Influence of the glycaemic index of mixed meals on postprandial and exercise metabolism in men and women

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INFLUENCE OF THE GLYCAEMIC INDEX OF MIXED MEALS ON POSTPRANDIAL AND EXERCISE METABOLISM IN MEN AND WOMEN

by

Emma Jane Stevenson

A Doctoral Thesis

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

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Abstract

The benefits of carbohydrate ingestion before and after prolonged exercise are well known to scientists, coaches and athletes alike. However, the type of carbohydrate consumed can have a significant effect on substrate metabolism both at rest and during exercise. The glycaemic index (GI) is a method of classifying carbohydrate-containing foods according to their postprandial glycaemic responses. This is a useful tool to aid the selection of appropriate carbohydrates for both pre- and post-exercise meals. This thesis examined the influence of ingesting mixed meals with different glycaemic indices both before exercise and during the post-exercise recovery period in male and female subjects.

During recovery from prolonged endurance exercise, it is recommended that high GI (HGI) carbohydrates are consumed to increase the rate of muscle glycogen resynthesis and therefore improve recovery. However, the initial study (Chapter 4) in this thesis reported that the ingestion of a low GI (LGI) recovery diet significantly improved endurance capacity the following day compared to a HGI diet. To help explain this finding, the second investigation (Chapter 5) examined the metabolic responses to HGI and LGI isoenergetic mixed meals during the recovery period following prolonged endurance exercise.

The subsequent studies in this thesis focused on the GI of pre-exercise feedings. To examine the effect of two pre-exercise meals, subjects were provided with two HGI or two LGI meals over a 6 h period. Subjects then completed a 60 min run at 70% \( \text{VO}_2\text{max} \). Following ingestion of the second LGI meal, fat oxidation rates were significantly higher than in the HGI trial but no significant differences in substrate oxidation were reported during the run (Chapter 6). To examine whether differences in substrate oxidation occur following HGI and LGI meals in women, isocaloric HGI and LGI breakfasts were provided to females 3 h before a 60 min run at 65% \( \text{VO}_2\text{max} \) (Chapter 7). Much like the responses that have previously been reported in male subjects, reduced hyperglycaemia and hyperinsulinaemia occurred following the LGI breakfast.
and this resulted in a significantly higher rate of fat oxidation during the subsequent run compared to the HGI trial. To investigate whether the GI of an evening meal can affect the metabolic responses to breakfast and exercise the next day, male (Chapter 8) and female (Chapter 9) subjects were provided with a HGI or LGI evening meal on day 1 of the experimental trial. On the morning of day 2, subjects were provided with a standard HGI breakfast 3 h before exercising for 60 min at 65% $\text{VO}_2\text{max}$ The ingestion of a LGI evening meal significantly improved glucose tolerance the following morning in both male and female subjects No differences in the metabolic responses to subsequent exercise were reported

In summary, the degree of hyperglycaemia and hyperinsulinaemia following the ingestion of different carbohydrates significantly affects substrate oxidation at rest and during exercise in both men and women The GI of a meal can significantly affect the metabolic responses to a second meal, even after an overnight fast The consumption of LGI mixed meals therefore promotes the oxidation of fat at the expense of carbohydrate oxidation and can significantly improve glucose tolerance in the short term in male and female subjects

**Key Words:** exercise, carbohydrate, glycaemic index, fat oxidation, hyperglycaemia, hyperinsulinaemia
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"Alone we can do so little; together we can do so much "

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Publications

The findings of some of the studies reported in this thesis have been published as follows:


Stevenson E J , Williams C., and Nute M L. (2005). The influence of the glycaemic index of breakfast and lunch on substrate utilisation during the postprandial periods and subsequent exercise. *British Journal of Nutrition* 93 1-10

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Postprandial hyperglycaemia, hyperinsulinaemia and substrate metabolism
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Hyperglycaemia, hyperinsulinaemia and satiety
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Chapter 1

1 Introduction

Carbohydrate and fat are the principal substrates that fuel aerobic ATP synthesis in human skeletal muscle both at rest and during exercise (van Loon et al., 2001). Whereas fat stores are fairly abundant, carbohydrate sources are limited. Fatigue during endurance exercise is closely associated with muscle glycogen depletion. Therefore, the availability of carbohydrate as a substrate for muscle metabolism becomes a limiting factor in the performance of prolonged endurance exercise (Burke et al., 2004). Extensive research has focussed on the ingestion of carbohydrate before, during and after exercise with the aim of increasing the availability of blood glucose and muscle glycogen to improve exercise performance.

The intake of carbohydrate induces a substantial increase in insulin secretion which has a strong inhibiting effect on fat metabolism (Horowitz et al., 1997). Due to the abundance of fat stores within the body, the provision of this substrate has not been considered to be limiting to performance (Johnson et al., 2004). However, increasing the contribution of fat to total energy expenditure will reduce carbohydrate oxidation and so spare muscle glycogen stores. The nutritional and metabolic challenge is therefore to optimise the supply of carbohydrate to the muscle but to delay its depletion by using fat as an energy substrate for as long as possible.

