glucose&lt;3.89mmol/l</td>
<td>CP &gt;C, CC</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHO (C)</td>
<td></td>
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<tr>
<td></td>
<td>CHO+CHO (CC)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Hall et al 2000</td>
<td>CHO+PRO (CP)</td>
<td>1.67g/kg/l sucrose + 0.5g/kg/l PRO</td>
<td>4h</td>
<td>600ml bolus + 150ml every 15min</td>
<td>2min intervals at 90%W_{max} +50%W_{max} till exhaustion</td>
<td>CP, C&gt;W</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHO (C)</td>
<td></td>
<td></td>
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<tr>
<td>Water (W)</td>
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<th>Plasma FFA during exercise</th>
<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallowfield and Williams 1993</td>
<td>Control (C)</td>
<td>5.8g/kg bw</td>
<td>22.5 h</td>
<td>+16.5% glucose polymer drink in CHO group</td>
<td>70% VO\textsubscript{2} max running till exhaustion</td>
<td>-</td>
<td>No difference</td>
<td>-</td>
<td>CHO&gt;C</td>
</tr>
<tr>
<td></td>
<td>CHO (CHO)</td>
<td>8.8g/kg bw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHO restored endurance capacity</td>
</tr>
<tr>
<td>Fallowfield and Williams 1997</td>
<td>6.9% Glucose polymer (C)</td>
<td>1.0g/kg bw/2h</td>
<td>4 h</td>
<td>Feeding every 2 h</td>
<td>70% VO\textsubscript{2} max running till exhaustion</td>
<td>-</td>
<td>No difference</td>
<td>-</td>
<td>No difference</td>
</tr>
<tr>
<td></td>
<td>19.3% Glucose polymer (D)</td>
<td>3.0g/kg bw/2h</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fallowfield et al 1995</td>
<td>6.5% CHO electrolyte drink (CHO)</td>
<td>2.0g/kg bw</td>
<td>4 h</td>
<td>1.0g/kg bw immediately after R1 and 2h later</td>
<td>70% VO\textsubscript{2} max running till exhaustion</td>
<td>-</td>
<td></td>
<td></td>
<td>CHO&gt;P</td>
</tr>
<tr>
<td></td>
<td>Placebo (P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wong and Williams 2000</td>
<td>6.5% CHO electrolyte drink (CHO)</td>
<td>50g + ~167g</td>
<td>4 h</td>
<td>50g immediately after R1 in both trials. Ingestion either CHO or P every 30min</td>
<td>70% VO\textsubscript{2} max running till exhaustion</td>
<td>-</td>
<td>No difference</td>
<td></td>
<td>No difference</td>
</tr>
<tr>
<td></td>
<td>Placebo (P)</td>
<td></td>
<td></td>
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<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wong et al 2000</td>
<td>6.9% CHO electrolyte drink (CHO) Placebo (P)</td>
<td>200% of the fluid lost after T1</td>
<td>4 h</td>
<td>Every 30 min</td>
<td>70% VO₂ max running till exhaustion</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CHO&gt;P</td>
</tr>
<tr>
<td>Wong et al 1998</td>
<td>6.9% CHO electrolyte drink (CHO)</td>
<td>A: 1405ml 4h I. Ad libitum (A) 2. prescribed: from the body mass loss during T1(P)</td>
<td>4 h</td>
<td>1. Ad libitum (A) 2. prescribed: from the body mass loss during T1(P)</td>
<td>70% VO₂ max running till exhaustion</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>P&gt;A 16% longer</td>
</tr>
<tr>
<td>Burke et al 1993</td>
<td>HGI (C) C + fat + protein (FP) C+CHO (ME)</td>
<td>10g/kgbw</td>
<td>24 h</td>
<td>0, 4, 8 and 21 h post-exercise</td>
<td>Glycogen depletion: 2h at 75% VO₂ max + 4*30s sprints, cycling</td>
<td>HGI&gt;LGI</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Burke et al 1995</td>
<td>HGI (C) C + fat + protein (FP) C+CHO (ME)</td>
<td>C: 7g/kg/day C: 4.8g/kg</td>
<td>24 h</td>
<td>0, 4, 8 and 21 h post-exercise</td>
<td>Glycogen depletion: 2h at 75% VO₂ max + 4*30s sprints, cycling</td>
<td>-</td>
<td>-</td>
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<th>CHO oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
<th>Exercise Capacity or Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsintzas et al</td>
<td>Low CHO (L)</td>
<td>50g</td>
<td>4h</td>
<td>50g CHO immediately after exercise + every 30min till 180min 25g CHO for High CHO, placebo for Low CHO</td>
<td>70% VO$_2$ max for 90min treadmill running 15min run after 4 h recovery</td>
<td>H&gt;L</td>
<td>H=L</td>
<td>L&gt;H</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>High CHO (H)</td>
<td>175g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilzon et al</td>
<td>Low CHO (L)</td>
<td>55g</td>
<td>4h</td>
<td>55g CHO immediately after exercise + every 60min till 180min 55g CHO for High CHO, placebo for Low CHO</td>
<td>60% VO$_2$ max for 90min or till exhaustion treadmill running after 4 h recovery</td>
<td>H&gt;L</td>
<td>H&gt;L</td>
<td>L&gt;H</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>High CHO (H)</td>
<td>220g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilzon et al</td>
<td>CHO (C)</td>
<td>138±12g</td>
<td>4h</td>
<td>0, 1, 2, 3 hour during 4 hour recovery</td>
<td>60% VO$_2$ max for 90min or till exhaustion running after 4 h recovery</td>
<td>C&gt;P</td>
<td>-</td>
<td>-</td>
<td>No difference (exercise at 35°C)</td>
</tr>
<tr>
<td>2000</td>
<td>Placebo (P)</td>
<td>0g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jentjens et al</td>
<td>CHO (C)</td>
<td>1.2g/kg</td>
<td>3h</td>
<td>0, 1, 3 hour</td>
<td>2min intervals at 90%W$<em>{max}$=50%W$</em>{max}$ at till exhaustion</td>
<td>CP&gt;C</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>CHO+protein(CP)</td>
<td>1.2g/kg + 0.4g/kg</td>
<td></td>
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CHAPTER 3
GENERAL METHODS

3.1 Introduction
This chapter describes the equipment, procedures and tests employed in the four studies reported this thesis. Loughborough University Ethical Advisory Committee approved all protocols and procedures used in these studies (a sample of application form see Appendix A). All subjects were informed of the nature, purposes and the possible risks in all studies before they provided written consent. Also, subjects were required to complete a health screen questionnaire (Appendix B) and a physical activity questionnaire (Appendix C) providing information about their medical history and their current training status. They then signed an informed consent in the presence of a witness. All the experimental trials were in conformity with the “Code of Practice for Persons Having Contact with Human Body Fluids” regulation, and conducted in the Exercise Physiology Research Laboratory in School of Sport and Exercise Sciences at Loughborough University.

3.2 Experiment design
All the experimental trials were randomised and counterbalanced in the four studies. In study 1, subjects completed three experimental trials, each separated by one week. Subjects consumed the test meals (Table 3.1) 3 hour before a 60 min run at 65% \( \dot{V}O_2 \text{max} \) on a motorized treadmill (Fig 3.1).

In study 2, subjects completed two experimental trials separated by at least 7 days. Subjects consumed the two test meals 3 hours before running to exhaustion at 70% \( \dot{V}O_2 \text{max} \) on a motorized treadmill (Fig 3.2).
In study 3, subjects were asked to undertake two experimental trials, which were separated by at least one week. Each trial consisted of two runs: (i) Run 1 (R₁): subjects ran on a treadmill at 70% \( \dot{V}O_2_{\text{max}} \) for 90 min or until fatigue which included 5 min warm up at 60% \( \dot{V}O_2_{\text{max}} \). (ii) Run 2 (R₂): after 4 hours recovery (REC) subjects ran on treadmill at 70% \( \dot{V}O_2_{\text{max}} \) for 30 min after 5 min warm up at 60% \( \dot{V}O_2_{\text{max}} \). Subjects consumed either HGI meal or LGI meal, as described in Table 3.1, during the recovery period (Fig 3.3).

In study 4, each subject undertook two experimental trials in a counterbalanced design separated by at least one week. Each trial included two runs: (i) Run 1 (R₁): subjects ran on a treadmill at a speed corresponding to 70% \( \dot{V}O_2_{\text{max}} \) for 90 min or until fatigue after a standardized 5 min warm up at 60% \( \dot{V}O_2_{\text{max}} \). (ii) Run 2 (R₂): after a 4 hour recovery (REC), subjects ran on a treadmill at 70% \( \dot{V}O_2_{\text{max}} \) until volitional fatigue. Subjects consumed either HGI meal or LGI meal, as described in Table 3.1, during the recovery period (Fig 3.4).

3.3 Preliminary test
3.3.1 Body mass and height
Body mass of the subjects was measured using a calibrated balance (Avery 3306 ABV) and height was measured using a stadiometer.

3.3.2 Treadmill
A motorised treadmill (Technogym®, Italy) was used in all exercise tests. The treadmill was calibrated at each time prior to each study by measuring the treadmill
belt length and the time to complete 50 revolutions at various speeds (5, 10, 15, 20 and 25 km·h⁻¹).

3.3.3 Determination of maximal oxygen uptake
An uphill treadmill running test was used to determine subjects' maximal oxygen uptake ($\dot{V}O_{2max}$). The treadmill speed was constant whereas the initial incline was set at 3.5%. Thereafter, the gradient was increased every 3 min by 2.5%. One min expired gas samples were collected from 1:45 to 2:45 min of each stage of the test and when subjects signalled their final minute. Subjects were supported by strong verbal encouragement throughout the test (Taylor et al. 1995; Williams et al. 1990).

3.3.4 Determination of running economy
A 16-min level treadmill running test was used to determine running economy of each subject. The initial running speed was set between 8 to 9 km·h⁻¹, dependent on subjects' running ability. The speed was then increased by 1-1.6 km·h⁻¹ every 4 min. Expired gas samples were collected at the final min of each stage of the test. A linear regression equation was used to calculate the relationship between running speed and oxygen uptake ($\dot{V}O_2$).

3.3.5 Familiarisation run
In all four studies, subjects were required to become familiar with the main trial procedures and running speed before the main trial. In study 1, subjects completed a 30min familiarisation run at 65% $\dot{V}O_{2max}$. In study 2, subjects completed a 45min familiarisation run at 70% $\dot{V}O_{2max}$. In study 3 and 4, subjects completed a 60min familiarisation run at 70% $\dot{V}O_{2max}$. Expired gas samples were collected every 15 min during the run in order to adjust the running speed to match the target running intensity in each study. This test also ensured that all subjects were fully familiar with...
the whole procedures and measurements used during the main experimental trials.

### 3.4 Main experimental trials

Subjects recorded their dietary intake using a food weighing method for 2 days before the first main trial and repeated the same diet before subsequent trials. In addition to the dietary record, subjects were asked to consume the last meal at the same time before all main trials. This dietary control has resulted in similar muscle glycogen concentration and avoided different timing of previous meal effects on glycaemic response (Tsintzas et al. 1995). All subjects were instructed to refrain from heavy physical activities two days before each main trial. All subjects were also asked to abstain from alcohol and caffeine consumption for 24 hours before all main trials.

On the day of main experimental trials, subjects reported to the laboratory between 0800-0900am after a 12-hour overnight fast. Nude body weights were obtained after subjects voided urine. Resting venous blood samples were obtained from an ante-cubital vein via an indwelling cannula (Venflon 18G, Sweden) in studies 1, 2 and 3, and via a venous puncture in study 4. Fasting expired air samples were collected for 5 min while subjects were sitting on a comfortable chair. The collection time for blood samples and expired air samples are summarised in Fig 3.1 for study 1, Fig 3.2 for study 2, in Fig 3.3 for studies 3 and Fig 3.4 for study 4.

In studies 3 and 4, subjects consumed 8ml\,kg\(^{-1}\) body mass of water before they started the first run (R\(_1\)). During the recovery period (studies 3 and 4), subjects ingested a volume of water equal to 150% of their body mass loss from R\(_1\) to ensure they were fully rehydrated (Wong et al. 1998). Each subject received 2g\,kg\(^{-1}\) body mass of water every 30 min during the performance tests (studies 2 and 4) to avoid severe
dehydration.

In studies 1 and 2, the test meal (described in the following section) was served after basal blood samples and expired air samples were obtained. Subjects were asked to finish the test meals within 20 min. Once subjects finished eating the test meal, the 3 hour postprandial observation period began.

In studies 3 and 4, the test meal was served 30 min after the first 90 min run during the recovery period. Subjects were allowed 20 min to consume the meal in study 3. In study 4, subjects were asked to finish the test meals during the first 60 min of 4 hour recovery i.e. the postprandial period was 3 hours, which was similar to study 1 and 2.

In studies 2 and 4, exhaustion was defined as the point when the subjects were no longer able to maintain the assigned running speed. Near the end of the run, subjects were allowed to slow down the treadmill twice for 2 min to a walking speed 5 km h⁻¹. This was to ensure that the subjects truly reached metabolic fatigue. Running times were not revealed to subjects until to the end of their last trial to avoid them using this information in subsequent trials e.g. goal setting.
Fig 3.1 Experimental design for study 1 (chapters 4)

- : 3 hours Postprandial period
- : Run at 65% $V_{\text{O}_2\text{max}}$

EA: Expired Air Collection
VS: Venous Sample
CA: Cannulation
W: Nude body weight
Meal: Test meal
F: Replacement fluid (Study 2 only)
Fig 3.2 Experimental design for study 2 (chapters 5)
Fig 3.3 Experimental design for study 3 (chapters 6)

- Warm up at 60% VO$_{2\text{max}}$ for 5 min
- Run at 70% VO$_{2\text{max}}$
- 4 hour recovery

EA: Expired Air Collection
VS: Venous Sample
Meal: Test meal
W: Nude body weight
F: Fluid replacement (water)
CA: Cannulation
Fig 3.4 Experimental design for study 4 (chapters 7)
3.5 Test meals
The test meals in all four studies comprise commercial products and natural foods which were all purchased at the same supermarket. The macronutrient composition of the commercial products were provided by the companies. Glycaemic index values were obtained from Foster-Powell and Miller (1995). Mixed meal GI values were calculated using the method proposed by Wolever (1986), which was described in the previous chapter (see chapter 2.3). The high glycaemic index meal (HGI) consisted of skimmed milk, Corn Flakes (Kellog's), white bread, jam, Lucozade original (GlaxoSmithKline, Brentford, UK), and water, with a calculated GI value of 77. The low glycaemic index meal (LGI) consisted of skimmed milk, All Bran (Kellog's), peaches, apples, and apple juice, with a calculated GI value of 37. Both meals contained the same macronutrients and water. Subjects consumed the equivalent of 2g kg\(^{-1}\) body weight of carbohydrate in both meals. For example, for a 70kg man would consume 141g carbohydrate, 20g protein, and 3g fat in both test meals (Table 3.1). In studies 2, 3 and 4 (Chapter 5, 6 and 7), two test meals were used. In study 1 (chapter 4), an additional control trial was included (fast) i.e. subjects consumed water only and the water content was the same as in the HGI and in the LGI meals (Table 3.1).
### Table 3.1 Characteristics of test meals (for 70kg subject)

<table>
<thead>
<tr>
<th>Meal</th>
<th>Description</th>
<th>Macronutrient content</th>
</tr>
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<tbody>
<tr>
<td><strong>HGI</strong></td>
<td>60g Corn Flakes + 280ml skimmed milk + 77 g white bread + 20g raspberry jam +167ml Lucozade original drink  +400ml water</td>
<td>2.79MJ, 141g CHO, 20g protein, 3g fat, 820ml water estimated GI=77*</td>
</tr>
<tr>
<td><strong>LGI</strong></td>
<td>80g All Bran + 200ml skimmed milk + 280g canned peach in own juice + 160g apples + 400ml unsweetened apple juice</td>
<td>2.79MJ, 141g CHO, 20g protein, 3g fat, 820ml water estimated GI=37*</td>
</tr>
<tr>
<td><strong>FAST</strong></td>
<td>820ml water</td>
<td>0 MJ estimated GI=0</td>
</tr>
</tbody>
</table>

* calculated by method described in Wolever et al. (1986) with GI values taken from Foster-Powell and Miller (1995).
3.6 Measurement of expired air samples

In all studies, expired air samples were collected by using the Douglas bag method. Expired gas samples were collected for one min during exercise and 5 min during rest. Each expired gas sample was collected via a one-way low resistant valve and a lightweight, wide bore tubing (Falconia Ltd.) into a Douglas bag. Samples from the Douglas bag were pumped through a gas analyser (Servomax 1440, England) for 3 min for determination of oxygen and carbon dioxide content. The gas analyser was calibrated before use, using two gases known of concentration gases, one was nitrogen against zero, and the other 16% of oxygen and 4% of carbon dioxide. The remaining volume in the Douglas bag was measured using a dry gas meter (Harvard Apparatus). Dry gas meter was calibrated using a Hans Rudolph 3 litre syringe (Series 5330). The coefficient of variation \([(\text{Standard Deviation}/\text{Mean}) \times 100]\) of the gas meter is shown in Table 3.2.