Alterning the type of carbohydrate consumed can have a significant effect on the degree of hyperglycaemia and hyperinsulinaemia that occurs following their ingestion. Traditionally, carbohydrates were described as either simple or complex according to their chemical structure. However, in 1981 Jenkins and colleagues proposed the glycaemic index (GI) as a method of classifying carbohydrates according to their postprandial glycaemic response (Jenkins et al., 1981).

Although originally devised to aid individuals with diabetes to control the glycaemic impact of their diet, the glycaemic index has become a useful tool within sports nutrition. Thomas and colleagues were the first to apply the GI concept to pre-exercise feedings. They reported that the ingestion of low glycaemic index (LGI)
carbohydrates 1 h before exercise prolonged endurance time by 20 min compared to the HGI trial (Thomas et al., 1991) Following ingestion of the LGI carbohydrate, the degree of hyperglycaemia and hyperinsulinaemia was significantly lower than following the ingestion of the high glycaemic index (HGI) carbohydrate. Such metabolic and hormone responses promoted more stable blood glucose and maintained higher free fatty acid (FFA) concentrations during the exercise period.

The findings of this pioneering study have led to several subsequent investigations into the influences of the GI of pre-exercise carbohydrate feedings on metabolism and performance. Although not all studies have reported performance benefits, increased free fatty acid concentrations and increases in fat oxidation rates have consistently been reported during exercise following the ingestion of LGI carbohydrates (Thomas et al., 1994, Febbraio & Stewart 1996, Sparks et al., 1998, Wee et al., 1999b; Stannard et al., 2000; Wu et al., 2003). Interestingly, in the majority of these studies the subjects were fed single foods in the hour before commencing exercise. In reality, most people, including athletes, are more likely to consume a high carbohydrate meal 3-4 hours before exercise. A recent study by Wu and colleagues reported that the ingestion of a LGI mixed meal 3 h before exercise resulted in a significantly higher rate of fat oxidation during the exercise period compared to when a HGI pre-exercise mixed meal was consumed (Wu et al., 2003). The results of this study also highlight that the metabolic effects of HGI and LGI meals are still present several hours after their ingestion.

During the recovery period following prolonged strenuous exercise, athletes are advised to consume carbohydrates with a HGI. The elevated glucose and insulin concentrations that result from the ingestion of HGI foods facilitate muscle glycogen resynthesis and therefore accelerates the recovery process. Despite these recommendations, research on the GI of post-exercise feedings is limited. Consuming HGI carbohydrates increases muscle glycogen resynthesis compared to LGI carbohydrates (Burke et al., 1993, Jozsi et al., 1996). However, it is only presumed that this will improve subsequent exercise performance because the influence of a HGI recovery diet on exercise capacity has not been reported.

Carbohydrates have recently received a great deal of attention from a clinical
Chapter 1

Introduction

The apparent failure of low fat diets to reduce the prevalence of overweight and obesity in Americans has led to studies of other dietary factors (Ball et al., 2003). Adherence to standard dietary advice to reduce fat intake has led to a concomitant increase in carbohydrate consumption. A typical Western diet is now high in HGI carbohydrates which are digested and absorbed rapidly, resulting in increased insulin secretion (Brand-Miller et al., 2002). The chronic ingestion of such carbohydrates has therefore been related to diseases such as obesity, type 2 diabetes mellitus and coronary heart disease. Currently, there is much interest in the potential of low glycaemic index carbohydrate diets in the management of obesity. It has been hypothesised that LGI diets may help in weight regulation by promoting fat oxidation at the expense of carbohydrate oxidation and also promoting feelings of satiety (Warren et al., 2003).

It appears that the majority of studies reported in the GI literature have used male rather than female subjects. It is well known that patterns of substrate oxidation differ between genders both at rest and during exercise. Females are generally reported to oxidise greater proportions of fat than males during exercise (Tarnopolsky et al., 1995, Horton et al., 1998; Venable et al., 2005) and at rest, the reverse has been reported (Jones et al., 1998). Several studies have also reported that the cyclical variations in the female sex hormones can also induce changes in substrate oxidation (Campbell et al., 2001, Zderic et al., 2001; Redman et al., 2003). Therefore it is possible that the metabolic responses to HGI and LGI foods at rest and during exercise may not be the same in men and women. Further research in this area is required to investigate whether such differences do occur.