<table>
<thead>
<tr>
<th>Assigned volume (L)</th>
<th>36</th>
<th>72</th>
<th>108</th>
<th>144</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test volume (L)</td>
<td>37.47±0.15</td>
<td>73.52±0.14</td>
<td>110.08±0.13</td>
<td>146.0±0.25</td>
</tr>
<tr>
<td>C.V.</td>
<td>0.4</td>
<td>0.19</td>
<td>0.12</td>
<td>0.17</td>
</tr>
<tr>
<td>Errors %</td>
<td>4.08</td>
<td>2.11</td>
<td>1.92</td>
<td>1.39</td>
</tr>
</tbody>
</table>

A thermometer (Edale Instruments, model C) was used to measure temperature of the expired air samples. Barometric pressure was obtained from a barometer (Griffen and George Ltd.). Oxygen uptake (\(\dot{V}O_2\)) and carbon dioxide production (\(\dot{V}CO_2\)), ventilation rate (\(\dot{V}E\)) and RER were calculated using the method previously described (Williams et al. 1990). Substrate utilisation, CHO oxidation rate (g·min\(^{-1}\)) and fat oxidation rate (g·min\(^{-1}\)), were calculated using non-protein stoichiometric equations (Frayn, 1983):

\[
\text{CHO oxidation rate (g·min}^{-1}\text{)} = 4.585 \times \dot{V}CO_2 - 3.226 \times \dot{V}O_2
\]
Chapter 3

Fat oxidation rate (g min\(^{-1}\)) = 1.695 \* \(\dot{V}O_2\) - 1.701 \* \(\dot{V}CO_2\)

\(\dot{V}O_2\) and \(\dot{V}CO_2\) were measured in l min\(^{-1}\). The total CHO and fat oxidation were calculated from the area under the CHO and fat oxidation rate versus time curve during resting and during exercise.

3.7 Treatment and analysis of blood samples

In study 4, the fasting blood samples were obtained by veni-puncture. The rest of blood samples in all the other studies were obtained from an indwelled cannula (Venflon 18G, Sweden) in the antecubital vein. The cannula was connected to a 3-way stopcock (Connecta Ltd., Sweden) with a 10cm extension tube. The cannula was frequently flushed with a sterile sodium chloride solution (0.9% w/v) to keep it patent throughout the experiment. Blood samples were obtained from subjects in the sitting position during the postprandial period (studies 1 and 2) and the recovery period (studies 3 and 4).

Ten millilitres of blood were collected at each sampling time. Each sample was dispensed into an EDTA tube and a serum tube (Sarstedt, Germany). Haemoglobin concentration was determined by the cyanmethaemoglobin method (Boehringer Mannheim, FRG) using a spectrophotometer (UV mini 1240, Shimazu, Japan). Haematocrit values were determined in triplicate on samples of whole blood by microcentrifugation (Micro-Haematocrit Centrifuge, Hawksley, England). Changes in plasma volume were estimated from changes in haemoglobin concentration and haematocrit values (Dill and Costill, 1974). A duplicated 20 \(\mu\)l blood sample was deproteinised in 200 \(\mu\)l of 2.5% perchloric acid and then centrifuged for 3 min at 13000rpm (Eppendorf 5415C, Germany) before being frozen at -20\(^\circ\)C for later analysis of lactate (Maughan, 1982) using a fluorometer (Locarte, Model 8-9, UK or
RF-1501 Spetrofluorometer, Shimazu, Japan) (Appendix D). A 7ml blood sample was put into an EDTA tube and centrifuged (Burkard Ltd.) at 3000rpm for 10 min to obtain plasma for analysis of free fatty acid (FFA) (ACS-ACOD method, Wako NEFA C, Germany), glucose (GOD-PAP method, Randox, Ireland) and glycerol (GPO-PAP method, Randox, Ireland) using an automatic photometric analyser (Cobas-Mira Plus, Roche, Switzerland). In study 3, plasma glycerol concentrations were analysed using a fluorometric method (Laurrell and Tibbling, 1966)(Appendix E). The remaining blood (~3ml) was placed into a non-heparinized tube and left to clot for 1 hour. Serum samples were then obtained after centrifugation (Burkard Ltd.) at 3000rpm for 10 min. The aliquoted serum was stored at -70°C and later analysed for insulin by radioimmunoassay (Coat-Count Insulin, ICN Ltd.) using a gamma counter (Packard, Cobra 5000).

The coefficient of variation \([\text{Standard Deviation/Mean}*100]\) of the blood, plasma and serum assays are shown in Table 3.3.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
<th>C.V.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lactate</td>
<td>3.0 mmol/l</td>
<td>1.1</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>5.67 mmol/l</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>8.39 mmol/l</td>
<td>0.6</td>
</tr>
<tr>
<td>Plasma FFA</td>
<td>0.6 mmol/l</td>
<td>0.8</td>
</tr>
<tr>
<td>Plasma glycerol</td>
<td>0.1 mmol/l</td>
<td>1.0</td>
</tr>
<tr>
<td>Plasma triglycerides</td>
<td>1.0 mmol/l</td>
<td>2.6</td>
</tr>
<tr>
<td>Serum insulin</td>
<td>105.8 mIU/l</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>31.8 mIU/l</td>
<td>5.6</td>
</tr>
</tbody>
</table>
3.8 Other measurements

Heart rate monitoring

In all preliminary tests, familiarisation run and main experimental trials, heart rate was monitored throughout exercise by a short-range telemetry (Polar Electro Sports Tester, PE3000).

Rating of perceived exertion (RPE)

Rating of perceived exertion was measured using the Borg’s scale (Borg, 1973) during exercise in all preliminary tests, familiarisation run and main trials.

Thirst feeling and Gut fullness feeling

Thirst feeling and gut fullness feeling were measured using a 6-20 scale (Appendix F and G) at each time of expired air sampling time.

Environmental condition

The dry bulb and wet bulb temperature were recorded every 30 min during resting and recovery stage, and every 15 min during exercise tests. All the trials in the same study were controlled in the same laboratory, using the same equipment to avoid environmental effects.

Objective observation

In all studies, we observed changes in the subjects’ physical conditions e.g. facial expression and asked their feelings during the running test.
CHAPTER 4
THE INFLUENCE OF HIGH CARBOHYDRATE MEALS WITH DIFFERENT GLYCAEMIC INDICES ON SUBSTRATE UTILISATION DURING SUBSEQUENT EXERCISE

4.1 Introduction

Ingestion of a high carbohydrate (CHO) food before exercise can increase liver and muscle glycogen concentrations (Nilsson and Hultman, 1973). It also affects the metabolic response and substrate utilisation during subsequent exercise (Horowitz et al. 1997; Coyle et al. 1997; Wee et al. 1999). Several studies have observed depressed rates of fat oxidation following ingestion of CHO (Horowitz et al. 1997; Coyle et al. 1997; Wee et al. 1999). This is mainly due to hyperinsulinaemia during the postprandial period (Horowitz et al. 1997; Coyle et al. 1997). Therefore, ingestion of high CHO foods before exercise without inducing high insulin secretion may cause a shift in substrate utilisation toward fat oxidation during the subsequent submaximal exercise.

A number of studies have examined the influence of pre-exercise CHO ingestion on fat metabolism by using monosaccharides, disaccharides, oligosaccharides, whole food rich in CHO or the addition of the other macronutrients to a CHO source (Hargreaves et al. 1987; Ivy et al. 1988; Horowitz et al. 1997). Few studies have considered the effects of different glycaemic indices (GI) (Jenkins et al. 1981) foods ingested before exercise (Thomas et al. 1991; Thomas et al. 1994; Febbraio & Stewart, 1996; Sparks et al. 1998; DeMarco et al. 1999; Wee et al. 1999; Febbraio et al. 2000b; Kirwan et al. 2001a). Thomas et al. (1991) first reported that the ingestion
of a single LGI food 45 min before exercise improved endurance capacity on a cycle ergometer compared to that of a single HGI food. Nevertheless, subsequent studies have not shown differences in performance between the two type of foods (Thomas et al. 1994; Febbraio & Stewart, 1996; Sparks et al. 1998; Wee et al. 1999; Febbraio et al. 2000b). The inconsistency in the results may be due to the difference in the timing of food ingestion, the quantity of CHO ingested, and the type of exercise employed. However, these studies demonstrated that the ingestion of low GI foods before submaximal exercise reduces the postprandial glycaemia and insulinaemia. This is accompanied by higher concentrations of plasma free fatty acid (FFA) during exercise compared with the responses to a HGI food (Thomas et al. 1994; Febbraio & Stewart, 1996; Sparks et al. 1998; Wee et al. 1999; Febbraio et al. 2000a; Febbraio et al. 2000b). These responses favour higher rates of fat oxidation than is the case following the consumption of a HGI meal (Thomas et al. 1994; Febbraio & Stewart, 1996; Sparks et al. 1998; DeMarco et al. 1999; Wee et al. 1999; Febbraio et al. 2000a; Febbraio et al. 2000b). The metabolic changes following ingestion of a single GI food seemed clear, however, it is not custom to consume single GI foods in daily life. A method for calculating the GI of mixed meals has been proposed by Wolever and Jenkins (1986). Research into the metabolic responses to a mixed meal containing foods with different GI values is much needed. Therefore, the aim of the present study was to compare two meals with different glycaemic indices on substrate utilization during subsequent exercise.

4.2 Methods

Nine male recreational runners (age: 26.8±1.1 yrs, body mass: 74.7±2.4 kg, $\dot{V}O_2\text{max}$: 58.1±1.7 ml·kg$^{-1}$·min$^{-1}$) participated in this study. The protocol was approved by the Loughborough University Ethical Advisory Committee and all subjects gave their
written informed consent. Preliminary tests and main experimental trials were as described in Chapter 3. Briefly, each subject undertook three experimental trials. Subjects were provided with one of three test meals. They were (i) a high glycaemic index meal (HGI), (ii) a low glycaemic index meal (LGI) and (iii) water (FAST) as described in Table 3.1. Subjects consumed the test meals 3h before a 60 min run at 65% \( \text{VO}_2\text{max} \) on a motorized treadmill. A counterbalance design was applied to this study, and the order of the trials was randomised, and each trial separated by one week. All trials were performed under similar experimental and environmental conditions. Ambient temperature and relative humidity were recorded at 30 min intervals during the postprandial period and at 15 min intervals during exercise, using a hydrometer (Zeal, England). Temperature was maintained between 20-25°C and humidity was between 50-60% in all trials. While running on the treadmill, subjects were cooled by electric fans and wet sponges were also available for use ad libitum. All subjects were instructed to refrain from heavy physical activities for two days before each main trial. Subjects were asked to record a two-day diet by a food weighing method before the first main trial and to consume exactly the same diet before the second main trial. All subjects were asked to abstain from alcohol, caffeine and tobacco consumption for 24 hours before each main trial.

All data are presented as Mean ± SEM. A two-way (time and treatment) ANOVA with repeated measure was used to analyse overall differences in the physiological and biochemical responses to the three main trials. When a significant difference was found, the Tukey post hoc test was used to locate the differences in means. For non-time dependent variables, Student's t-test for paired observations was used. Significance was set at the 0.05 level of confidence.
4.3 Results

Plasma Glucose and Serum Insulin

Following ingestion of the HGI and LGI meals, plasma glucose concentration increased sharply and peaked at 15 min during the postprandial period in both conditions (6.8±0.5 mmol·l⁻¹ and 6.2±0.3 mmol·l⁻¹, respectively; Fig 4.1). Thereafter, plasma glucose decreased below the FAST concentration after 60 min in the LGI (FAST: 4.5±0.2 mmol·l⁻¹ and LGI: 4.13±0.22 mmol·l⁻¹; F₂,₁₆=6.65, p<0.05) and after 180 min in the HGI trial (FAST: 4.5±0.2 mmol·l⁻¹ and LGI: 3.9±0.2 mmol·l⁻¹). Fifteen minutes after the start of exercise, plasma glucose further declined to 3.5±0.1 mmol·l⁻¹ and 4.1±0.1 mmol·l⁻¹ in the HGI and LGI trials, respectively.

HGI and LGI serum insulin concentration peaked at 15 min after ingestion of the meals (139.4±18.4 mIU·l⁻¹ and 131.0±22.8 mIU·l⁻¹, respectively, Fig 4.2). The concentrations were significantly higher than in the FAST trial in the first two hours during the postprandial period in both the HGI and LGI trials (F₂,₁₆=24.14, p<0.05). Thereafter, serum insulin concentration decreased, such that it was similar to the fasting concentration.

The incremental area under the curve (IAUC), over the postprandial period, for both glucose (108.7 mmol·l⁻¹·min⁻¹ v.s. 48.9 mmol·l⁻¹·min⁻¹) and insulin (12146 IU·l⁻¹·min⁻¹ v.s. 8654 IU·l⁻¹·min⁻¹) was higher during the HGI than LGI trial. The ratio of plasma glucose IAUC between HGI and LGI was 2.2:1, which was similar to the estimated GI ratio of 2.1:1.

Plasma Free Fatty Acid and Glycerol

Plasma FFA concentrations were suppressed following the HGI and LGI meals
compared to the FAST trial (Fig 4.3). The FAST trial FFA concentrations were higher from 15 min into the postprandial period to the end of the exercise ($F_{2,16}=25.24$, $p<0.05$). At the onset of exercise, plasma FFA increased gradually throughout the exercise period in both the HGI and LGI trials. Although plasma FFA concentrations were higher in the LGI trial than the HGI trial during exercise, they remained lower than the values in the FAST trial ($p<0.05$).

Plasma glycerol showed a similar response to that of plasma FFA (Fig 4.4). The FAST trial plasma glycerol concentrations were higher from 60 min into the postprandial period to the end of the exercise ($F_{2,16}=22.58$, $p<0.05$). At the onset of exercise, plasma glycerol concentrations increased gradually throughout the exercise period in both the HGI and LGI trials. There were no significant differences between two test meal trials during the postprandial period, although at 60 min of exercise, glycerol concentration was higher during the LGI trial compared with the HGI trial ($p<0.05$).

**Blood Lactate**

Following the ingestion of LGI and HGI meals, blood lactate concentrations were significantly elevated ($F_{2,16}=31.55$, $p<0.05$) and peaked at 30 min during the postprandial period (Fig 4.5). Blood lactate concentration was significantly higher in the first 2 hours during the postprandial period in the LGI compared with the HGI and FAST trials ($p<0.05$). At the start of exercise, blood lactate values returned to resting concentrations in HGI and LGI trials. During exercise, blood lactate increased sharply in all trials. However, there was no significant difference between trials.

**Estimated Carbohydrate and Fat Oxidation Rate**

The estimated total amounts of CHO oxidation were significantly higher in the HGI
and LGI trials than that of FAST trial during the 3 hour postprandial period (HGI: 51.8±3.2 g·3hr⁻¹; LGI: 52.8±3.8 g·3hr⁻¹; FAST: 22.7±5.4 g·3hr⁻¹; F₂,₁₆=23.89, p<0.01).

In contrast, the calculated total amount of fat oxidised was lower in the HGI and LGI trials compared with FAST trials during the postprandial period (HGI: 9.5±1.0 g·3hr⁻¹; LGI: 9.9±1.0 g·3hr⁻¹; FAST: 17.5±1.6 g·3hr⁻¹; F₂,₁₆=, p<0.05).

A higher fat oxidation rate and lower CHO oxidation rate was observed in the FAST compared to the HGI and LGI trials during exercise (fat: 28.6±4.4 g·hr⁻¹; CHO: 146.0±8.2 g·hr⁻¹; p<0.05). The RER values were lower during postprandial period and exercise in the FAST than in LGI and HGI trials (Table 4.1, F₂,₁₆=11.94, p<0.05). There was a trend for lower RER values in LGI compared to HGI (p=0.09). However, there were differences in the calculated rates of fat and CHO oxidation (Fig 4.6 & 4.7, p<0.05) (Frayn, 1983). A higher amount of fat oxidation and a lower quantity of CHO oxidation were observed in the LGI trial compared to that of the HGI during the exercise (fat: HGI: 10.5±2.7 g·hr⁻¹, LGI: 19.3±3.2 g·hr⁻¹; CHO: HGI: 201.5±6.5 g·hr⁻¹, LGI: 175.8±6.6 g·hr⁻¹; F₂,₁₆=17.173, p<0.05).

Plasma Volume

There was a significant decrease in plasma volume during exercise in all three trials (HGI: 8.9%, LGI: 11.9%, and FAST: 11.6%) (p<0.05), however, there were no differences between trials.

Heart Rate and Rating of Perceived Exertion

There were no significant differences in heart rate (HR) or rating of perceived exertion (RPE) between the three trials (Table 4.2).
Gut Fullness and Thirst Scales

Following ingestion of the HGI and LGI meals, the perception of gut fullness significantly increased compared to that in the FAST trial ($F_{2,16}=24.80, p<0.05$) (Table 4.2). The peak value occurred 15 min after the meal in both trials and it remained higher than the FAST trial during the postprandial period ($p<0.05$). There were no significant differences in the ratings of thirst between the three trials (Table 4.2).

4.4 Discussion

The main finding from the current study was that the calculated amount of fat oxidation was significantly higher during exercise which commenced 3 hour following a LGI meal compared to that of a HGI meal. The results also demonstrated that the HGI meal resulted in a greater glycaemic and insulinaemic response during the postprandial period compared with the LGI meal. Several studies have reported a higher rate of fat oxidation after ingesting low GI single foods during subsequent submaximal exercise (Thomas et al. 1994; Febbraio & Stewart, 1996; Wee et al. 1999). However, there appear to be no reported studies on fat oxidation rates in response to low GI meals in relation to fasting.