The concept of classifying carbohydrates in terms of their GI is a method gaining popularity in both the clinical and sporting domains (Burke et al., 1998a). However, the GI has been heavily criticised by some authors who believe that the concept is too complicated to be practical. Furthermore, they argue that the differences in glycaemic indices of foods are lost once they are ingested as part of a mixed meal (Coulston et al., 1987). The aim of this thesis was to further investigate the role of the glycaemic index in sport and exercise nutrition using mixed meals which would be realistic to the habitual dietary practices of most people including athletes and recreational exercisers.
This thesis reports the results of six main studies. Chapter 4 aimed to investigate the influence of the GI of post-exercise recovery diets on endurance performance the following day and Chapter 5 more closely examines the metabolic responses to HGI and LGI meals during the early recovery period following glycogen depleting exercise. The remaining Chapters focus on pre-exercise feeding and subsequent substrate utilisation. It is fairly common practice for individuals to consume two meals (breakfast and lunch) before exercising in the early evening. Chapter 6 therefore examines the effect of two HGI meals or two LGI meals on substrate metabolism throughout the day and during exercise at the end of the day. As previously mentioned, the ingestion of a LGI meal 3 h before exercise has been demonstrated to result in an increased rate of fat oxidation compared to a HGI pre-exercise meal in male subjects (Wu et al., 2003). Chapter 7 therefore investigates whether a LGI pre-exercise meal also results in a higher rate of fat oxidation in female subjects. The final two studies consider whether changing the GI of an evening meal can affect the metabolic responses to a standard breakfast and subsequent exercise the following morning in both male (Chapter 8) and female (Chapter 9) subjects. Finally, the General Discussion summarises the main findings of these investigations and discusses their possible implications within sport and exercise nutrition. Four of the studies presented in this thesis have been accepted for publication therefore Chapters 4 – 9 have been modified from manuscript format.
2 Review of Literature

2.1 Introduction

The relative contribution of fat and carbohydrate to energy production at rest, during exercise and during recovery from exercise can be influenced by a number of factors. In particular, the ingestion of exogenous substrates has an extensive effect on substrate oxidation. Nutritional interventions have been widely used in both clinical and sports nutrition to alter substrate oxidation for health and performance benefits.

Altering the glycaemic index of carbohydrates is one such nutritional intervention that has been used in both clinical and sports nutrition. This chapter provides a review of the current literature on carbohydrate and fat oxidation during rest, exercise and recovery with a particular focus on the effects of the glycaemic index of carbohydrates on substrate metabolism. The differences in substrate oxidation between men and women are also discussed.

The role of the glycaemic index in the prevention and treatment of disease is discussed briefly in this Chapter.
2.2 Carbohydrate and fat metabolism during rest and exercise

Carbohydrates and fat are the most important fuel sources both at rest and during exercise. Carbohydrate is stored as glycogen in muscle and liver. The liver typically contains 80-100g of glycogen in the post-absorptive state whereas muscle glycogen can vary from 50g after strenuous exercise to 900g in a well-fed, well-trained individual (Bergstrom & Hultman 1967). Fat is predominantly stored in the form of triacylglycerol in subcutaneous adipose tissue and in intra-abdominal stores but approximately 300g can be found in muscle as intramuscular triacylglycerol (IMTG). Fat stores, in comparison to carbohydrate stores, are relatively large and there are considerable inter-individual differences. At rest and during exercise, substrates are mobilised and utilised mainly in skeletal muscle. In most conditions, fat and carbohydrate are oxidised simultaneously but the relative contribution of the two fuels is dependent on a number of factors, many of which are discussed below.

2.2.1 Substrate oxidation at rest: the postabsorptive and postprandial state

Following an overnight fast (postabsorptive state), plasma concentrations of insulin are at their lowest and non-esterified fatty acid concentrations are high. Circulating free fatty acids (FFA) originate almost solely from lipolysis in adipose tissue and provide the vast majority of circulating lipid fuel. During this time, FFA are therefore the primary fuel for muscle, liver, heart and renal cortex (Jensen 2003). Blood glucose concentrations are maintained around 5 mmol/l and glucose enters the blood from the breakdown of liver glycogen and from hepatic gluconeogenesis (Frayn 2003). A large proportion of the glucose is taken up by the brain and completely oxidised whereas very little is utilised by skeletal muscle due to the low glucose and insulin concentrations. The post-absorptive state is usually interrupted by the intake of a meal. The metabolic changes that occur in the postprandial state are largely determined by the content of the meal and also by the activity level of the individual. The time course in which different nutrients are absorbed and enter the circulation is also variable. Glucose and amino acids enter the portal vein and then the general circulation within about 15-30 mm, although after ingestion of some carbohydrate meals, plasma glucose may remain elevated for 3-4 hours (Frayn 2003). The pancreas responds rapidly to the rise in glucose concentrations by releasing insulin which increases in parallel with the rise in glucose concentrations. The carbohydrate-induced
rise in insulin inhibits the mobilisation and hence availability of circulating FFA and inhibits the rate of long-chain fatty acid entrance into the mitochondria for β-oxidation (Sidossis & Wolfe 1996, Coyle et al., 1997). Due to the decline in FFA concentrations, glucose uptake is stimulated in the muscle and glycolysis increases. Thus, an increase in carbohydrate oxidation and an increase in lactate production occurs.