The GI concept is based on the incremental area under the blood glucose curve (IAUC) following the ingestion of carbohydrate rich foods compared with that of a reference food, i.e. glucose or white bread (Jenkins et al. 1981). The validity of the GI values of mixed meals has been questioned in several studies (Coulston et al. 1984a; Coulston et al. 1984b; Hollenbeck et al. 1988). In the present study, subjects consumed $2g\ CHO\cdot kg\ \cdot 1$ body mass, approximately three times the amount of CHO used in the standard GI values test (50g CHO). However, the estimated GI ratio between the two test meals in the present study was 2.1:1.0 (77.4/36.9), which is similar to the actual
measured value of 2.2:1.0. This offers support for calculation of the GI values for mixed meal suggested by Wolever and Jenkins (Wolever and Jenkins, 1986).

Although the serum insulin IAUC was higher in the HGI trial than in the LGI, the magnitude of the insulinaemic response was not as pronounced as between the HGI and LGI meals in previous studies (Thomas et al. 1991; Febbraio & Stewart, 1996; Thomas et al. 1994; Sparks et al. 1998; Wee et al. 1999). In the current study, insulin concentration peaked at 15 min following the ingestion of either the HGI or LGI meal. This observation has not been reported in previous studies, possibly because single foods rather than meals were used (Thomas et al. 1991; Febbraio & Stewart, 1996; Thomas et al. 1994; Sparks et al. 1998; Wee et al. 1999). Some earlier studies observed a linear correlation between the GI and insulineamic index (II) (Wolever and Bolognesi, 1996; Bjorck et al. 2000), which was not observed in the early stage of postprandial period in the present study. However, several studies have indicated that additional protein, especially dairy products, in a CHO-containing meal could stimulate higher insulin secretion (Burke et al. 1995; Tarnopolsky et al. 1997). In the current study, both test meals contained skimmed milk, classified as a low GI index food, but which may induce insulinaemia during the postprandial period (Bjorck et al. 2000). The high insulin responses in the LGI trial may also explain the rapid decrease in the plasma glucose concentration during the early postprandial period.

Interestingly, blood lactate was elevated during the postprandial period following the ingestion of the HGI and LGI meals. Indeed in the LGI trial, blood lactate concentration peaked at 2.6 mmolL\(^{-1}\) which was approximately fourfold higher than the resting values. Several studies have shown elevated lactate concentrations following the ingestion of CHO foods (Wee et al. 1999; Stannard et al. 2000).
Although the mechanism is unclear, high lactate concentration following the ingestion of a fructose solution has been observed in several studies (Decombaz et al. 1985; Fielding et al. 1987; Hargreaves et al. 1987; Murray et al. 1989a; Murray et al. 1989b; Ventura et al. 1994). In the current study, the LGI meal contained more fructose (26g/70kg man) than the HGI meal (4g/70kg man); this may explain the higher blood lactate concentration in the LGI trial during the postprandial period. The proportion of fructose and glucose contents of the meals was <20% of the energy content of the meal. During exercise there were no differences in the blood lactate concentrations during the two trials.

A higher rate of fat oxidation was observed in the LGI compare with the HGI during exercise in the present study: this confirms the results of an earlier study (Wee et al. 1999). The exercise intensity elicits the maximal rate of fat oxidation was recently reported as being approximately 64% $\text{VO}_{2\text{max}}$ (Achten et al. 2002), and this was very close to the intensity used in the present study (65% $\text{VO}_{2\text{max}}$). However, this exercise intensity may be somewhat different when subjects are fed rather than fasting, because pre-exercise ingestion of CHO suppressed fat oxidation. The exercise intensity that stimulates optimum fat oxidation in fed rather than in fasting individuals has yet to be reported. Nevertheless, fasting and exercise are the most effective ways of increasing fat oxidation. The observation that carbohydrate ingestion results in the reduction in fat oxidation has been reported in several studies (Horowitz et al. 1997; Coyle et al. 1997). However, low GI CHO induces lower insulin secretion during postprandial period, and this may be accompanied by a reduced suppression of fat oxidation during subsequent exercise. Therefore, ingestion of low GI high CHO meal may provide the CHO required in the subsequent exercise without adversely affecting fat oxidation. More recently, it has been reported that following the ingestion of a
medium GI food, muscle glycogen used during subsequent exercise was similar to that in the control (fasting) trial (Kirwan et al. 2001b). Although muscle glycogen was not measured in the present study, it is reasonable to speculate that the higher fat oxidation may spare muscle glycogen during exercise and may also contribute to an increased loss of body fat mass.

In summary, ingestion of pre-exercise CHO meals resulted in lower rates of fat oxidation during subsequent exercise (65% VO_{2\max}) than when subjects performed exercise in the fasting state. However, the low GI meal resulted in a higher rate of fat oxidation during exercise than following the consumption of the high GI meal. Furthermore, the greater rate of fat oxidation in the LGI meal may be beneficial in improving endurance performance in men by delaying the depletion of muscle glycogen.
Table 4.1 Oxygen uptake (\(\dot{V}O_2\)), carbon dioxide expired (\(\dot{V}CO_2\)), and the respiratory exchange ratio (RER) during HGI, LGI and FAST trials; values are mean ± SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meal</th>
<th>Resting</th>
<th>Postprandial Period</th>
<th>Exercise Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\dot{V}O_2) (l min(^{-1}))</td>
<td>HGI</td>
<td>0.27±0.01</td>
<td>0.31±0.01*</td>
<td>2.79±0.10</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.28±0.01</td>
<td>0.32±0.01*</td>
<td>2.82±0.10</td>
</tr>
<tr>
<td></td>
<td>FAST</td>
<td>0.28±0.01</td>
<td>0.27±0.01</td>
<td>2.77±0.10</td>
</tr>
<tr>
<td>(\dot{V}CO_2) (l min(^{-1}))</td>
<td>HGI</td>
<td>0.22±0.01</td>
<td>0.28±0.01*</td>
<td>2.70±0.08*</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.23±0.02</td>
<td>0.29±0.01*</td>
<td>2.62±0.08*</td>
</tr>
<tr>
<td></td>
<td>FAST</td>
<td>0.22±0.01</td>
<td>0.22±0.01</td>
<td>2.48±0.10</td>
</tr>
<tr>
<td>RER</td>
<td>HGI</td>
<td>0.82±0.02</td>
<td>0.90±0.01*</td>
<td>0.96±0.01*</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.81±0.03</td>
<td>0.90±0.01*</td>
<td>0.94±0.01*</td>
</tr>
<tr>
<td></td>
<td>FAST</td>
<td>0.79±0.03</td>
<td>0.79±0.02</td>
<td>0.90±0.01</td>
</tr>
</tbody>
</table>

* Significantly different from FAST, p<0.05.
Table 4.2 Heart rate (HR), rating of perceived exertion (RPE), gut fullness (GF), and thirst scale (TS) during HGI, LGI and FAST trials; values are mean±SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meal</th>
<th>Resting</th>
<th>Postprandial Period (min)</th>
<th>Exercise Period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>HR (beats\cdot\text{min}^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGI</td>
<td>55±3</td>
<td>56±3</td>
<td>56±2</td>
<td>59±3</td>
</tr>
<tr>
<td>LGI</td>
<td>56±2</td>
<td>60±3</td>
<td>63±5</td>
<td>62±3</td>
</tr>
<tr>
<td>FAST</td>
<td>57±3</td>
<td>56±3</td>
<td>56±4</td>
<td>55±3</td>
</tr>
<tr>
<td>RPE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGI</td>
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</tr>
<tr>
<td>LGI</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FAST</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGI</td>
<td>7±0</td>
<td>14±1*</td>
<td>13±1*</td>
<td>12±1*</td>
</tr>
<tr>
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<td>16±1*†</td>
<td>15±1*†</td>
<td>14±1*</td>
</tr>
<tr>
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<td>9±1</td>
<td>8±0</td>
<td>8±0</td>
</tr>
<tr>
<td>TS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGI</td>
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<td>7±0</td>
<td>8±0</td>
<td>9±1</td>
</tr>
<tr>
<td>LGI</td>
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<td>8±1</td>
<td>8±1</td>
<td>9±1</td>
</tr>
<tr>
<td>FAST</td>
<td>11±1</td>
<td>8±0</td>
<td>8±0</td>
<td>8±0</td>
</tr>
</tbody>
</table>

* Significantly different from Fast, p<0.05.
† Significantly different from HGI, p<0.05.
Fig 4.1 Plasma glucose concentration (mmol·l⁻¹) during the HGI (○), LGI (●) and FAST (△) trials (mean ± SEM).

* Significantly different from FAST, p<0.05.
† Significantly different from LGI, p<0.05.
Fig 4.2  Serum insulin concentration (mIU l⁻¹) during the HGI (○), LGI(●) and FAST(△) trials (mean ± SEM).

* Significantly different from FAST, p<0.05.
† Significantly different from LGI, p<0.05.
Fig 4.3 Plasma FFA concentration during the HGI (○), LGI(●) and FAST(△) trials (mean ± SEM).
* Significantly different from HGI, p<0.05.
† Significantly different from LGI, p<0.05.
Fig 4.4 Plasma glycerol concentration during the HGI (○), LGI(●) and FAST(△) trials (mean ± SEM).
* Significantly different from HGI, p<0.05.
† Significantly different from LGI, p<0.05.
Fig 4.5 Blood lactate concentrations (mmol/l) during the HGI (○), LGI(●) and FAST(△) trials (mean ± SEM).
* Significantly different from FAST, p<0.05
† Significantly different from HGI, p<0.05
Fig 4.6 The rate of CHO oxidation (g min⁻¹) during the HGI (○), LGI (●) and FAST (△) trials (mean ± SEM).

* Significantly different from FAST, p<0.05
† Significantly different from LGI, p<0.05
Fig 4.7  The rate of fat oxidation (g min⁻¹) during the HGI (○), LGI(●) and FAST(△) trials (mean ± SEM).
* Significantly different from LGI and HGI, p<0.05
† Significantly different from HGI, p<0.05
CHAPTER 5
THE INFLUENCE OF PRE-EXERCISE HIGH CARBOHYDRATE BREAKFAST WITH DIFFERENT GLYCAEMIC INDICES ON RUNNING ENDURANCE CAPACITY AND SUBSTRATE UTILIZATION IN MEN

5.1 Introduction

Ingestion of carbohydrate (CHO) before exercise, as opposed to exercising in the fasted state, has been demonstrated to improve exercise performance (Nilsson and Hultman, 1973; Neufer et al. 1987; Hargreaves et al. 1987; Sherman et al. 1989; Wright et al. 1991). It is well known that the ingestion of CHO depresses fat oxidation and increases CHO oxidation (Horowitz et al. 1997; Coyle et al. 1997; Wee et al. 1999). These metabolic responses are mainly due to an increase in insulin secretion during the postprandial period. Therefore, by choosing pre-exercise CHO food that does not induce hyperinsulinaemia should lead to a greater rate of fat oxidation, and so sparing the limited glycogen stores in skeletal muscles. Hence the reduced hyperinsulinaemia may lead to a greater endurance capacity.

Carbohydrate-rich foods can be classified by glycaemic index (GI) (Jenkins et al. 1981). Several studies examined the effects of ingesting different GI foods on subsequent exercise performance (Thomas et al. 1991; Thomas et al. 1994; Febbraio and Stewart, 1996; Kirwan et al. 1998; Sparks et al. 1998; DeMarco et al. 1999; Febbraio et al. 2000a; Kirwan et al. 2001a; Kirwan et al. 2001b), although the results were inconsistent. The inconsistency in the results might be due to the differences in the amount of the CHO ingested, timing of food ingested, GI values and the type of exercise performance tests employed.
Furthermore, most of these earlier studies were designed using either single foods or drinks, rather than using meals. The uses of single foods or drinks as pre-exercise meals are not common practice in daily life. Therefore, the aim of this study was to examine the effects of ingestion high carbohydrate meals with different glycaemic indices on endurance running capacity.

5.2 Method

Eight healthy recreational runners (age: 28.9±1.5 yrs; weight: 70.5±2.2 kg; Height: 175.0±1.9 cm; \( \dot{V}O_{2\max} \): 60.6±1.5 ml kg\(^{-1}\) min\(^{-1}\)) voluntarily participated in this study. All subject were informed the possible risks of the study and completed health questionnaires before the informed consent signed. Preliminary tests and main experimental trials were as described in Chapter 3. Briefly, each subject undertook two experimental trials in a counterbalance design separated by at least 7 days. Subjects consumed the test meals, as described in chapter 3, 3 hours before running to exhaustion at 70% \( \dot{V}O_{2\max} \) on a motorized treadmill. To prevent dehydration, subjects were supplied with 2 ml/kg body mass water every 15 min during the endurance capacity test. Ambient temperature and relative humidity were recorded at 30 min intervals during the postprandial period and at 15 min intervals during exercise using a hydrometer (Zeal, England). Temperature was maintained between 15-25°C and humidity was between 46-60% in all trials. While running on the treadmill, subjects were cooled by electric fans and wet sponges were also available for use ad libitum. The subjects were instructed to refrain from heavy physical activities and also were asked to abstain from alcohol, caffeine and tobacco consumption for 24 hours before each main trial. All subjects were asked to record their diet for two days before the first main trial using food weighing method, and were asked to repeat the same diet for two days before the second main trial. The definition of exhaustion was that
subjects were no longer able to maintain the assigned running speed. Near the end of the run, subjects were allowed to slow down the treadmill twice for 2 min to a walking speed at 5km/h. This was to ensure that the subjects truly reached metabolic fatigue.

A two-way (time and treatment) ANOVA with repeated measure was used to analyse overall differences in the physiological and biochemical responses to the two main trials. When a significant difference was found, the Tukey post hoc test was used to locate the differences in means. For non-time dependent variables, Student’s t-test for paired observations was used. Significance was set at the 0.05 level of confidence. Results are presented as mean±SEM.

5.3 Results

Endurance capacity and subjective observation

All subjects completed two experimental trials. Six out of eight subjects ran longer in the LGI trial. The average running time of LGI trial (108.8±4.1min) was significantly longer than HGI trial (101.4±5.2min) (p=0.038). Five subjects were observed to be pale and sweating profusely during the first 30 min of exercise in HGI trial (glucose concentration: 3.3±0.1mmol/l). Six subjects expressed hunger in the early stage of exercise during HGI trial. Two subjects, who ran shorter in the LGI trial, claimed abdominal discomfort during exercise.

Plasma Glucose and Serum Insulin Responses

Following ingestion of the HGI and LGI meals, plasma glucose concentrations increased sharply and in both trials peaked at 15 min during the postprandial period (Fig 5.1). The plasma glucose concentration in the HGI trial was significantly higher at 30, 60 and 90min during postprandial period compared to the LGI trial (F_{1,7}=8.75, p<0.05; Fig 5.1).
The LGI trial maintained higher concentrations of plasma glucose during the first 30 min into exercise (p<0.05; Fig 5.1).

Serum insulin concentration peaked at 15 min after ingestion of both test meals (HGI: $140.9\pm15.2$ mIU$^{-1}$; LGI: $133.4\pm12.5$ mIU$^{-1}$; p>0.05, Fig 5.2). There were significantly higher serum insulin concentrations in the HGI trial at 30, 60, 90, and 120min during the postprandial period compared to the LGI trial ($F_{1,7}=7.58$, p<0.05; Fig 5.2). There were no differences between the two trials in serum insulin concentrations during exercise.

The incremental area under the curve (IAUC), over the postprandial period, for both glucose (HGI: 134.2 mmol$^{-1}$·3h$^{-1}$; LGI: 57.5 mmol$^{-1}$·3h$^{-1}$) and insulin (HGI: 13298 mIU$^{-1}$·3h$^{-1}$; LGI: 7445 mIU$^{-1}$·3h$^{-1}$) were higher during the HGI than LGI trial. The ratio of glucose IAUC between HGI and LGI was 2.3:1, which was similar to the estimated test meal GI ratio of 2.1:1.

**Plasma Free Fatty Acid (FFA) and Glycerol Responses**

Plasma FFA concentrations were suppressed following ingestion of both test meals (Fig 5.3). There were no significant differences between two trials during the postprandial period. At onset of exercise, plasma FFA concentrations were increased gradually in both HGI and LGI trials. However, the plasma FFA concentrations were higher during the LGI trial than during the HGI trial throughout the exercise period ($F_{1,7}=13.86$, p<0.05; Fig 5.3).

Plasma glycerol showed similar responses to that of plasma FFA (Fig 5.4). There were no significant differences between trials during the postprandial period. However during exercise, plasma glycerol concentrations were higher during the LGI trial at 45, 60,
90min and at fatigue than that of the HGI trial (F_{1,7}=6.23, p<0.05; Fig 5.4).

_Blood Lactate_

Blood lactate concentrations increased significantly after ingestion of the LGI meal (Fig 5.5). The values were higher in the LGI trial than in the HGI trial at 15, 30, 60, and 90min during the postprandial period (F_{1,7}=14.72, p<0.01; Fig 5.5). There were no significant differences between trials during exercise.

_Substrate Utilisation_

The estimated fat oxidation rates were significantly higher in the LGI trial at 15, 45, 60, 75, and 90min during exercise than the HGI trial (F_{1,7}=8.24, p<0.05; Fig 5.6). In contrast, the estimated CHO oxidation rates were lower in the LGI trial during exercise (F_{1,7}=9.07, p<0.05; Fig 5.7). The fat oxidation rates were increased, and the CHO oxidation rates decreased gradually during the exercise (Fig 5.6 & Fig 5.7). However, here were no differences between two trials in both fat and CHO oxidation rate during postprandial period (p>0.05; Fig 5.6 & 5.7).

\(\dot{V}O_2\), \(\dot{V}CO_2\) and _Respiratory Exchange Ratio (RER)_

There were no significant differences between two trials in \(\dot{V}O_2\) and \(\dot{V}CO_2\) during postprandial period nor during exercise (p>0.05; Table 5.1). However, there was a higher RER value during the first 90min of exercise in the HGI trial (t=3.616, p<0.05; Table 5.1).