This general course of events may be repeated several times a day following each meal and frequent feeding (every 3-4 h) will prevent a return to the post-absorptive state until after the last meal of the day. The type and amount of different nutrients in a meal can significantly change the metabolic responses during the postprandial period. The studies in this thesis investigate the effect of altering the type of carbohydrate in a meal on the metabolic responses during the postprandial period.

2.2.2 Substrate oxidation during endurance exercise: the interaction between carbohydrate and fat

With the onset of exercise, the pathways that metabolise carbohydrate and fat to produce ATP must be heavily up-regulated to meet the increased demand for energy (Spriet 2002). Although amino acids can be utilised during exercise, their contribution to total energy metabolism is minimal (Jeukendrup 2003). Carbohydrate is often thought to be the more versatile fuel as it can contribute to ATP production both aerobically and anaerobically (Spriet & Watt 2003). However, carbohydrate stores within the body can become depleted with 1-2 hours of intense exercise (Hermansen et al., 1967) thus any 'sparing' of carbohydrate by fat oxidation would be beneficial so that the utilisation of glycogen stores is as economic as possible.

While both fuels are important during endurance exercise, the relative contribution of fat and carbohydrate to total energy expenditure is dependent on a number of factors including intensity and duration of the exercise, availability of endogenous and exogenous fat and carbohydrate sources, gender (see Section 2.3) and training status of the individual. It has long been known that, in the absence of dietary interventions, increasing the intensity of exercise exacerbates the reliance on carbohydrate whereas increasing the duration of low to moderate intensity exercise augments the reliance on
Nevertheless, the mechanisms that regulate these shifts in fuel utilisation are still unclear.

Romijn and colleagues used isotope tracer techniques to investigate substrate oxidation during 30 min of exercise at 25, 65 and 85% VO$_2$ max (Romijn et al., 1993). Plasma glucose uptake and muscle glycogen oxidation increased in relation to exercise intensity. Lipolysis increased as a function of power output at 25% and 65% VO$_2$ max. Plasma FFA concentrations remained high during exercise at these two intensities; therefore, FFA delivery to the working muscle increased as exercise intensity increased. Adipose tissue lipolysis was not decreased during exercise at 85% VO$_2$ max but was maintained at the same rate as when exercise intensity was 65% VO$_2$ max. Yet plasma FFA concentrations decreased by ~50%. The authors speculated that in spite of a maintenance of lipolysis at the higher exercise intensity, decreasing adipose tissue blood flow prevented much of the released FFA from reaching the blood, therefore accounting for the lower blood FFA concentrations. To assess the importance of FFA delivery during exercise, Romijn and colleagues artificially maintained FFA concentrations at the 65% VO$_2$ max level during exercise at 85% VO$_2$ max. Although plasma FFA uptake and oxidation were higher when FFA concentrations were maintained, fat oxidation was only partially restored compared with the levels observed during exercise at 65% VO$_2$ max in the previous study (Romijn et al., 1995). This suggests that other factors, related to intramuscular events, also play an important role in determining the rate of plasma FFA uptake and oxidation at higher exercise intensities.

More recently, van Loon and colleagues used stable isotope methodology in combination with muscle biopsy sampling to accurately quantify substrate utilisation and study the regulation of muscle fuel selection during exercise (van Loon et al., 2001). 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$_{\text{max}}$). Muscle glycogen and plasma glucose oxidation rates increased with every increment in exercise intensity. The relative contribution of fat oxidation to total energy expenditure did not change from resting conditions to exercise, and remained
unchanged when exercise was increased up to 55 \% W_{\text{max}}. However, as exercise intensity was further increased up to 75 \% W_{\text{max}}, total body fat oxidation rate decreased by 34\% compared to that at 55 \% W_{\text{max}}. This decline involved a decrease in the oxidation rate of both plasma FFA and other fat sources (intramuscular and lipoprotein-derived triacylglycerol). In contrast to the data collected by Romijn and colleagues (1995), plasma FFA availability did not seem to play a role in reducing fat oxidation rates. The authors proposed that a mechanism involving the down-regulation of carnitine palmitoyl transferase I (CPT I), the protein involved in FFA transfer into the mitochondria, was the most likely candidate to lead to the reduction of fat oxidation during high intensity exercise. Despite this conclusion, the factors that down-regulate fat metabolism during high intensity exercise are still incompletely understood (Spriet 2002). For a detailed review of the mechanisms thought to be responsible for this regulation readers are referred to Spriet (2002).