_Heart Rate and Rating of Perceived Exertion_

There were no significant differences in heart rate (HR) or rating of perceived exertion (RPE) between two trials (Table 5.2).


**Gut Fullness (GFS) and Thirst Feeling (TS)**

After ingestion of LGI meal, gut fullness was higher at 15, 30 and 60 min during the postprandial period ($F_{1,7}=8.75$, $p<0.05$; Table 5.2). Gut fullness tended to be higher during exercise in the LGI trial ($p<0.05$ at 75 min; Table 5.2). There were no significant differences in thirst sensation between the two trials during the experimental trials (Table 5.2).

### 5.4 Discussion

The main finding of present study was that ingestion of the LGI high carbohydrate breakfast resulted in a greater endurance capacity during running than after the ingestion of the HGI breakfast. This study also showed a greater glycaemic and insulinaemic response during the postprandial period after the HGI than after the LGI meal. In contrast, the LGI meal induced higher fat oxidation rate, plasma FFA and plasma glycerol responses during exercise than following the HGI meal.

Several studies have examined the GI effects of pre-exercise feeding on exercise performance (Thomas et al. 1994; Febbraio and Stewart, 1996; Kirwan et al. 1998; Sparks et al. 1998; DeMarco et al. 1999; Febbraio et al. 2000a; Kirwan et al. 2001a; Kirwan et al. 2001b; Wong et al. 2003). Some of the studies claimed that ingestion of low GI foods improves performance (Thomas et al. 1991; Kirwan et al. 1998; DeMarco et al. 1999; Kirwan et al. 2001a; Wong et al. 2003) as a result of lower insulin production and so a higher rate of fat oxidation which might spare muscle glycogen. In the current study, we also demonstrated a higher fat oxidation rate during exercise in the LGI trial compared to the HGI trial (Fig 5.6). Although we did not measure muscle glycogen, it is reasonable to speculate that the “muscle glycogen sparing effect” in the LGI trial...
contributed to the improve endurance capacity. This effect of higher fat oxidation is also supported by the increase concentrations of plasma FFA and glycerol concentrations during exercise in the LGI trial (Fig 5.3 and 5.4). It has been reported that when FFA concentrations are increased, muscle glycogen utilization is reduced (Hickson et al. 1977; Costill et al. 1977).

In the present study, subjects who ran for less time in the LGI trial, experienced abdominal discomfort during exercise. Some studies reported cases of gut discomfort after ingestion of high doses of fructose (Hargreaves et al. 1987; Thomas et al. 1991; Costill and Hargreaves, 1992). Indeed, the LGI meal contained more fructose than the HGI meal. This might have caused those subjects, who were not used to eating fruits, gut discomfort. Although these two subjects elicited higher fat oxidation during exercise in the LGI trial, the gut discomfort might have offset the metabolic advantages of this meal.

In addition to a muscle glycogen sparing effect, a number of studies demonstrated that exercise performance might be enhanced following CHO feeding by preventing hypoglycaemia and increasing CHO availability to muscles (Coyle et al. 1985; Neufer et al. 1987; Wright et al. 1991; Sherman et al. 1991). Our findings show that ingestion of the LGI meal 3 hour before exercise resulted in a more stable plasma glucose concentration than following ingestion of the HGI meal. At 15 and 30 min into exercise, the plasma glucose in the HGI trial was significantly lower than the LGI trial (Fig 5.1). During this initial period of exercise, five subjects were observed to be pale and sweating profusely which suggested hypoglycaemia symptoms. Therefore, ingestion of the HGI meal 3 hours before exercise might be disadvantageous for endurance performance. Nevertheless, a high GI CHO meal before exercise results in a greater endurance running capacity than no-meal at all (Chryssanthopoulos et al. 2002).
In an earlier study, we investigated the effects of different GI foods on endurance running capacity using a similar experimental design (Wee et al. 1999). However, this study did not find differences in endurance capacity between the HGI and LGI trials. The main difference between the earlier study by Wee et al. (1999) and the current study was the nature of the foods used. In the present study, the test meals were designed to be as close to a normal breakfast as possible. In the earlier study the test meals consisted of lentils as LGI food and mixed potatoes, tuna, sweet corn, and crumpets as HGI foods. Therefore the different pre-exercise food may account for the differences between two studies. Furthermore, in the previous study, the plasma FFA, plasma glycerol, and estimated fat oxidation rate were higher during the postprandial period in the LGI trial. These results are different from our current study where we did not find any difference in plasma FFA and glycerol concentrations, nor fat oxidation rate during postprandial period. One difference between the two studies is particularly worthy of mention. In the previous study, the postprandial plasma glucose responses were extremely low during the LGI trial. Furthermore, the experimental GI values failed to follow the estimated GI values which the experimental GI ratio between two meals were 5.8:1.0 and 2.8:1.0 for the estimated values (Wee et al. 1999). The high experimental ratio was due to the extremely low glycaemia response in the LGI trial. We speculate that the cooking process, especially the level of gelatinisation of lentils, affects glycaemic responses. As a result, the un-gelatinised starch might not be able to be absorbed in the small intestine. As a consequence the ingested CHO might have not been taken up by muscle or liver in LGI trial during the postprandial period. This might have been the reason behind the lack of improvement in endurance capacity despite inducing the consistently high fat oxidation rate both during the postprandial period and during exercise.
An interesting study recently reported that ingestion of low GI cereal (All Bran, Kellogg's) which was also used in the current study, showed a high rate of glucose disappearance leading to a lower glycaemic response (Schenk et al. 2003). They suggested that the traditional view that low GI foods have a characteristically low absorption rate was probably not the case for the low GI cereal. Our data also showed an unexpected hyperinsulinaemia in the early stages of the postprandial period in the LGI trial (Fig 5.2). Nevertheless, insulin concentrations declined following the decrease in glucose concentrations whereas in the study by Schenk et al. (2003), using All Bran plasma insulin concentrations remained high for 90 minutes. Although the underlying mechanism is still unclear, the glycaemia and insulinaemia are consistent with our previous study on pre-exercise feeding (chapter 4).

In summary, ingestion of mixed low glycaemic index high CHO meal 3 hour before exercise resulted in a greater endurance capacity than following the ingestion of a mixed high glycaemic index carbohydrate meal. The differences in endurance capacity may be a consequence of the greater rate of fat oxidation during exercise following the low glycaemic index carbohydrate meal.
Table 5.1 Oxygen uptake (\( \dot{V}O_2 \)), carbon dioxide expired (\( \dot{V}CO_2 \)), and the respiratory exchange ratio (RER) during HGI and LGI trials; values are mean ± SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meal</th>
<th>Resting</th>
<th>Postprandial Period</th>
<th>Exercise Period (to 90min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \dot{V}O_2 ) (l( \text{min}^{-1} ))</td>
<td>HGI</td>
<td>0.25±0.01</td>
<td>0.30±0.01</td>
<td>2.93±0.10</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.28±0.01</td>
<td>0.31±0.01</td>
<td>3.01±0.11</td>
</tr>
<tr>
<td>( \dot{V}CO_2 ) (l( \text{min}^{-1} ))</td>
<td>HGI</td>
<td>0.22±0.01</td>
<td>0.28±0.01</td>
<td>2.87±0.09</td>
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<td>0.28±0.01</td>
<td>2.81±0.09</td>
</tr>
<tr>
<td>RER</td>
<td>HGI</td>
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<td>0.95±0.02</td>
<td>0.98±0.01*</td>
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<tr>
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<td>0.81±0.03</td>
<td>0.93±0.01</td>
<td>0.94±0.01</td>
</tr>
</tbody>
</table>

* Significantly different from LGI, p<0.05.
Table 5.2 Heart rate (HR), rate of perceived exertion (RPE), gut fullness (GF), and thirst scale (TS) during HGI and LGI trials; values are mean±SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meal</th>
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<th>Postprandial Period (min)</th>
<th>Exercise Period (min)</th>
<th>Fatigue</th>
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<td>15</td>
<td>30</td>
<td>60</td>
</tr>
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<td>-</td>
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<td>-</td>
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<tr>
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<td>LGI</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RPE</td>
<td>HGI</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>GF</td>
<td>HGI</td>
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<td>13±1*</td>
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<td>11±1</td>
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<td>LGI</td>
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<td>7±0</td>
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</table>

* Significantly different from LGI, p<0.05.
Fig 5.1 Plasma glucose concentration (mmol/l\(^{-1}\)) during the HGI and LGI trials (mean ±SEM). * Significantly different from LGI, \(p<0.05\).
Fig 5.2 Serum insulin concentration (mIU l⁻¹) during the HGI and LGI trials (mean±SEM). * Significantly different from LGI, p<0.05.
Fig 5.3 Plasma FFA concentration (mmol/l) during the HGI and LGI trials (mean±SEM). * Significantly different from HGI, p<0.05.
Fig 5.4 Plasma glycerol concentration (μ molL⁻¹) during the HGI and LGI trials (mean ± SEM). * Significantly different from HGI, p<0.05.
Fig 5.5 Blood lactate concentrations (mmol·l⁻¹) during the HGI and LGI trials (mean ±SEM). * Significantly different from HGI, p<0.05.
Fig 5.6 The rate of fat oxidation (g min⁻¹) during the HGI and LGI trials (mean±SEM).

* Significantly different from HGI, p<0.05.
Fig 5.7 The rate of CHO oxidation (g/min⁻¹) during the HGI and LGI trials (mean±SEM). * Significantly different from LGI, p<0.05.
CHAPTER 6

INFLUENCE OF ISOCALORIC CARBOHYDRATE MEALS ON SUBSTRATE UTILISATION DURING SUBMAXIMAL EXERCISE FOLLOWING A 4 HOUR RECOVERY IN MEN

6.1 Introduction

Carbohydrate is the main energy source during prolonged strenuous endurance exercise. One of the main goals of nutrition after strenuous endurance exercise is to refuel the body’s energy stores in order to meet energy requirement during the subsequent exercise. Carbohydrate (CHO) feeding immediately after prolonged exercise improves the resynthesis rate of muscle glycogen (Blom et al. 1987; Ivy et al. 1988a; Ivy et al. 1988b; Krssak et al. 2000; van Hall et al. 2000b). However, carbohydrate oxidation rate is increased following a high CHO meal and fat oxidation is reduced (Horowitz et al. 1997; Coyle et al. 1997). These changes are a consequence of the insulinogenic responses to the CHO intake (Horowitz et al. 1997; Coyle et al. 1997).

The nature of CHO rich foods can be described in terms of their glycaemic responses during the postprandial period. Jenkins and co-workers established the glycaemic index (GI) concept on CHO rich foods in the early 1980s (Jenkins et al. 1981). Low GI foods induce lower blood glucose and insulin responses compared to high GI foods. Thomas and her colleagues first conducted CHO feeding studies using high and low GI foods before prolonged submaximal exercise (Thomas et al. 1991). Interestingly, they found low GI food improved endurance capacity and they suggested that this was the result of the lower blood glucose concentration. However, subsequent studies did
not show the same significant improvement in endurance capacity or performance (Thomas et al. 1994; Febbraio and Stewart, 1996; Sparks et al. 1998; Wee et al. 1999). Nevertheless insulin and glucose responses to the low GI foods were significantly lower than that of high GI foods (Burke et al. 1993; Thomas et al. 1994; Febbraio and Stewart, 1996; Wee et al. 1999; Febbraio et al. 2000b). Our recent study on pre-exercise feeding showed that ingestion of low GI food resulted in higher fat oxidation compare to that of high GI foods (Wee et al. 1999). Therefore, low GI CHO rich food may favour fat oxidation and so spare endogenous CHO usage during the post-exercise recovery period and subsequent exercise.

Nevertheless, most of the recent studies on CHO feeding either before or after exercise used single food or CHO drinks rather than normal meals (Fallowfield et al. 1995; Fallowfield and Williams, 1997; Wee et al. 1999; Wong et al. 1998; Wong and Williams, 2000). However, solid and liquid forms of CHO rich meals after prolonged exercise appeared to be equally effective in restoring muscle glycogen after 24-hour recovery (Keizer et al. 1987). Under normal circumstances athletes eat meals rather than single foods. In a 24-hour recovery study, Burke et al. (1993) fed subjects CHO meals with different GI values after glycogen-depleting submaximal cycling. They suggested that the high GI CHO food produced the most rapid increase in muscle glycogen during 24-hour recovery. However, the literature is lacking in information on the GI effects on the short time recovery from strenuous endurance exercise, especially so on the metabolic responses during subsequent exercise.

Therefore, the aim of the present study was to assess the influence of two isocaloric carbohydrate meals of different glycaemic indices on substrate utilisation during subsequent exercise after a 4 hour recovery from prolonged running.
6.2 Methods

Eight healthy male recreational runners (age: $24.1 \pm 1.9$ yrs; height: $176.3 \pm 1.9$ cm; body mass: $68.1 \pm 1.3$ kg; $\dot{V}O_2\text{max} \ 65.8 \pm 3.1$ ml$\cdot$kg$^{-1}$$\cdot$min$^{-1}$; body mass index: $21.8 \pm 0.4$ kg$\cdot$m$^{-2}$) volunteered to participate in the study. Preliminary tests and main experimental trials were as described in Chapter 3. Briefly, subjects were asked to undertake two experimental trials, the order of the trials was randomised, which were separated by at least one week. Each trial consisted of two runs: (i) Run 1 ($R_1$): subjects ran on a treadmill at $70\% \ \dot{V}O_2\text{max}$ for 90 min or until fatigue which included 5 min warm up at $60\% \ \dot{V}O_2\text{max}$ . (ii) Run 2 ($R_2$): after a 4 hour recovery (REC) and following a 5 min warm up at $60\% \ \dot{V}O_2\text{max}$ subjects ran on a treadmill at $70\% \ \dot{V}O_2\text{max}$ for 30 min. Subjects consumed either HGI meal or LGI meal (Table 3.1) during the recovery period.

All trials were performed under similar experimental and environmental conditions. Temperature was maintained between 20-25°C and relative humidity was between 50-60%. The subjects were instructed to refrain from heavy physical activities and to consume exactly the same diet for two days before each main trial. All subjects were also asked to abstain from alcohol, caffeine and tobacco consumption for 24 hours before each main trial.

On the day of experiment, subjects reported to the laboratory after a 12 hour overnight fast. Then subjects were asked to void urine before nude body mass was obtained. Nude dried body mass was also measured before and after $R_1$. After weighing, a cannula was inserted into a forearm vein while the subject was lying on an examination couch. A short range telemeter (Sports Tester PE300, Polar Electro,
Finland) was then attached to monitor the subjects' heart rates (HR).

The first blood sample was obtained after subject had stood for 10 min. A 5 min warm up at 60% \( \dot{V}O_2\text{max} \) then began, thereafter the treadmill speed was increased to the pace equivalent to 70% \( \dot{V}O_2\text{max} \) for each subject. The first run (R₁) lasted 90 min, or until volitional fatigue, which ever happened first. The 4 hour recovery period (REC) began immediately after R₁. The test meals were served and the subjects were asked to consume them within 15 min following the first 30 min of recovery. Nude body mass was obtained in order to calculate the body mass change from exercise-induced dehydration, before and after test meal. Thereafter, subjects consumed water in an amount equivalent to 150% of body weight loss (g=ml) during REC (Wong et al. 1998). During REC the subjects sat on a comfortable chair and avoided any physical activity. Following the 4 hour REC, the subjects were required to perform R₂ using the same protocol as previously described. During each run, the subjects were cooled by an electric fan and wet sponges were also available for use ad libitum.

Two-way analysis of variance (ANOVA) repeated measures on two factors (experimental treatments and time was used to analyse overall differences between physiological and biochemical responses to two main trials. When the significant difference was found, the Tukey post hoc test was used to locate the differences in means. For non-time dependent variables, Student’s t-test for paired observations was used. Significance was set at the 0.05 level of confidence. Results are presented as mean±S.E.M..
6.3 Results

Exercise Time and Percentage \(\dot{V}O_2\text{max}\)

All subjects completed the 90 min run during \(R_1\) and 30 min run during \(R_2\) in both trials. The mean relative exercise intensities during \(R_2\) in the HGI and LGI trials were 71.1±2.0% and 70.5±2.0%, respectively (NS).

RER and Substrates Utilization

There were no significant differences in RER between the two trials. There were no significant difference in \(\dot{V}E\), \(\dot{V}O_2\) and \(\dot{V}CO_2\) (Table 6.1). There were no significant differences in the rate of CHO and fat oxidation (Fig 6.1 and 6.2) between the two trials throughout the experiments.

Heart Rate, Gut Fullness (GFS), Thirst scale (TS) and RPE

There were no significant differences in heart rate, RPE, and perceived thirst between the two trials. Nevertheless, in LGI trial the subjects reported higher gut fullness at REC-60 after the meal than after the HGI meal. (p<0.05) (Table 6.2)

Plasma Glucose and Serum Insulin

In both trials, plasma glucose concentration increased sharply at REC-60 in response to the test meals ingested after \(R_1\). Thereafter, the plasma glucose concentration in the LGI trial declined rapidly compared to that of HGI trial. Plasma glucose concentration decreased gradually and returned to the pre-exercise value at the end of recovery in the both trials (Fig 6.3). There were significantly higher plasma glucose concentrations at REC-90, REC-120 and REC-180 in HGI trial compare to that of LGI trial during the postprandial period (\(F_{1,7}=6.54\), p<0.05). The incremental area under the curve (IACU) for plasma glucose was significantly higher in HGI trial
(367.6±87.1 mmol/l⁻¹) than for the LGI trial (170.9±27.7 mmol/l⁻¹) during postprandial period (p<0.05).

The pattern of change in serum insulin concentration was similar to that of plasma glucose concentration (Fig 6.4). Serum insulin concentration increased in both trials after ingesting the meals. It decreased more slowly in HGI trial than in LGI trial, and only in the LGI trial returned to the pre-exercise value before R₂. There were significantly higher serum insulin concentrations at REC-90, REC-120, REC-180, and REC-240 in the HGI trial than the LGI trial (F₁,₇=8.05, p<0.05). The incremental area under the curve for serum insulin was higher in the HGI trial (6384.4±992.2 mIU L⁻¹) than that for the LGI trial (3984.3±491.3 mIU L⁻¹) during the postprandial periods (p<0.05).

Plasma FFA and Glycerol
In both trials, plasma FFA and glycerol concentrations increased following R₁ and the peak values appeared at REC-30 before the meal was ingested. Then the values decreased followed the meal and reached the lowest point at REC-180. There were no significant differences between two trials in plasma FFA and glycerol concentrations (Fig 6.5 and 6.6).

Blood Lactate
There were no significant differences in blood lactate concentration between trials during R₁ and R₂. However after the LGI meal, blood lactate increased sharply, and peaked at R-90 then decreased gradually to the pre-exercise value at the end of recovery. The values were higher at REC-60, REC-90 and REC-120 during the LGI trial than during the HGI trial (F₁,₇=9.47, p<0.01) (Fig 6.7).
Plasma Volume

Plasma volume decreased after R₁ in HGI and LGI trials (-1.9±1.3% vs. -3.3±2.0%, respectively, N.S.) During REC, plasma volume was restored in both trials and remained at a level above the pre-exercise value until the end of R₂. There were no significant differences in the changes in plasma volume between the two trials.

6.4 Discussion

The main finding of the present study was that there were no differences in the substrates utilization during the 30min run after a 4 hour recovery when subjects ingested either HGI or LGI meal containing of 2g CHO·kg⁻¹ body mass meals.

Sports men training in the early morning without having a breakfast, i.e. after an overnight fast, may have a meal 3-4 hours before the next training session in the afternoon. The present study demonstrated the influences of ingesting isocaloric high and low GI CHO meals during a 4 hour recovery on substrate utilization during subsequent exercise. Although the results did not show the shift in the substrate utilisation during subsequent exercise compared to that of pre-exercise feeding studies after ingestion of low GI meal (Wee et al. 1999). The underlying mechanism is likely to be different because of the duration of the R₂. However, lower RER values were observed in the present study compared to that of pre-exercise feeding studies both during the postprandial period and subsequent exercise (Febbraio and Stewart, 1996; Wee et al. 1999; Febbraio et al. 2000b). The means of RER during R₂ were between 0.88-0.90 in the current study compare to the pre-exercise feeding were at 0.94-0.98 at commencement of the exercise (Wee et al. 1999). This could indicate a higher fat metabolism during the post-exercise recovery period and subsequent exercise even if
subject ingestion of high CHO meal after a prolonged endurance exercise. As expected, a higher fat metabolism has been observed when an energy deficit exists. The estimated energy expenditure during R1 was approximated 1300 kcal. The energy content of the test meal was ~690 kcal (for 70kg man) and so would not have compensated for the total energy expenditure during R1. We speculated that the short-term energy shortage might facilitate fat oxidation even if the post-exercise meal contained high CHO. As a consequence, high fat metabolism may be beneficial to body fat loss.

In the current study, rates of carbohydrate and fat oxidation were not significantly different between the two trials (Fig 6.1). High carbohydrate diets depress fat oxidation during the postprandial period as can be seen in the current study. Plasma FFA and glycerol concentrations were decreased after both test meals during the recovery period. Nevertheless, pre-exercise feeding data has shown that consuming LGI rather than HGI food could increase the fat oxidation both in the postprandial period and the subsequent submaximal exercise (Wee et al. 1999). However, these pre-exercise studies fed subjects with single foods rather than real mixed meals as in the present study. In the present study, the nature of the CHO in the pre-exercise (R2) meal did not produce differences in the plasma FFA concentrations nor in fat oxidation rates. The food interaction and the impact of post-prolonged strenuous exercise on the physiologic and metabolic aspect are still not clear.

Ingestion of CHO foods before exercise resulted in hyperinsulinemia and increased CHO oxidation (Costill et al. 1977; Koivisto et al. 1985; Hargreaves et al. 1987; Montain et al. 1991; Febbraio and Stewart, 1996; Wee et al. 1999; Febbraio et al. 2000b). In the present study, insulin response was lower after ingestion of high CHO
meals either HGI or LGI compared to that of pre-exercise feeding studies (Wee et al. 1999; Febbraio et al. 2000b). In most of the pre-exercise studies subjects were fed in the early morning which is different from the mid-morning post-exercise feeding in the present study. It has been reported that the time of the day may induce different effects on the glycaemia and insulinaemia responses to a meal (Wolever et al. 1997). Furthermore, post-prolonged exercise could increase muscle glucose uptake associating with translocation of glucose transporter-4 (GLUT-4) (Hansen et al. 1998). GLUT-4 translocation rate was increased for facilitating glucose uptake due to a low glycogen content in the muscle cell. Nevertheless, insulin secretion can activate GLUT-4 translocation in order to increase glucose uptake in muscle. This response could elicit lower insulin secretion during the post-exercise CHO feeding (Richter et al. 1998). In a 24 h recovery study, Burke et al. (1993) found no differences in glucose and insulin IAUC following ingestion of the first meal containing either high or low GI foods.

In the present study, glycaemic index were based on the postprandial blood glucose incremental area under the curve following the ingestion of carbohydrate rich food. The estimated GI values of test meals were calculated by the mixed meal method. In the current study, we observed an unexpected high plasma glucose concentration at REC-60 during the recovery period in the LGI trial which was similar to the HGI meal response. The GI values of carbohydrate rich food is based on 2h postprandial blood glucose response after ingestion of 50 g CHO content of the food. The current study fed subjects with 2g CHO kg\(^{-1}\) body mass carbohydrate meals which were over 2.5 times higher than the standard GI test. This could cause high glucose response in the early postprandial period. However, the real ratio of the glucose IAUC during the postprandial period between two meals is 2.15, which is similar to the estimated GI
ratio, 2.11 (78/37). This suggests that the mixed meal GI calculation method was applicable in a mixed high CHO meal.

After glycogen depleting exercise, athletes have been suggested to consume high CHO diet in order to replenish muscle and liver glycogen stores, in particular during first 24 hour post-exercise (Zachwieja et al. 1993; Conlee et al. 1987; Burke, 1997). There are two phases in muscle glycogen resynthesis following its depletion by exercise: non-insulin dependent and insulin dependent (Price et al. 1994). The first stage is non-insulin dependent, which has been found to last for 30-60 min. The second phase is insulin dependent that is directly related to the insulin response to the supplement (Zawadzki et al. 1992). Ingestion of complex CHO rich, high GI foods associated with high insulin secretion as well as single high GI CHO foods. Therefore, using GI values as an indicator to choose food can be extensively applied to common practise for athletes to replenish glycogen storage after prolonged intensive exercise during early recovery period. Although the insulin-AUC in the HGI trial is higher than in the LGI trial in the present study this could induce higher muscle glycogen resynthesis in the insulin dependent phase.

Interestingly, blood lactate concentrations increased significantly after consuming LGI meal and were elevated for 1.5 hours in the present study. Carbohydrate ingestion may induce high lactate production during the postprandial period (Wee et al. 1999; Stannard et al. 2000). Although the mechanism is still unclear, a high lactate concentration following fructose solution ingestion has been observed in several studies(Decombaz et al 1985; Fielding et al. 1987; Murray et al. 1989a; Murray et al. 1989b; Hargreaves et al. 1987; Ventura et al. 1994). In the current study, the LGI meal contains more fructose than the HGI meal, and as a consequence a high lactate
production during the postprandial period. Resynthesis of muscle glycogen is a high metabolic priority after muscle glycogen depletion (Kiens and Richter, 1998). Krssak et al (Krssak et al. 2000) hypothesised that there is transfer of glycogen by glucose-lactate and glucose-alanine cycle from resting muscle to recovering muscle after running exercise. However, the high level of blood lactate concentration could be a disadvantage prior to intensive exercise.

In summary, our findings suggested that there was no difference in the substrate utilisation during the 30min run after 4 hour recovery, when subjects ingested either HGI or LGI of 2.0g·kg⁻¹ body wt carbohydrate meals. Furthermore, the subjects were also able to tolerate the meal size as reflected by no reports of gastrointestinal disturbance during exercise following the short recovery period.
Table 6.1 Respiratory exchange ratio (RER), $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$ during Run 1, recovery and Run 2 in the HGI and LGI trials; values represent Mean±SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meal</th>
<th>Resting</th>
<th>Run 1 (min)</th>
<th>Recovery Period (min)</th>
<th>Run 2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>45</td>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGI</td>
<td>0.84±0.08</td>
<td>0.90±0.01</td>
<td>0.91±0.02</td>
<td>0.78±0.06</td>
<td>0.82±0.05</td>
</tr>
<tr>
<td>LGI</td>
<td>0.82±0.06</td>
<td>0.89±0.01</td>
<td>0.88±0.01</td>
<td>0.79±0.04</td>
<td>0.91±0.03</td>
</tr>
<tr>
<td>$\dot{V}E(l)*$</td>
<td>HGI</td>
<td>53.2±7.3</td>
<td>72.4±2.5</td>
<td>77.5±3.0</td>
<td>60.0±7.0</td>
</tr>
<tr>
<td>LGI</td>
<td>51.1±4.1</td>
<td>69.6±3.1</td>
<td>72.0±2.5</td>
<td>57.6±3.0</td>
<td>62.5±1.8</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (ml·min$^{-1}$·kg$^{-1}$)</td>
<td>HGI</td>
<td>4.9±0.3</td>
<td>46.6±1.6</td>
<td>47.0±1.3</td>
<td>5.5±0.2</td>
</tr>
<tr>
<td>LGI</td>
<td>5.5±0.4</td>
<td>45.8±1.3</td>
<td>46.4±1.2</td>
<td>5.4±0.2</td>
<td>5.8±0.2</td>
</tr>
<tr>
<td>$\dot{V}CO_2$ (ml·min$^{-1}$·kg$^{-1}$)</td>
<td>HGI</td>
<td>4.2±0.7</td>
<td>42.0±1.5</td>
<td>42.5±1.4</td>
<td>4.4±0.5</td>
</tr>
<tr>
<td>LGI</td>
<td>4.4±0.4</td>
<td>40.8±0.9</td>
<td>40.7±0.9</td>
<td>4.3±0.3</td>
<td>5.3±0.2</td>
</tr>
</tbody>
</table>

* $\dot{V}E$ values represent 5 min collection during recovery period.
Table 6.2 Heart rate (HR), rate of perceived exertion (RPE), gut fullness (GF), and thirst scale (TS) during HGI and LGI trials; values are mean±SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meal</th>
<th>Resting</th>
<th>Run 1 (min)</th>
<th>Recovery Period (min)</th>
<th>Run 2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>45</td>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>HR (beats·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGI</td>
<td>6±±2</td>
<td>159±3</td>
<td>165±3</td>
<td>97±3</td>
<td>91±2</td>
</tr>
<tr>
<td>LGI</td>
<td>59±3</td>
<td>158±3</td>
<td>165±4</td>
<td>96±2</td>
<td>91±2</td>
</tr>
<tr>
<td>RPE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGI</td>
<td>12±1</td>
<td>13±1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGI</td>
<td>11±1</td>
<td>12±1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGI</td>
<td>10±1</td>
<td>8±1</td>
<td>7±1</td>
<td>7±1</td>
<td>11±1</td>
</tr>
<tr>
<td>LGI</td>
<td>8±1</td>
<td>8±1</td>
<td>7±1</td>
<td>8±1</td>
<td>15±1</td>
</tr>
<tr>
<td>TS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGI</td>
<td>7±1</td>
<td>11±1</td>
<td>14±1</td>
<td>11±1</td>
<td>9±1</td>
</tr>
<tr>
<td>LGI</td>
<td>8±1</td>
<td>10±1</td>
<td>12±1</td>
<td>12±1</td>
<td>9±1</td>
</tr>
</tbody>
</table>

* Significantly different from LGI, p<0.05.
Fig 6.1 The rate of fat oxidation (g·min⁻¹) during the HGI and LGI trials (mean±SEM).
Fig 6.2 The rate of CHO oxidation (g·min⁻¹) during the HGI and LGI trials (mean±SEM).
Fig 6.3 Plasma glucose concentration (mmol/l⁻¹) during the HGI and LGI trials (mean ±SEM). * Significantly different from LGI, p<0.05.
Fig 6.4  Serum insulin concentration (mIU l⁻¹) during the HGI and LGI trials (mean ±SEM).* Significantly different from LGI, p<0.05.
Fig 6.5 Plasma FFA concentration (mmol/l⁻¹) during the HGI and LGI trials (mean±SEM).
Fig 6.6 Plasma glycerol concentration (umol l⁻¹) during the HGI and LGI trials (mean±SEM).
Fig 6.7  Blood lactate concentrations (mmol\textsuperscript{-1}l\textsuperscript{-1}) during the HGI and LGI trials (mean ±SEM). * Significantly different from LGI, p<0.01.
CHAPTER 7
THE INFLUENCE OF HIGH CARBOHYDRATE MEALS WITH DIFFERENT GLYCAEMIC INDICES ON SUBSTRATE UTILIZATION AND ENDURANCE CAPACITY AFTER A FOUR-HOUR RECOVERY IN MEN

7.1 Introduction
It is well known that the onset of fatigue during prolonged heavy exercise is associated with glycogen depletion in skeletal muscles. Therefore to recover quickly and restore exercise capacity it is essential that carbohydrate is consumed as soon as possible in order to successfully restore glycogen stores in muscle and in the liver. The nature of the carbohydrate consumed also appears to influence the rate and amount of glycogen resynthesised a day after prolonged heavy exercise (Burke et al. 1993). Ingesting high CHO meals consisting mainly of high glycaemic index (HGI) foods during the 24 hour recovery from prolonged heavy continuous exercise and prolonged high intensity exercise successfully restores exercise capacity (Fallowfield and Williams, 1993; Nicholas et al. 1997).

Consumption of carbohydrate immediately after exercise increases the rate of glycogen resynthesis during the several hours of recovery (Ivy et al. 1988a). Ivy and colleagues recommend a carbohydrate intake of approximately 1 to 1.2g·kg\textsuperscript{-1} CHO immediately after exercise and at 2 hour intervals thereafter or until the next high carbohydrate meal (Ivy, 1998b). This recommendation has been shown to improve exercise capacity, following a 4-hour recovery, to a significantly greater extent than when only water is consumed in this recovery period (Fallowfield et al. 1995; Wong
Paradoxically, ingesting larger amounts of carbohydrate in the recovery period does not necessarily lead to a greater endurance capacity during subsequent exercise. Fallowfield and colleagues reported that the ingestion of 3g·kg⁻¹ CHO immediately after 90 min of submaximal running and again 2 hour later had no greater benefit on endurance capacity at the end of a 4 hour recovery period than ingesting only 1g·kg⁻¹ CHO (Fallowfield and Williams, 1997). A similar response was also reported when the intake of carbohydrate was either 50g or ~167g CHO during a 4 h recovery period (Wong and Williams, 2000). Why the improvement in endurance capacity was not in proportion to the CHO intake during recovery is a question that remains to be answered. One possibility is that the carbohydrate provided was enough to depress fat oxidation but was insufficient to cover the deficit in substrate caused by the decreased contribution of fat to exercise metabolism. Therefore, the optimum strategy for recovery of performance during short-term recovery would be to consume foods that provide CHO but do not fully suppress fat oxidation during subsequent exercise.

Low glycaemic index carbohydrate (LGI) foods do not produce such a large increase in blood glucose nor in serum insulin concentrations as HGI foods (Wolever et al. 1991; Wolever and Bolognesi, 1996). Furthermore, the ingestion of a LGI pre-exercise meal results in a higher rate of fat oxidation and a lower rate of CHO metabolism during submaximal running than when the meal consisted of HGI foods (Wee et al. 1999).

Therefore, the aim of this study was to investigate the effects of consuming high CHO meals with different glycaemic indices during a 4-hour recovery from prolonged...
exercise on subsequent endurance running capacity.

7.2 Methods

Eight male recreational male runners (age: 26.8±1.5 yrs; height: 174.3±1.6 cm; body mass: 68.6±2.3 kg; \(\dot{VO}_2\)max: 59.9±1.5 ml\(\text{min}^{-1}\cdot\text{kg}^{-1}\); body mass index: 22.6±0.6 kg\(\text{m}^{-2}\)) volunteered to participate in this study. All subjects were fully informed about the possible risks before giving their written informed consent. Preliminary tests and main experimental trials were as described in Chapter 3. Briefly, each subject undertook two experimental trials. Each trial included two runs: (i) Run 1 (R1): subjects ran on a treadmill at a speed corresponding to 70% \(\dot{VO}_2\) max for 90 min or until fatigue after a standardized 5 min warm up at 60% \(\dot{VO}_2\) max. (ii) Run 2 (R2): after a 4 hour recovery (REC), subjects ran on a treadmill at 70% \(\dot{VO}_2\) max until volitional fatigue. During REC, subjects consumed either a high glycaemic index meal (HGI) or a low glycaemic index meal (LGI) as described in Table 3.1. The order of the trials was randomised. All trials were performed under similar experimental and environmental conditions. Ambient temperature and relative humidity were recorded every 30 min using a hydrometer (Zeal, England) during the main trials. Temperature was maintained between 15-18°C and relative humidity was between 45-58%. All subjects were instructed to refrain from heavy physical activities for two days before each main trial. Subjects were asked to record a two-day diet by a food weighing method before the first main trial and to consume exactly the same diet before the second main trial. All subjects were asked to abstain from alcohol, caffeine and tobacco consumption for 24 hours before each main trial.