Increasing the availability of FFA will increase the rate of fat oxidation and therefore slow the rate of muscle glycogen utilisation. As pre-exercise muscle glycogen content is strongly correlated to subsequent endurance capacity, it is not surprising that a number of nutritional interventions have been tested to increase fatty acid availability and promote lipolysis. Increases in FFA concentrations have been achieved through the ingestion of high fat meals before exercise or the infusion of Intralipid both before and during the exercise period. In a recent review by Hargreaves (Hargreaves et al., 2004), it was reported that only one study has observed an improvement in endurance capacity when plasma FFA concentrations were elevated before exercise (Pitsiladis et al., 1999). Although other studies have successfully increased FFA concentrations, no performance benefit from such an intervention was observed (Starling et al., 1997, Whitley et al., 1998; Burke & Hawley 2002, Hawley 2002).

Recent improvements in the ability to measure intramuscular triacylglycerol (IMTG) stores have confirmed that IMTG acts as a significant fuel source during prolonged exercise (Johnson et al., 2004). For a detailed review on the use of IMTG as a substrate during exercise in humans, the reader should refer to van Loon (van Loon 2004). The typical high carbohydrate, low fat diet that is consumed by most athletes successfully increases muscle glycogen concentrations but will fail to replenish IMTG stores. This has been demonstrated during short term recovery (Decombaz et al.,
Chapter 2

Review of Literature

van Loon et al., 2003b) and also over a longer period of time (Coyle et al., 2001). Coyle and colleagues (2001) studied seven endurance-trained cyclists over a 3-wk period during which time they exercised 2 h a day at 70% \( \text{VO}_2 \text{max} \) and consumed approximately 4,400 kcal per day. During the first week, the subjects were provided with a diet consisting of 32% of total energy as fat. During weeks 2 and 3, they were randomly assigned to eat 2 or 22% of energy from fat. The 2% fat diet significantly lowered IMTG concentrations and increased muscle glycogen concentrations at rest compared to the 22% fat diet. Metabolism was studied during 1 h of exercise at 67% \( \text{VO}_2 \text{max} \) performed in the fasted state. Subjects consuming the 2% fat diet reduced calculated non-plasma FFA oxidation by 40% in association with a 19% reduction in whole body lipolysis compared to the subjects who had consumed the 22% fat diet. An increase in the calculated muscle glycogen oxidation was also observed in the 2% fat diet compared to the 22% fat diet.

It is clear that muscle glycogen is not the only muscle substrate metabolised during exercise. However, unlike muscle glycogen, there is no conclusive evidence that IMTG depletion is limiting to exercise performance (Johnson et al., 2004). The observation that IMTG is accumulated rapidly under conditions that promote minimal glycogen resynthesis suggests that the role of IMTG is to maintain a readily available substrate for moderate intensity exercise when glycogen availability is not optimal (Johnson et al., 2004). In light of this, it may be necessary to review current dietary guidelines for athletes to ensure that both muscle glycogen and triacylglycerol concentrations are restored following prolonged exercise.

Several studies have demonstrated that increasing carbohydrate availability both before and during exercise increases carbohydrate oxidation and decreases fat oxidation (Coyle et al., 1997, Horowitz et al., 1997). The precise mechanism/s mediating the decrease in fat oxidation are not fully understood (Coyle et al., 1997; Febbraio et al., 2000a). It has been suggested that the maintenance of plasma insulin concentrations may account for this response because hyperinsulinaemia is associated with a decrease in adipose tissue lipolysis (Campbell et al., 1992; Horowitz et al., 1997). Recently, Watt and colleagues reported that glucose ingestion before and during 120 min cycling at 60% \( \text{VO}_2 \text{max} \) blunted the activity of hormone sensitive
lipase (HSL), the rate-limiting enzyme for the hydrolysis of IMTG (Watt et al., 2004).

The intake of carbohydrate during the pre-exercise period and its influence on subsequent exercise metabolism and performance has been extensively investigated. A full review of this literature can be found in Section 2.7.