The meal was finished within one hour after R1. Nude body mass was obtained before the test meal was consumed in order to calculate the amount of water for rehydration.
Thereafter, subjects consumed water that was equivalent in volume to 150% of body mass loss (g=ml) during R₁. During the recovery, subjects sat on a comfortable chair and avoided unnecessary physical activity.

Four hours later, subjects performed R₂ using the same protocol as previously described. During each run, subjects were supplied with 2ml·kg⁻¹ body mass of water every 30 min to minimise dehydration. Electric fans and wet sponges were also available for use ad libitum.

All data are presented as Mean±SEM. A two-way ANOVA (time and treatment) for repeated measures was use to analyse differences in the physiological and biochemical responses. The Tukey post-hoc test was applied to locate the differences between means when a significant difference was found. Student’s paired t-test was used for non-time dependent variables. Significance was set at the 0.05 level of confidence.

7.3 Results

Running Time and Objective Observations

All subjects completed the 90min run (R₁). There were no significant differences in running times to fatigue between HGI (70.5±4.4min) and LGI (75.1±5.6min) during R₂. Five subjects were observed to be pale and sweating profusely during the first 15 min of R₂ during the HGI trial (plasma glucose concentration: 3.53±0.05mmol·l⁻¹). Three of those five subjects claimed to have felt dizzy in the early stages of R₂ during the HGI trial.
Substrate Utilisation

There was no significant difference in substrate utilisation during R₁ nor during the recovery period. During R₂, the estimated rate of fat oxidation was significantly higher in the LGI trial than in the HGI trial ($F_{1,7} = p<0.05$)(Fig 7.1). Conversely, the estimated rates of CHO oxidation tended to be higher in the HGI trial than the LGI during R₂ (Fig 7.2).

Plasma Glucose and Serum Insulin Responses

Plasma glucose concentrations increased after ingestion of both test meals. There were significantly higher values in the HGI trial compared with the LGI trial at REC₁₂₀, REC₁₅₀ and REC₁₈₀ ($F_{1,7}=12.17$, $p<0.05$)(Fig 7.3). During R₂, plasma glucose concentrations tended to be lower in the HGI trial than the LGI trial. The incremental area under the curve (IAUC) for plasma glucose concentration during the postprandial period was significantly higher for HGI ($250±41$ mmol⁻¹·h⁻¹) trial than for LGI ($154±19$ mmol⁻¹·h⁻¹) ($p<0.05$).

The trend of the serum insulin responses was similar to plasma glucose responses during the postprandial period. Serum insulin concentrations were significantly higher in the HGI than LGI trials at REC₁₂₀, REC₁₅₀ and REC₁₈₀ ($F_{1,7}=23.55$, $p<0.05$)(Fig 7.4), but there were no significant differences during R₂. Serum insulin IAUC was significantly higher for the HGI trial ($9224±2323$ mIU⁻¹·h⁻¹) than for the LGI trial ($3409±910$ mIU⁻¹·h⁻¹) ($p<0.01$) during the postprandial period.

Plasma FFA, Glycerol and Triglycerides

There were no significant differences in plasma FFA concentrations between the two trials during the recovery period. However, plasma FFA concentrations were higher in
the LGI trial than in the HGI trial at 15, 30, 45 and 60 min during R2 \((F_{1,7}=7.05, p<0.05)\) (Fig 7.5).

As well as plasma FFA, plasma glycerol concentrations were also higher in the LGI trial compared with the HGI trial at 30, 45 and 60 min during R2 \((F_{1,7}=8.09, p<0.05)\) (Fig 7.6).

Plasma triglyceride concentrations were higher after 3h of recovery and throughout R2 in the LGI trial compared with the HGI trial \((F_{1,7}=12.15, p<0.05)\) (Fig 7.7).

**Blood lactate**

Blood lactate concentrations were significantly elevated after ingestion of the LGI meal at REC75, REC90 and REC120 during the recovery period \((F_{1,7}=11.05, p<0.05)\) (Fig 7.8). There were no significant differences between trials during R2.

\(\dot{V}O_2\), \(\dot{V}CO_2\) and RER

There were no significant differences in \(\dot{V}O_2\), \(\dot{V}CO_2\) and RER between the two trials (Table 7.1).

**Heart Rate and Rating of Perceive Exertion**

There were no significant differences between trials in heart rate or RPE through the experimental period (Table 7.2).

**Gut fullness and Thirst Scales**

After ingestion of the LGI meal, gut fullness was higher during the recovery period compared to that of HGI \((F_{1,7}=13.92, p<0.05)\) (Table 7.2). There were no significant
differences in thirst between the two trials during the experimental period (Table 7.2).

### 7.4 Discussion

The main finding of the present study was that there were no differences in endurance running capacity 4 hours after ingesting a recovery meal containing either low or high GI CHO foods. However, there was a higher rate of fat oxidation during R₂ in the LGI trial than during R₂ in the HGI trial.

Even though there was a higher rate of fat oxidation during R₂ in the LGI trial than during R₂ in the HGI trial, this was not reflected in a greater endurance capacity. Higher fat metabolism spares muscle glycogen during endurance exercise and therefore helps delay the onset of fatigue. For example, ingesting a high fat pre-exercise meal, followed by the administration of heparin, increases the availability of fatty acids for oxidation by muscle during subsequent exercise. Using this pre-exercise preparation resulted in longer run times to exhaustion than when the pre-exercise meal contained mostly carbohydrates (Pitsiladis et al. 1999). In the present study the LGI meal resulted in a significantly higher rate of fat oxidation during exercise but no greater endurance running capacity. One reason why the increase in fat oxidation rate is not reflected in an improvement in endurance capacity is that the amount of fat oxidised was not large enough to replace a significant proportion of the ongoing CHO metabolism. Indeed, several pre-exercise CHO feeding studies using high and low GI CHO foods have also reported similar increases in fat oxidation but without improvements in endurance exercise capacity (Thomas et al. 1994; Febbraio and Stewart, 1996; Wee et al. 1999).

The ingestion of CHO early in recovery from exercise increases the rate of muscle
glycogen resynthesis (Bergstrom and Hultman, 1967; Ivy et al. 1988; van Hall et al. 2000a). It is clear that an increase in insulin concentration is essential for the optimum replacement of muscle glycogen (Price et al. 1994; Burke et al. 1995). In the present study, the total postprandial insulin response, as reflected by the integrated areas under the response curve, was significantly higher in HGI than that of LGI trial. Although muscle glycogen was not measured in the current study, it is reasonable to suggest that there was a higher muscle glycogen resynthesis rate in the HGI trial compared to the LGI trial during recovery. However, this was not reflected in the run times in the post-recovery run to exhaustion. One possibility is that the higher pre-exercise muscle glycogen concentration may have resulted in a greater rate of glycogen degradation during R₂. Indirect support for this speculation is available from the calculated rates of CHO oxidation that were higher during R₂ in the HGI trial. If this was the case then the advantage of beginning the second run with higher muscle and liver glycogen concentrations may have been blunted because more CHO was used during exercise.

Furthermore, we observed that five of the eight subjects in the current study experienced hypoglycaemic symptoms in the early stage of R₂ during the HGI trial. This was confirmed when glucose analyses revealed lower plasma glucose concentrations for these subjects in the HGI trial but not during R₂ in the LGI trial. Pre-exercise feeding studies have also demonstrated low plasma glucose concentrations early in exercise when subjects were fed with HGI foods (Thomas et al. 1991; Wee et al. 1999). Hypoglycaemia is one of the contributory factors to fatigue during endurance exercise (Coyle et al. 1983; Coyle and Coggan, 1984; Okano et al. 1988; Rutherford, 1990). This might suggest that ingestion of HGI foods 3 hours before exercise, either pre-exercise or post-exercise, might not be beneficial for
maintaining stable plasma glucose concentrations during subsequent submaximal exercise in some people.

The post-exercise insulin concentrations were lower than the values we obtained following pre-exercise feeding of similar foods in an earlier study (Wee et al. 1999). This apparent increase in insulin sensitivity, that has also been reported by others (Hughes et al. 1993) may be explained by the mobilisation of the muscle glucose transporter proteins (GLUT 4) that play a central role in the translocation of glucose into muscle after exercise (Hughes et al. 1993; Ivy and Kuo, 1998; Hansen et al. 1998a). Nevertheless, it is reasonable to assume that the elevated post-exercise insulin concentrations found in the present study contributed to an increased glycogen resynthesis rate and depressed fat oxidation (Burke et al. 1995; Hansen et al. 1998a; Thorell et al. 1999).

We observed high glycaemic and insulinaemic responses to the LGI meal during the early postprandial period that is not found in studies using low GI single foods (Thomas et al. 1991; Thomas et al. 1994; Febbraio and Stewart, 1996; Wee et al. 1999). Glycaemic responses are affected by many factors, including total amount of CHO ingested, macronutrient interactions, and the presence of dietary fibre (Wolever, 1990; Wolever and Bolognesi, 1996). Several studies have reported that ingestion of CHO and protein mixtures induced higher glycaemia and insulinaemia than the ingestion of CHO alone (Zawadzki et al. 1992; Ivy et al. 2002). The meals in the current study not only contained CHO, but also contained protein, fat and milk, which have been reported as highly insulinaemic (Bjorck et al. 2000). Therefore, this might explain the high plasma glucose and serum insulin responses in the early stage of the postprandial period in the LGI trial. Nevertheless, the overall glycaemic responses to
the LGI meal were still lower than the responses after the HGI meal.

One of the interesting responses to the two meals during the postprandial period was the greater increase in blood lactate concentration following the LGI meal. Ingesting CHO per se has been shown to elevate blood lactate concentrations (Wee et al. 1999; Stannard et al. 2000). Although the mechanism is unclear, a high lactate concentration following the ingestion of a fructose solution has been observed in several studies (Decombaz et al. 1985; Fielding et al. 1987; Hargreaves et al. 1987). Indeed, in the present study, the LGI meal contained more fructose than the HGI meal and so the higher lactate concentration during the postprandial period may have been a consequence of a higher rate of fructose metabolism.

Reported sensations of gut fullness during postprandial period were greater during the LGI trial than in the HGI trial even though both test meals were isocaloric. Several other studies have also reported that the ingestion of LGI foods produced higher satiety (Kaplan and Greenwood, 2002; Brand-Miller et al. 2002) and this may have advantages under circumstances where the pre-exercise meal must be consumed well in advance of exercise.

In summary, there were no differences between endurance running capacity following the ingestion of high and low GI recovery meals even though there was a significantly higher rate of fat oxidation during exercise after the LGI meal. Based on the findings of this study we recommend the consumption of LGI meals during short-term recovery because (a) they provide the sensation of satiety for longer than the HGI meal, (b) they do not produce the symptoms of transient hypoglycaemia nor a significant decrease in blood glucose concentrations during the onset of subsequent
exercise, and (c) they result in higher rates of fat oxidation than after consuming HGI meals.
Table 7.1 Oxygen uptake (\(\dot{V}O_2\)), carbon dioxide expired (\(\dot{V}CO_2\)), and the respiratory exchange ratio (RER) during HGI and LGI trials; values are mean ± SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meal</th>
<th>Resting</th>
<th>(R_1)</th>
<th>Recovery Period</th>
<th>(R_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\dot{V}O_2) (l·min(^{-1}))</td>
<td>HGI</td>
<td>0.29±0.01</td>
<td>2.80±0.11</td>
<td>0.33±0.02</td>
<td>2.78±0.12</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.30±0.02</td>
<td>2.82±0.10</td>
<td>0.33±0.01</td>
<td>2.88±0.10</td>
</tr>
<tr>
<td>(\dot{V}CO_2) (l·min(^{-1}))</td>
<td>HGI</td>
<td>0.27±0.02</td>
<td>2.56±0.10</td>
<td>0.28±0.01</td>
<td>2.59±0.10</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.29±0.04</td>
<td>2.58±0.09</td>
<td>0.30±0.02</td>
<td>2.60±0.08</td>
</tr>
<tr>
<td>RER</td>
<td>HGI</td>
<td>0.88±0.04</td>
<td>0.91±0.01</td>
<td>0.86±0.03</td>
<td>0.93±0.01</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.87±0.06</td>
<td>0.91±0.02</td>
<td>0.87±0.04</td>
<td>0.91±0.01</td>
</tr>
</tbody>
</table>
Table 7.2 Heart rate (HR), rate of perceived exertion (RPE), gut fullness (GF), and thirst scale (TS) during HGI and LGI trials; values are mean ± SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meal</th>
<th>Resting</th>
<th>Recovery Period (min)</th>
<th>R2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>75</td>
</tr>
<tr>
<td>HR (beats·min⁻¹)</td>
<td>HGI</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPE</td>
<td>HGI</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GF</td>
<td>HGI</td>
<td>9±1</td>
<td>8±5</td>
<td>11±1*</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>9±1</td>
<td>8±5</td>
<td>15±1</td>
</tr>
<tr>
<td>TS</td>
<td>HGI</td>
<td>11±0</td>
<td>11±0</td>
<td>10±1</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>10±1</td>
<td>11±1</td>
<td>8±1</td>
</tr>
</tbody>
</table>

* Significantly different from LGI, p<0.05.
Fig 7.1 The rate of fat oxidation (g min\(^{-1}\)) during the HGI and LGI trials (mean±SEM).

* Significantly different from HGI, \(p<0.05\).
Fig 7.2 The rate of CHO oxidation (g min\(^{-1}\)) during the HGI and LGI trials (mean±SEM).
Fig 7.3 Plasma glucose concentration (mmol/l) during the HGI and LGI trials (mean ±SEM). * Significantly different from LGI, p<0.05.
Fig 7.4  Serum insulin concentration (mIU l⁻¹) during the HGI and LGI trials (mean± SEM). * Significantly different from LGI, p<0.05.
Fig 7.5 Plasma FFA concentration (mmol l⁻¹) during the HGI and LGI trials (mean±SEM). * Significantly different from HGI, p<0.05.
Fig 7.6  Plasma glycerol concentration (umol\,l$^{-1}$) during the HGI and LGI trials (mean±SEM).* Significantly different from HGI, p<0.05.
Fig 7.7 Plasma triglyceride concentration (mmol\(L^{-1}\)) during the HGI and LGI trials (mean ± SEM). * Significantly different from HGI, \(p<0.05\).
Fig 7.8  Blood lactate concentrations (mmol/l⁻¹) during the HGI and LGI trials (mean ±SEM). * Significantly different from HGI, p<0.05.
CHAPTER 8
GENERAL DISCUSSION

The aim of this final chapter is try to integrate the observations from all studies in this thesis in order to develop an overall view of the influences of CHO meals with different GI values on postprandial and subsequent exercise metabolism before and after exercise.

Postprandial glycaemic and insulinaemic responses are one of the most important factors influencing subsequent exercise metabolism. Many studies have examined the effects of different types of CHO ingestion on pre- and post-exercise metabolism. However, these studies focused on single foods or single nutrient effects on exercise metabolism and performance (see Chapter 2). Moreover, most of pre-exercise feeding studies were designed to ingest test foods within an hour before exercise. Normally, athletes are recommended to consume a moderate size meal about 3 hours before undertaking exercise or competition (Burke et al. 1998). Only a few studies have examined the effects of GI foods hours before exercise (Burke et al. 1998; Wee et al. 1999; Wong 2003). However, the test foods used in these studies were not similar to a real meal, e.g. breakfast. Thus, the results of these studies seemed less applicable to the real world of daily training and competition.

Many athletes often undertake two training sessions in a day, one session in the morning after an overnight fast and the other in the afternoon, with a short break of about 4 hours between. The choice of CHO ingested during short-term recovery is a critical factor in restoring fuel stores for the next training session. Several studies have examined the effects of CHO-electrolyte solutions or CHO mixed with other nutrients
on short-term recovery (see Chapter 2). Again, consuming only a sports drink or single foods between two bouts of trainings or competitions is not a common practise. Therefore, this thesis examined the effects of a mixed high CHO meal, rather than single foods, on substrate utilisation and exercise performance.

8.1 The effects of pre-exercise mixed GI carbohydrate meals on substrate metabolism and endurance performance

Pre-exercise CHO ingestion increases muscle and liver glycogen stores which may benefit subsequent endurance performance by increasing CHO availability during exercise (Bergstrom et al. 1967; Astrand, 1967; Karlsson and Saltin, 1971; Nilsson, 1973). Subsequent performance studies have shown that ingestion of CHO prior to exercise generally improves subsequent endurance exercise performance when compared to the ingestion of a placebo (Sherman et al. 1991; Chryssanthopoulos and Williams, 1997; Schabort et al. 1999; Chryssanthopoulos et al. 2002). However, CHO ingestion tends to increases in CHO oxidation and decreases in fat oxidation i.e. increases muscle glycogen usage (Hickson et al. 1977; Costill et al. 1977) that may impair endurance performance. Although there is little evidence to support that the consumption of CHO before exercise does impair subsequent exercise capacity, the depression of fat oxidation in relation does not result in the optimum fuel mixture. The ideal metabolic condition would be one in which there is a maximum amount of fat oxidation with an economic contribution from the limited carbohydrate stores. Therefore, a nutritional strategy for supplying CHO without severely suppressing fat oxidation is an important challenge to exercise nutrition.