### 2.2.3 Substrate oxidation during recovery from endurance exercise

During the early phase of recovery from prolonged endurance exercise, glycogen concentrations in the exercised muscle are very low. Resynthesis of muscle glycogen will start to occur immediately after exercise and even in the absence of a dietary supply of carbohydrate it will occur at a very slow rate (Ivy 1998). A significant decrease in the glycogen content of non-exercising muscle has been observed during the post-exercise recovery period suggesting that there is also a transfer of glycogen (in the form of glucose) from the resting muscle to the recovering muscle (Krssak et al., 2000). The exercise-induced low muscle glycogen concentrations and the decrease in circulating insulin concentrations during this time stimulate an increase in fat oxidation by increasing fatty acid metabolism, perhaps until carbohydrate stores are replenished (Dionne et al., 1999). Several studies have reported an increase in whole body lipid oxidation after exercise, as demonstrated by a decrease in respiratory exchange ratio (RER) (Bielinski et al., 1985, Kiens & Richter 1998; Kimber et al., 2003). It has been suggested that intramuscular triacylglycerol (IMTG) stores are an important substrate source during the post-exercise period. A study carried out by Kiens & Richter (1998) demonstrated that IMTG content decreased significantly during the initial 18 h of recovery in well-trained individuals, despite a large intake of carbohydrate. The authors suggested that during recovery, muscle glycogen resynthesis is of high metabolic priority resulting in the preferential utilisation of IMTG and circulating lipids by the recovering skeletal muscle (Kiens & Richter 1998). Subsequent studies have reported no change (Decombaz et al., 2000; Decombaz et al., 2001) or an increase (Larson-Meyer et al., 2002) in IMTG during recovery from prolonged endurance exercise. It is still controversial whether IMTG stores are utilised during the recovery period following exercise and differences in the methods used to measure IMTG (muscle biopsy or nuclear magnetic resonance spectroscopy) may explain discrepancies in the results.
It is now common practice to consume carbohydrates (CHO) after exercise in order to restore muscle glycogen as quickly as possible and therefore restore endurance capacity during a subsequent exercise session. Intake of carbohydrate would suggest an increase in CHO oxidation and so it might be expected that there would be no need for significant fat oxidation (Kiens & Richter 1998). The increase in insulin concentration that occurs when CHO is ingested would also lead to a suppression of fat oxidation (Campbell et al., 1992). Indeed, Dionne and colleagues (Dionne et al., 1999) found that the immediate compensation of the substrate mix oxidised during 60 min running at 50% VO2 max, especially carbohydrates, rapidly replenishes glycogen stores and attenuates the shift to fat oxidation that normally follows exercise. However, in contrast to this, Kiens and Richter and more recently, Kimber and colleagues both reported an increase in fat oxidation during recovery from glycogen depleting exercise despite a large intake of CHO (8-10 g CHO·kg⁻¹ BM day⁻¹) (Kiens & Richter 1998).

The intake of carbohydrate during the recovery period following prolonged exercise has, and continues to be extensively investigated. A review of the current literature is found in Section 2.8.

2.3 Gender differences in substrate oxidation at rest and during exercise

Gender differences in post-absorptive substrate oxidation have been reported, with women having lower absolute and relative rates of fat oxidation compared to men (Toth et al., 1998). In addition, it has been reported that these differences persist in the postprandial state, with a trend towards greater absolute net fat oxidation in men than in women attributed at least in part, to a greater oxidation of exogenous fat (Jones et al., 1998).

Several studies have investigated whether a gender difference exists in the relative utilisation of carbohydrates and lipids as fuel sources during sub-maximal exercise (Roepstorff et al., 2002). The majority of studies have reported that females derive a relatively larger contribution from lipids to oxidative metabolism during exercise than
males (Tamopolsky et al., 1990; Tamopolsky et al., 1995; Friedlander et al., 1998; Horton et al., 1998; Knechtle et al., 2004; Venables et al., 2005). However, a small number have observed a similar relative utilisation of carbohydrates and lipids in females and males exercising at the same relative workload (Mittendorfer et al., 2002; Roepstorff et al., 2002). Although total carbohydrate and fat oxidation were reported to be similar in these studies, differences in the source of substrate were reported. Mittendorfer and colleagues (2002) reported a higher plasma FFA tissue uptake and oxidation in women than men and therefore a lower utilisation of non-plasma sources in the male subjects. In contrast, Roepstorff and colleagues (2002) reported significantly higher resting concentrations and significantly higher utilisation of myocellular triacylglycerol (MCTG) during exercise in females compared to males. This is in agreement with the findings of Steffensen and colleagues who also reported that MCTG resting content and use during exercise were higher in females than males (Steffensen et al., 2002).

The findings of a recent study by Kïens and colleagues may help to explain gender differences in lipid metabolism. They reported that in the untrained state, women had higher muscle mRNA levels for several proteins related to muscle lipid metabolism compared to men. In the endurance trained, gender differences in mLPL mRNA existed and levels of FAT/CD36 protein in muscle were higher in women than in men irrespective of training status (Kïens et al., 2004).

It is clear that gender differences in substrate oxidation do exist but are still not fully understood. This highlights the importance of studying males and females separately and not applying research findings in males to the female population and vice versa. Further research is required to fully understand the metabolic responses to exercise in females.

2.4 Effect of the menstrual cycle on substrate oxidation at rest and during exercise

Throughout the menstrual cycle, women are continuously exposed to changing female steroid hormones (Janse de Jonge, 2003). The cycle can be divided into two distinct
hormone environments or phases that are separated by ovulation. Throughout the pre-ovulation or follicular phase, plasma oestrogen and progesterone concentrations are low, while during the post-ovulation or luteal phase, both are increased several fold (Redman et al. 2003). Although traditionally known for their role in reproduction, there is increasing consensus that the ovarian hormones have important roles in metabolism (Campbell et al., 2001). Indeed, evidence suggests that the normal cyclical variations in oestrogen and progesterone can affect a number of aspects of lipid and carbohydrate metabolism (Horton et al., 2002).