8.1.1 Substrate metabolism and endurance performance

Ingestion of low GI meal 3 hours before exercise induced lower postprandial
insulinaemic responses leading to a higher fat oxidation during subsequent exercise when compared to that of a high GI meal in studies 1 and 2 (Chapter 4 and 5). A number of studies have reported lower insulinaemic responses and higher fat oxidation during exercise after ingestion of low GI CHO (Thomas et al. 1991; Thomas et al. 1994; Sparks et al. 1998; Wee et al. 1999; DeMarco et al. 1999) but some found no difference in substrate utilisation (Febbraio and Stewart, 1996; Burke et al. 1998).

An increase in fat oxidation during prolonged exercise has the potential to spare the limited muscle glycogen and so delay the onset of fatigue (Hickson et al. 1977; Costill et al. 1977). Study 2 (Chapter 5) demonstrated a better endurance capacity in the low GI trial than that of the high GI trial, which supported the above hypothesis. Plasma FFA concentrations (Fig 5.3) and the estimated rate of fat oxidation (Fig 5.6) were both higher in the low GI trial compared to that of the high GI trial during exercise. The higher free fatty acid availability may depress glucose oxidation, i.e. glycogenolysis (Hickson et al. 1977; Costill et al. 1977). The “muscle glycogen sparing effect” might be introduced by ingestion of low GI meals which promoted a higher rate of fat oxidation during subsequent submaximal exercise.

8.1.2 Glycaemia response during exercise

In studies 1 and 2, hypoglycaemic symptoms appeared in some subjects in the early stage of exercise, 3 hour after the HGI meal. Some subjects complained of having extreme feeling of hunger and were observed to sweat profoundly in the early stage of exercise during high GI trials but not during low GI trials. Several pre-exercise feeding studies have also reported a fall blood glucose concentrations early in exercise when subjects consumed high GI CHO food before exercise but this ‘hypoglycaemic’
response did not appear to occur following the consumption of low GI CHO foods (Decombaz et al. 1985; Hargreaves et al. 1987; Fielding et al. 1987; Guezennec et al. 1989; Thomas et al. 1991; Guezennec et al. 1993; Sparks et al. 1998; Wee et al. 1999). Postprandial hyperinsulinaemia might play an important role during subsequent exercise. It is observed that the serum insulin concentrations have returned to fasting values in most low GI trials prior to exercise. In contrast, during the high GI trials the insulin concentrations were still higher than the fasting values at the end of postprandial period. These high insulin concentrations are probably responsible for the low plasma glucose concentrations in the early stage of exercise (Decombaz et al. 1985; Hargreaves et al. 1987; Fielding et al. 1987; Guezennec et al. 1989; Thomas et al. 1991; Guezennec et al. 1993; Sparks et al. 1998; Wee et al. 1999) which might be disadvantageous to subsequent exercise performance for those individuals who become hypoglycaemic.

8.2 The effect of post-exercise mixed GI carbohydrate meals on substrate metabolism and subsequent exercise performance

Studies 3 and 4 examined the relative merits of high and low GI CHO meals, ingested during a 4 hour recovery, on metabolism and endurance capacity during subsequent exercise (Chapter 6 and 7). Despite higher fat oxidation induced in the low GI trials during the second bout of endurance exercise, there were no differences in endurance capacity (Chapter 7). Although the underlining mechanism is still unclear, a similar result was obtained when subjects consumed either glucose (high GI) or sucrose (medium GI) during 4 hours recovery (Casey et al. 2000).

Sports nutritionists suggested that after glycogen depleting exercise, muscle glycogen could be fully restored by ingesting 8-10g CHO\textsuperscript{kg\textsuperscript{-1}} body mass within 24 hours
(Burke et al. 1993; 1967; Kochan et al 1979; Keizer et al 1987; Casey et al 1995; Burke, 1997). However, muscle glycogen stores cannot be replaced during short-term recovery. A number of studies reported that ingestion of CHO drinks during 4 hour recovery enhances the subsequent exercise performance when compared to placebo (Fallowfield et al. 1995; Wong et al. 1998; Wong et al. 2000; Casey et al. 2000). It is well known that muscle glycogen resynthesis requires sufficient CHO supplement during the recovery period, especially in the early stage of recovery. During the insulin-dependent phase, the rate of muscle glycogen resynthesis is directly related to the insulin response to the supplement (Zawadzki et al. 1992; Price et al 1994). A 24 hour recovery study (Burke et al. 1993) reported a higher glycogen resynthesis when subjects consumed high GI CHO compared to low GI CHO. In study 3 and 4, ingestion of high GI meal induced higher insulinaemic and glycaemic responses than the low GI meal. Although it is speculated that the high GI meal resynthesis more muscle glycogen than low GI meal during the 4 hour recovery period, a higher rate of CHO oxidation might eliminate this advantage during subsequent endurance capacity.

8.3 An overview of pre-exercise and post-exercise GI meals ingestion on metabolism

8.3.1 Glycaemic and insulinaemic response

Several studies have questioned the validity of the estimation of GI values of mixed meals (Coulston et al. 1984a; Coulston et al. 1984b; Hollenbeck et al. 1988). To assess the glycaemic responses of the CHO meals used in the studies reported in this thesis the ratio of the ‘incremental area under the curves’ (IAUC) for blood glucose for the high and low GI meals were compared. Thus the glucose IAUC ratio (HGI / LGI) between two test meals during pre-exercise feeding was 2.2:1.0 (studies 1 and 2) and 1.9:1.0 respectively during post-exercise feeding (studies 3 and 4). The overall
average ratio was 2.1:1.0 in the real measurement that was nearly identical to our predicted GI meal’s ratio 2.1:1.0 (HGI: 77 / LGI: 37). Although we did not perform GI tests for the test meals, the overall average ratio seems consistent with the Wolever and Jenkin’s mixed GI meal calculation method (Wolever and Jenkins, 1986), even though subjects consumed a large amount of CHO.

In the pre-exercise feeding studies (studies 1 and 2) the insulinaemic responses were higher than those found in the post-exercise feeding studies even though the meals were of the same composition (studies 3 and 4) (Table 8.1). When muscle glycogen is severely reduced or depleted following prolonged heavy exercise then glycogen resynthesis becomes a priority during the recovery period (Price et al. 1994). In the normal resting state, it takes insulin to activate the translocation of the glucose transporter protein (GLUT-4) to the cell membrane for transporting glucose into muscle cells. Immediately after exercise there is an increase in the migration of GLUT 4 transporter proteins to the plasma membrane of muscle that increases the rate of glucose uptake. This occurs in the early part of recovery and the glucose uptake is ‘insulin independent’. Later in recovery the role of insulin becomes more important in either maintaining the GLUT 4 transporters active or indeed recruiting more transporters from a separate pool (Ivy and Kuo, 1998; Kuo et al. 1999; Thorell et al. 1999). In addition, exercise training studies also report an improvement in insulin sensitivity presumably as a result of an increased complement of glucose transporters (Frost et al. 1998; Wojtaszewski et al. 2000). Collectively these changes within previously exercised muscle may explain the reduced insulin response to a standard CHO meal.
Table 8.1: Plasma glucose and serum insulin concentration expressed as the incremental area under the curve (IAUC) in four studies

<table>
<thead>
<tr>
<th>Trials</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3*</th>
<th>Study 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGI</td>
<td>Glucose IAUC (mmol/l/3h)</td>
<td>108.7</td>
<td>134.2</td>
<td>367.6</td>
</tr>
<tr>
<td></td>
<td>Insulin IAUC (mIU/l/3h)</td>
<td>12146</td>
<td>13298</td>
<td>6384</td>
</tr>
<tr>
<td>LGI</td>
<td>Glucose IAUC (mmol/l/3h)</td>
<td>48.9</td>
<td>57.5</td>
<td>170.9</td>
</tr>
<tr>
<td></td>
<td>Insulin IAUC (mIU/l/3h)</td>
<td>8654</td>
<td>7445</td>
<td>3984</td>
</tr>
</tbody>
</table>

HGI: High glycaemic index trial
LGI: Low glycaemic index trial
*Study 3 IAUC values were calculated over 3.5 hours duration.

8.3.2 Postprandial substrate utilisation

In all studies, there were no differences in substrate utilisation between two test meals during the postprandial period. The results were different from those of Wee et al (1999), who found significantly higher fat oxidation during postprandial period. The inconsistent results might be due to the nature of the test meals used which has been described in Chapter 5. All studies indicated that insulin concentrations during LGI trials were as high as those during high GI trials 15 min into the postprandial period (Fig 4.1; Fig 5.1; Fig 6.3; Fig 7.3). This phenomenon was not observed in previous studies (Thomas et al. 1991; Thomas et al. 1994; Febbraio and Stewart, 1996; Wee et al. 1999; DeMarco et al. 1999). Glycaemic index and insulin index (II) are highly correlated except for dairy products (Wolever and Bolognesi, 1996). Most of dairy products are described as having low GI values, however their II is higher than expected. Several studies have demonstrated that ingestion of protein and CHO mixture can induce higher insulin response than ingestion of CHO alone (Zawadzki et
In addition to the effect of dairy products, a recent study reported that the ingestion of a cereal, Kellogs All Bran, which was part of the low GI meal used in all the studies reported in this thesis, induced unexpectedly high insulin responses (Schenk et al. 2003). The acute insulinaemia in the early stage of the postprandial period might result in the same amount of suppression fat oxidation as the ingestion of a high GI CHO food. This is an interesting result that requires further investigation because even though All Bran was a component of the meals used in the studies reported in this thesis, the overall GI of the low GI meals were close to predicted values.

8.3.3 Glycaemic index meal and Satiety

Ingestion of low GI CHO foods have been reported to produce higher ratings of satiety when compared to high GI CHO foods (Holt and Miller, 1994; Brand-Miller et al. 2002; Ball et al. 2003; Roberts, 2003). The sensation of gut fullness was reported to be greater during the postprandial period in the LGI trial than during the HGI trial in studies 1 and 2 (Chapter 4 and 5) even though both test meals were isocaloric. From a public health point of view this may help those who want to lose weight because by choosing low GI CHO foods as CHO sources they may maintain the sense of satiety for longer period of time (Holt and Miller, 1994; Brand-Miller et al. 2002; Ball et al. 2003; Roberts, 2003).

8.4 Conclusion

The rate of fat oxidation during exercise is affected by postprandial insulinaemic and glycaemic responses. Therefore, consumption of 2g·kg⁻¹ body mass of LGI CHO meals 3 hour before exercise or during 4 hour recovery provides the sensation of
Chapter 8

satiety for longer than after the high GI meal, and does not produce the symptoms of hypoglycaemia nor a significant decrease in blood glucose concentrations during the onset of subsequent exercise. Furthermore there is a higher rate of fat oxidation following the consumption of a low GI CHO meal than after a high GI meal. In addition, the ingestion of a low GI CHO meal 3 hour before exercise enhances subsequent endurance capacity during running. However, it does not result in an improvement in endurance running capacity when consumed during recovery following prolonged exercise even though the rate of fat oxidation is greater than following the consumption of a high GI CHO meal.

8.5 Future studies

A few studies recently demonstrated relationships between muscle glycogen concentrations and muscle derived interleukin-6 (IL-6) (Steensberg et al. 2001; Keller et al. 2001; Steensberg et al. 2002; Helge et al. 2003; Febbraio et al. 2003; Keller et al. 2003; Steensberg et al. 2003). We measured plasma IL-6 concentrations using an ELISA method in study 2 (Chapter 4). As can be seen in Table 8.2, the plasma IL-6 concentrations increased following exercise, although there were no differences between HGI and LGI trials during the exercise period. Interleukin-6 concentration has recently received attention because of its effect on glucose and fat metabolism, especially within the muscle (Steensberg et al. 2001; Keller et al. 2001; Steensberg et al. 2002; Helge et al. 2003; Febbraio et al. 2003; Keller et al. 2003; Steensberg et al. 2003; van Hall et al. 2003). It is hypothesized that an increased IL-6 concentration is related to glycogen depletion and up-regulated fat metabolism (Pedersen et al. 2003; Febbraio and Pedersen, 2002). Nevertheless, these studies compared the fed and the fasted state which was different from comparison of two feed states in the present study (study 2). Indeed, it was observed that the peak IL-6 concentrations at fatigue
reached ~8ng\textsuperscript{1}\textsuperscript{l}, and are similar to values reported at fatigue by other authors (Steensberg et al. 2001; Keller et al. 2001; Febbraio et al. 2003; Keller et al. 2003). The current study did not measure muscle glycogen content and so it is difficult to link changes in IL-6 to changes in muscle glycogen concentrations. However, there is significant rise in IL-6 concentrations at metabolic fatigue. Therefore, it is possible that IL-6 concentration may be an indicator of muscle glycogen concentrations (Febbraio and Pedersen, 2002; Pedersen et al. 2003).

Table 8.2 Interlukin-6 (IL-6) concentrations during HGI and LGI trials in study 2; values are mean±SEM.

<table>
<thead>
<tr>
<th>Time</th>
<th>IL-6 HGI</th>
<th>IL-6 LGI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fast</td>
<td>Pre-ex</td>
</tr>
<tr>
<td>0.17±0.11</td>
<td>0.49±0.22</td>
<td>1.08±0.28</td>
</tr>
<tr>
<td>0.13±0.05</td>
<td>0.56±0.14</td>
<td>1.08±0.32</td>
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Am J Clin Nutr 40, 965-70.


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Febbraio, M.A., Chiu, A., Angus, D.J., Arkinstall, M.J. and Hawley, J.A. (2000a)


References

882-8.


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RESEARCH PROPOSAL FOR HUMAN BIOLOGICAL OR PSYCHOLOGICAL AND SOCIOLOGICAL INVESTIGATIONS

1. Project Title
The effect of pre-exercise high carbohydrate meals with different glycaemic indices on running endurance capacity in men.

2. Brief lay summary of the proposal for the benefit of non-expert members of the Committee
It has been well documented that a high carbohydrate (CHO) meal can increase CHO oxidation. This is due to the secretion of insulin that suppresses fat oxidation and so shifts metabolism towards CHO. The nature of CHO rich food can be classified by its glycaemic response during the postprandial period. Jenkins and co-workers introduced the glycaemic index (GI) concept as a method of classifying CHO rich foods in the 1980s. They found that low GI food induced lower blood glucose and insulin responses during the postprandial period. Our previous study demonstrated that ingestion of a low GI meal elicited higher fat metabolism during subsequent submaximal exercise compared to a high GI meal. This may be improve endurance capacity.

Therefore, the aim of this study is to compare the influence of high CHO meals with different glycaemic indices on running endurance capacity in men.

3. Details of responsible investigator (supervisor in case of student projects)

<table>
<thead>
<tr>
<th>Title</th>
<th>Professor</th>
<th>Surname</th>
<th>Williams</th>
<th>Forename</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>School of Sport and Exercise Sciences</td>
<td><a href="mailto:C.Williams@lboro.ac.uk">C.Williams@lboro.ac.uk</a></td>
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<td>Personal experience of proposed procedures and/or methodologies.</td>
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<td>Professor Clyde Williams has over 30 years experience of conducting similar studies and he will perform the cannulations on the participants.</td>
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4. Names, experience, department and email addresses of additional investigators
Mr. Ching-Lin Wu, PhD student, School of Sport and Exercise Sciences, has been trained to undertake the procedures described and has conducted other similar studies during the past three years.C.L.Wu1@lboro.ac.uk

Mrs. Maria Nute, Research Assistant, School of Sport and Exercise Science, has over 20 years experience of conducting similar studies.M.L.Nute@lboro.ac.uk
5. **Proposed start and finish date and duration of project**

| Start date | 25.11.2002 | Finish date | 25.02.2003 | Duration | 3 months |

6. **Location(s) of project**

This work will be carried out in the Sports Science Research Laboratories in the School of Sport and Exercise Sciences, Loughborough University.

7. **Reasons for undertaking the study (eg contract, student research)**

This study will be undertaken as a part of a PhD thesis.

8. **Do any of the investigators stand to gain from a particular conclusion of the research project?**

No.

9. **Is the project being sponsored?**

Yes ☐ No ☑

If yes, please state source of funds including contact name and address.

10. **Aims and objectives of project**

It has been well documented that a high carbohydrate (CHO) meal can increase CHO oxidation. This is due to the secretion of insulin that suppresses fat oxidation and so shifts metabolism towards CHO. The nature of CHO rich food can be classified by its glycaemic response during the postprandial period. Jenkins and co-workers introduced the glycaemic index (GI) concept as a method of classifying CHO rich foods in the 1980's. They found that low GI food induced lower blood glucose and insulin responses during the postprandial period. Our previous study demonstrated that ingestion of low GI meal elicited higher fat metabolism during subsequent submaximal exercise compared to high GI meal. This may help increase endurance capacity.

Therefore, the aim of this study is to compare the influence of high CHO meals with different glycaemic indices on running endurance capacity in men.

11. **Brief outline of project**

A counterbalance design will be used in this study, and the order of the trials will be randomised. Participants will be asked to complete the preliminary tests in the week preceding the first main trial and then to participate in 2 main trials.

A weighed food dietary analysis will be conducted over the 2 days preceding the first trial and participants will be asked to adhere to this diet during the 2 days preceding the other trial.

The participants will be asked to undertake two experimental trials at least one week apart. Each participant will consume either a high GI CHO meal (HGI) or a low GI CHO meal (LGI) on each occasion. After a 3 hour postprandial period, participants will be asked to run on a motorized treadmill at 70%VO\textsubscript{2max} to exhaustion.
A) STUDY DESIGN

A counterbalance design will be applied to this study, and the order of the trials will be randomised. Participants will be asked to complete the preliminary test, detailed below, in the week preceding the first main trial and then to participate in two main trials.