2.4.1 Effect of the menstrual cycle on substrate oxidation at rest

The majority of studies investigating the effect of menstrual cycle phase on substrate oxidation at rest have reported no differences between the luteal and follicular phase. Melanson and colleagues reported that there was no effect of the phase of the menstrual cycle on postabsorptive or postprandial protein fat or carbohydrate oxidation following four different test meals, each with a different energy content (Melanson et al., 1996). No differences have been reported in resting whole body RER (Piers et al., 1995) and glucose turnover (Zderic et al., 2001) throughout the different phases of the menstrual cycle.

Insulin sensitivity has been reported to be affected by the sex steroids. Poorer glycaemic control has been reported during the luteal phase of the menstrual cycle in women with insulin-dependent diabetes mellitus (Widom et al., 1992). The effect of the menstrual cycle on insulin sensitivity, glucose effectiveness and acute insulin response to a glucose load in healthy young women was investigated by Pulido and colleagues. The authors reported a decrease in insulin sensitivity in the luteal phase of the menstrual cycle when the levels of oestrogen and progesterone were high but no differences in glucose tolerance (as measured by an intra-venous glucose tolerance test) (Pulido & Salazar 1999). The mechanisms by which sex steroids affect glucose metabolism are still unclear and no other study has reported such an effect on insulin sensitivity.

2.4.2 Effect of the menstrual cycle on substrate oxidation during exercise

There is growing consensus that oestrogen and progesterone have important roles in regulating substrate oxidation during exercise in women (D'Eon et al., 2002). Studies
in animals have demonstrated that oestrogen is a potent promoter of increased lipid oxidation during exercise, resulting in a sparing of muscle glycogen (Kendrick & Ellis 1991). In contrast, progesterone has been reported to antagonise the lipolytic effects of oestrogen and reduce fatty acid availability (Hatta et al., 1988).

Studies investigating exercise metabolism in different phases of the menstrual cycle in humans have produced equivocal results. Several studies have reported a shift toward reduced blood glucose use and increased fat oxidation during sub-maximal exercise in the luteal phase when oestrogen and progesterone concentrations are higher (Campbell et al., 2001; Zdenc et al., 2001, Redman et al., 2003) but others have reported no significant differences (Bailey et al., 2000, Horton et al., 2002). For a more complete understanding of substrate utilisation in exercising women, Zdenc and colleagues investigated whether glucose kinetics were different during exercise in different phases of the menstrual cycle. Participants exercised at 70% lactate threshold (LT) and 90% LT during the follicular and luteal phase of the menstrual cycle. At rest and during exercise at 70% LT there were no differences in rate of plasma glucose appearance (Ra) and disappearance (Rd) or CHO or fat oxidation. At 90% LT, Ra and Rd and CHO oxidation were lower in the luteal phase compared to the follicular phase (Zdenc et al., 2001). Campbell and colleagues reported that the variations in the ovarian hormone levels throughout the menstrual cycle only alter exercise metabolism when carbohydrate stores became depleted. When subjects were provided with glucose throughout prolonged cycling at 70% VO$_2$ max, variations in the ovarian hormones throughout the menstrual cycle did not alter exercise metabolism (Campbell et al., 2001). The intensity and duration of the exercise is therefore an important factor when investigating differences in substrate oxidation throughout the menstrual cycle. Variations in exercise metabolism induced by the menstrual cycle may be minimised by ensuring an adequate source of CHO either before or during exercise (Campbell et al., 2001).

High inter- and intra-subject variability inherent to studying the "natural" hormonal environment complicates the ability to draw clear conclusions on the effect of menstrual cycle on metabolism (D'Eon et al., 2002). The inconsistent findings regarding the interaction between menstrual cycle phase and exercise metabolism may also be a consequence of differences in the methods used to define normal
menstrual function and the different phases of the menstrual cycle (body temperature versus hormone assay), and the choice of phase studied (Redman et al., 2003). There is also difficulty in trying to assess the metabolic actions of a single hormone in vivo (Suh et al., 2002). However, determining how the menstrual cycle phase effects various aspects of metabolism is necessary to provide a comprehensive understanding of normal physiology in women (Horton et al., 2002).

2.5 Effect of oral contraceptives on substrate utilisation

Despite the extensive use of oral contraceptives by both sedentary and sports women, few investigators have evaluated the affects of these exogenous ovarian steroids on the metabolic responses to physical exercise (Casazza et al., 2004). Given the fact that there may be differences in metabolic responses between the follicular and luteal phases during exercise in normal menstruating women when steroid levels are low and high respectively, the presence of synthetic steroids in oral contraceptives may have an effect on carbohydrate and/or lipid metabolism during exercise (Bonen et al., 1991).