A weighed food dietary analysis will be conducted over the 2 days preceding the first trial and participants will be asked to adhere to this diet during the 2 days preceding the other trial.

B) MEASUREMENTS TO BE TAKEN

(1) Familiarization: a practice session for the participants to become familiar with treadmill running.
(2) VO_{2\text{-Speed}} test: a 16 min submaximal running test during which expired air will be collected using the Douglas bag method (Generic Protocol).
(3) VO_{2\text{max}} test: an incremental test for the determination of VO_{2\text{max}} (Generic Protocol).
(4) 45 min run: a 45 min practice run at 70% VO_{2\text{max}} for familiarizing participants with all procedures used in the experimental trials.
(5) Two experimental trials:
   The participants will be asked to undertake two experimental trials at least one week apart. After a 3 hour postprandial period, participants will be ask to run at 70% VO_{2\text{max}} to exhaustion. In each trial, participants will consume either HGI or LGI.

Expired gas samples will be collected over a one minute period every 15 min and at exhaustion during the treadmill run, and every 30 min of the 3 hour postprandial period using the Douglas bag method.

A short-range telemeter will be attached to the participant to monitor heart rates.

Ten ml blood samples will be taken before the meal, then at 15 min, 30 min, 60 min, 90 min, 120 min, 180 min post-meal, and at 15 min, 30 min, 45 min, 60 min and at fatigue during the run by means of an indwelling cannula. This will be inserted into one of the forearm veins and will be kept patent by frequent flushing with sterile saline. The total volume of blood be taken from participant is 130 ml per trial. The cannula will be inserted while the participant is lying on an examination couch.

12. Please indicate whether the proposed study:

Involves taking bodily samples

Involves procedures which are physically invasive

Is designed to be challenging (physically or psychologically in any way)

Involves dietary manipulation or supplementation

Prescribes intake of compounds additional to daily diet

Involves procedures which may cause embarrassment to participants

Involves collection of personal and/or potentially sensitive data

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<th>Yes</th>
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If Yes - please give specific details of the procedures to be used and arrangements to deal with adverse effects.

An indwelling cannula will be inserted into one of the forearm veins, which will be kept patent by frequent flushing with sterile saline. The cannula will be inserted while the participant is lying on an examination couch. Blood sampling via the cannula may cause minor bruising and carries a small risk of air or plastic embolism, as is usual in such procedure, but good practice minimizes the risk.

The test meals, either HGI or LGI, are designed to be similar to the daily breakfast. The HGI meal consists of corn flakes, skimmed milk, white bread, jam, a sports drink and water. The LGI meal consists of all bran, skimmed milk, peaches, apples, and apple juice. After trials are completed, participants will be fed before they leave the laboratory. This study requires participants to exercise to volitional fatigue. The discomfort is, therefore, by definition tolerable and when it becomes intolerable the participant will simply stop exercising. The participant can signal his wishes to the investigator, or stop the treadmill by striking the emergency button located within easy reach. The risks of injury are minimal, as it is a well-controlled procedure with the investigator standing alongside the participant throughout the test.

13. Participant Information

Details of participants (gender, age, special interests etc)

Eight healthy male participants will be recruited from the staff and student population of Loughborough University, and running clubs, to participate in this investigation.

Number of participants to be recruited: 8

How will participants be selected? Please outline inclusion/exclusion criteria to be used.

Healthy volunteers will be required to complete a questionnaire on their medical history. Individuals with diabetes mellitus will not be accepted for the study, neither will individuals who are on medication for any other medical condition.

How will participants be recruited and approached?

Recruitment will be through advertisements around the University campus and web site.

Please state demand on participants' time.

The standardization of diet and training for 48 hours preceding each trial will require the participants to keep a food diary. Each of the three preliminary tests will last approximately 1 hour and will be conducted over a 7-day period. The two main trials will each last approximately 5 hours and will be conducted as soon as possible after the preliminary tests, allowing a sufficient recovery period (7-10 days) between trials.

14. Control Participants

Will control participants be used? Yes ☐ No ☒

If Yes, please answer the following:

Number of control participants to be recruited:

How will control participants be selected? Please outline inclusion/exclusion criteria to be used.
How will control participants be recruited and approached?

Please state demand on control participants' time.

15. **Procedures for chaperoning and supervision of participants during the investigation**

Participants will weigh themselves in the nude in privacy, and report the weights to the investigators waiting outside the weighing room.

16. **Possible risks, discomforts and/or distress to participants**

All procedures will be carried out in accordance with the Code of Practice for Workers having Contact with Body Fluids. The determination of VO$_{2\text{max}}$ will cause breathlessness and temporary fatigue. Any vigorous exercise results in an increase in the risk of cardiovascular emergency above that present at rest. This risk is very small for individuals not exhibiting risk factors for coronary heart disease. This study requires participants to exercise to volitional fatigue. The discomfort is, therefore, by definition tolerable and when it becomes intolerable the participant will simply stop exercising. The participant can signal his wishes to the investigator, or stop the treadmill by striking the emergency button located within easy reach. The risks of injury are minimal, as it is a well-controlled procedure with the investigator standing alongside the participant throughout the test. The investigators are, at all times, vigilant in their observations of participants performing under the prescribed experimental conditions, and are ready to end the test should the participant report, or even appear, unduly stressed.

The cannula will be inserted under local anaesthesia (Lignocaine), which reduces any discomfort associated with the procedure. Blood sampling via the cannula may cause minor bruising and carries a small risk of air or plastic embolism, as is usual in such procedure, but good practice minimizes the risk.

17. **Details of any payments to be made to the participants**

None.

18. **Is written consent to be obtained from participants?**

If yes, please attach a copy of the consent form to be used.

If no, please justify.

19. **Will any of the participants be from one of the following vulnerable groups?**

<table>
<thead>
<tr>
<th>Group</th>
<th>Yes</th>
<th>No</th>
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<td>Children under 18 years of age</td>
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<td>People over 65 years of age</td>
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<td>People with mental illness</td>
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Appendix A

Prisoners/other detained persons
Yes [ ] No [ ]

Other vulnerable groups
Yes [ ] No [ ]

If you have selected yes to any of the above, please answer the following questions:

a) what special arrangements have been made to deal with the issues of consent?

b) have investigators obtained necessary police registration/clearance? (please provide details or indicate the reasons why this is not applicable to your study)

20. How will participants be informed of their right to withdraw from the study?
The right to withdraw from the study statement is stated on the informed consent form.

21. Will the investigation include the use of any of the following?

Audio/video recording
Yes [ ] No [ ]

Observation of participants
Yes [ ] No [ ]

If yes to either, please provide detail of how the recording will be stored, when the recordings will be destroyed and how confidentiality of data will be ensured?

22. What steps will be taken to safeguard anonymity of participants/confidentiality of personal data?
Data storage will adhere to the Data Protection Act so no participant’s confidentiality will be breached.

23. Will collection and storage of data comply with the Data Protection Act?
Yes [ ]

24. INSURANCE COVER:
It is the responsibility of investigators to ensure that there is appropriate insurance cover for the procedure/technique.

The University maintains in force a Public Liability Policy, which indemnifies it against its legal liability for accidental injury to persons (other than its employees) and for accidental damage to the property of others. Any unavoidable injury or damage therefore falls outside the scope of the policy.

Will any part of the investigation result in unavoidable injury or damage to participants or property?
Yes [ ] No [ ]

If yes, please detail the alternative insurance cover arrangements and attach supporting documentation to this form.
The University Insurance relates to claims arising out of all **normal** activities of the University, but Insurers require to be notified of anything of an unusual nature.

Is the investigation classed as **normal** activity? Yes [ ] No [ ]

If no, please check with the University Insurers that the policy will cover the activity. If the activity falls outside the scope of the policy, please detail alternative insurance cover arrangements and attach supporting documentation to this form.

25. **Declaration**

I have read the University's Code of Practice on Investigations on Human Participants and have completed this application. I confirm that the above named investigation complies with published codes of conduct, ethical principles and guidelines of professional bodies associated with my research discipline.

I agree to provide the Ethical Advisory Committee with appropriate feedback upon completion of my investigation.

**Signature of applicant:** .................................................................

**Signature of Head of Department:** ..................................................

**Date** ...........................................................................................

**PLEASE ENSURE THAT YOU HAVE ATTACHED COPIES OF THE FOLLOWING DOCUMENTS TO YOUR SUBMISSION.**

- Participant Information Sheet
- Informed Consent Form
- Health Screen Questionnaire
- Advertisement/Recruitment material*
- Checklist for Psychological and Sociological Investigations* (found at [http://www.lboro.ac.uk/admin/central_admin/policy/ethical/form.html](http://www.lboro.ac.uk/admin/central_admin/policy/ethical/form.html))
- Evidence of consent from other Committees*

* where relevant.
Appendix B

HEALTH SCREEN FOR STUDY VOLUNTEERS  

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, do you have any health problem for which you are:
   (a) on medication, prescribed or otherwise ................. Yes ☐ No ☐
   (b) attending your general practitioner ......................... Yes ☐ No ☐
   (c) on a hospital waiting list ...................................... Yes ☐ No ☐

2. In the past two years, have you had any illness which require you to:
   (a) consult your GP ...................................................... Yes ☐ No ☐
   (b) attend a hospital outpatient department ................. Yes ☐ No ☐
   (c) be admitted to hospital ......................................... Yes ☐ No ☐

3. Have you ever had any of the following:
   (a) Convulsions/epilepsy .............................................. Yes ☐ No ☐
   (b) Asthma .................................................................... Yes ☐ No ☐
   (c) Eczema .................................................................... Yes ☐ No ☐
   (d) Diabetes ................................................................. Yes ☐ No ☐
   (e) A blood disorder .................................................... Yes ☐ No ☐
   (f) Head injury ............................................................. Yes ☐ No ☐
   (g) Digestive problems ................................................ Yes ☐ No ☐
   (h) Heart problems ...................................................... Yes ☐ No ☐
   (i) Problems with bones or joints ................................. Yes ☐ No ☐
   (j) Disturbance of balance/coordination ..................... Yes ☐ No ☐
   (k) Numbness in hands or feet ..................................... Yes ☐ No ☐
   (l) Disturbance of vision ............................................. Yes ☐ No ☐
   (m) Ear / hearing problems .......................................... Yes ☐ No ☐
   (n) Thyroid problems ................................................ Yes ☐ No ☐
   (o) Kidney or liver problems ....................................... Yes ☐ No ☐
   (p) Allergy to nuts ..................................................... Yes ☐ No ☐

4. Has any, otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? .......... Yes ☐ No ☐

If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.) ..............................................................

Thank you for your cooperation!
Appendix C

Physical Activity Questionnaire

The following questions are designed to give us an indication of your current level of physical activity.

Name: ___________________ Date: ___/___/___

Do you practice ENDURANCE TRAINING?  YES ☐ NO ☐

If Yes, how many days each week do you usually train? ___________________

How many minutes does each session last? ___________________

What is your weekly mileage? ___________________

Do you practice the following training?

Weight training ☐ Interval training ☐ Skills training ☐

If Yes, how many days each week do you usually train? ___________________

How many minutes does each session last? ___________________
Appendix D

BLOOD LACATE ASSAY

Fluorometric method base upon Maughan (Maughan, 1982).

Principle:

\[ \text{Lactate} + \text{NAD}^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH} + \text{H}^+ \]

\[ \text{Pyruvate} + \text{Hydrazine} \rightarrow \text{Pyruvate hydrazone} \]

LDH = Lactate dehydrogenase

\( \text{NAD}^+ \) is converted to NADH in the amount proportional to amount of lactate pH=9.0 and the removal of pyruvate by hydrazine drive the reaction forward.

Reagent solutions:

1. **1.1M Hydrazine buffer, pH=9.0**

   One litre buffer contains:
   - 46.167 ml of hydrazine hydrate
   - 20.77g of hydrazinium sulphate
   - 0.37224g of EDTA

   Stable at room temperature.

2. **Lactate diluent**

   0.07M HCl solution

3. **Reagent mixture (make up at the start of assay)**

   2.0mg NAD
   10 μl LDH
   1 ml Hydrazine buffer

   \( \text{NAD}^+ \): Free acid, grade II, ~98%, MW=663.4, Boehringer Mannheim.
   \( \text{LDH} \): 5500U/ml, Boehringer Mannheim.
4. Standards

Made from 1 M L-Lactate solution (Boehringer Mannheim). A 10 mM standard is made and diluted with 2.5% perchloric acid (PCA) to make 0.5, 1, 2, 4, 6, 8 mM standards.

5. Samples collection

A 20 µl of whole blood sample by deproteinised by adding into 200 µl 2.5% PCA already dispensed in an eppendorf tube. The sample is thoroughly mixed and then centrifuged for 3 min at 13000 rpm before store at -20°C.

Procedures

1. Remove samples and standards form the freezer and allow to thaw at room temperature for at least one hour.

2. Mix samples thoroughly using Whirlimix, and centrifuge for 3 min at 13000 rpm.

3. Pipette 20 µl standards or supernatants (sample) into fluorometer tubes. And then add 200 µl of reaction mixture.

4. Mix tubes thoroughly (Whirlimix) and incubate for 30 min at room temperature. Covering the tubes with a cling film to prevent contamination.

5. Add 1 ml of lactate diluent to each tube and mix thoroughly.

6. Read fluorescence of the standards and samples on a fluorometer (RF1501, Shimazu, Japan).

7. The fluorescence readings and the lactate concentrations are linear relationships.

Reference

Appendix E

PLASMA GLYCEROL ASSAY

Fluorometric method based upon Laurell and Tibbling (Laurell and Tibbling, 1966).

**Principle:**

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate}
\]

\[
\text{Glycerol-3-phosphate} + \text{NAD}^+ \xrightarrow{\text{GDH}} \text{Dehydroxyacetone phosphate} + \text{NADH}
\]

GK = Glycerol kinase.

GDH = Glycerol-3-phosphate dehydrogenase.

\(\text{NAD}^+\) is converted to NADH in the amount proportional to amount of glycerol.

**Reagent solutions:**

1. **0.087M Zinc Sulphate (ZnSO}_4\)**

   6.25g of ZnSO\(_4\)-7H\(_2\)O (MW=287.54) dissolved in 250ml of distilled water. To be kept in room temperature.

2. **0.083M Barum hydroxide (Ba(OH)}_2\)**

   6.55g of Ba(OH\(_2\))-8H\(_2\)O (MW=315.4) dissolved in 250ml of distilled water. To be kept in room temperature.

3. **0.2M Cysteine**

   24.2mg of Cysteine dissolved in 1.0ml of 0.4M NaOH. To be prepared daily.

   (0.4M NaOH : 400mg of NaOH dissolved in 25ml of distilled water)

4. **1.0M Hydrazine-HCl buffer with 1.5M MgCl\(_2\)**

   12.15ml of hydrazine-hydrate (99% solution) and 76.2mg of MgCl\(_2\) dissolved in 250ml of distilled water. Adjust pH with 5M HCl to 9.4. To be kept at 3°C.

5. **Diluent: 0.01M NaOH with 1.0mM EDTA**

   400mg of NaOH and 372.24mg of EDTA dissolved in 1000ml of distilled water. To be kept at room temperature.

6. **Reagent mixture (make up at the start of the assay)**

   (100 \(\mu\)l of reaction mixture is required per test tube)

   Each reaction mixture contains:

   - 700 \(\mu\)l of 1.0M hydrazine-HCl buffer
   - 200 \(\mu\)l of distilled water containing 12mg of ATP and 20 mg of NAD (0.1M ATP
and 0.15M NAD)
-100 µl of 0.2M cysteine
-1.0 µl of glycerol kinase
-5.0 µl of glycerol-3-phosphate dehydrogenase
NAD⁺: Free acid, grade II, ~98%, MW=663.4, Boehringer Mannheim.
ATP: MW=605.2, Boehringer Mannheim.
GK: 1mg/ml, Boehringer Mannheim.
GDH: 2mg/ml, Boehringer Mannheim.

7. Standards
The standards are made from Glycerol AR stock solution (99% solution, wt/ml:1.259, MW=92.10). Working standards concentrations as follows,
Blank, 0.02 mM, 0.04 mM, 0.2 mM, 0.6 mM, 0.8 mM

Procedures
1. The samples, standards, and reagent solutions are allowed to thaw to room temperature.
2. The samples are then mixed using a whirlimixer and centrifuged for 2-3 min.
3. 250 µl of ZnSO₄ transferred into eppendorf tubes using an air-displacement piston pipette.
4. 50 µl of sample or standard then added.
5. 250 µl of Ba(OH)₂ is then added. Mix well immediately.
6. All samples and standards are chilled at -20°C for 5 min and then centrifuged for 5 min.
7. 200 µl of the supernatant transferred in to glass fluorometer test tubes.
8. 100 µl of reaction mixture then added. All samples are made in duplicate. Use water and reaction mixture as blank.
9. Mix thoroughly and allow to incubate for 60 min in room temperature.
10. 1.0 ml of diluent is then added to each tube. Mix well.
11. Read the fluorescence of the standards and samples on a spectrofluorometer (RF1501, Shimazu, Japan).
12. The glycerol concentrations of the samples are calculated using a linear regression model.
Appendix F

Thirst Scale

6

7 NOT Thirsty

8

9

10

11 Fairly Thirsty

12

13

14

15 Thirsty

16

17

18

19 Very Very Thirsty

20
Appendix G

Gut Fullness Scale

6

7  NOT Full

8

9

10

11  Fairly Full

12

13

14

15  Full

16

17

18

19  Very Very Full

20