Typically, investigators have used cross-sectional study designs to compare oral contraceptive users with non-users. A study by Bonen and colleagues examined hormone and substrate responses to mild and heavy treadmill exercise in women who were and were not taking oral contraceptives. It was reported that substrate patterns during exercise were not altered by the phase of the menstrual cycle or by oral contraceptive usage. Despite this, FFA concentrations were consistently higher during mild exercise in the oral contraceptive group compared to the control. Glucose concentrations were also lower during rest and during exercise in the oral contraceptive group (Bonen et al., 1991). In support of these findings, Bemben and colleagues observed significantly lower blood glucose concentrations in oral contraceptive users during exercise at 50% VO2 max (Bemben et al., 1992). However unlike Bonen and colleagues (Bonen et al., 1991), a significant decrease in RER during exercise was reported in oral contraceptive users vs. nonusers. In 2001, Boisseau and colleagues carried out a study to assess glucose tolerance during exercise in women using oral contraceptives and non-users. Subjects ingested 0.5g CHO kg⁻¹ BM at the onset of exercise (30 min at 60% VO2 max). No significant
differences in plasma glucose, insulin and catecholamines were reported during exercise between the two groups. It was concluded that glucose ingestion at the onset of exercise induced similar glucose tolerance in women taking or not taking oral contraceptives (Boisseau et al., 2001)

More recently, two longitudinal studies using stable isotopic tracers have been carried out to investigate the effects of oral contraceptives on blood glucose flux (Suh et al., 2003) and on triglyceride mobilisation (Casazza et al., 2004). In both studies, eight eumenorrheic women were tested during the follicular and luteal phase before and after 4 months of oral contraceptive usage. In each case, subjects were tested during 90 min of rest and during 60 min of leg ergometry cycling exercise at 45 and 65% VO₂ max and were tested 3 h after a standard breakfast. After 4 months of taking the contraceptive pill, there were significant reductions in glucose rates of appearance (Ra) and disappearance (Rd) during exercise at both intensities but not at rest (Suh et al., 2003). No phase effects on substrate oxidation were reported either at rest or during exercise (Suh et al., 2003). Casazza and colleagues reported that following 4 months of oral contraceptive use, dietary composition, exercise patterns, plasma glycerol concentrations, growth hormone concentrations and exercise respiratory exchange ratios did not change. However there was a significant increase in glycerol Ra at both exercise intensities after 4 months of contraceptive pill use. Plasma cortisol concentrations were also significantly higher at rest and during exercise (Casazza et al., 2004). Both studies concluded that exogenous ovarian hormones have a greater metabolic effect on metabolism during exercise than endogenous ovarian hormones. Although oral contraceptive use decreases glucose flux and increases lipolysis (glycerol Ra) no overall effect on carbohydrate or lipid oxidation during exercise was observed (Suh et al., 2003, Casazza et al., 2004)

2.6 The Glycaemic Index

The glycaemic index concept was first published in 1981 by Jenkins and colleagues to provide a numeric classification of carbohydrate foods according to their glycaemic responses (Jenkins et al., 1981). The concept was originally designed to aid individuals with diabetes mellitus in controlling the glycaemic impact of their diet and to supplement existing information on the chemical composition of foods given on
food labels

The glycaemic index is defined as the incremental area under the glucose response curve (IAUC) after consumption of 50g of carbohydrate from a test food divided by the IAUC after consumption of 50g of carbohydrate from a standard food (white bread or glucose, GI=100) as applied to the same individual.

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

The calculation of the IAUC was proposed by Wolever and Jenkins. The methods used incorporate the trapezoid rule and ignore the area below the fasting value during a 2 hour postprandial period (Wolever & Jenkins 1986)

Numerous methodological factors may influence the value and/or variability of the results obtained for a given test food. The Food and Agricultural Organisation and World Health Organisation Expert Consultation (FAO/WHO 1998) have therefore proposed a standardised protocol to determine the GI of a given food to provide a reliable and consistent measure of relative blood glucose responses. The guidelines state that the tests must be repeated in at least six subjects and the GI values averaged. Subjects are studied on separate days, in the morning following a 10-12 h fast. On each occasion, subjects consume a portion of the sample food which provides 50g of available carbohydrate. Blood glucose concentrations are determined from whole blood capillary samples collected over a 2 hour postprandial period. Plasma glucose may also be used however capillary blood is recommended because it yields results that are similar to those of arterial blood. The standardised method has been tested in seven centres internationally and has been found to be reliable, with the 95% confidence interval of the GI value obtained in 10 subjects being ± 15 (Wolever et al., 2003).

The GI concept allows the classification of foods into low (GI<55), medium (55<GI>70) and high (GI>70) CHO foods (when glucose is used as a standard). An
extensive database of GI values for individual foods consumed by both healthy and diabetic subjects is now available (Foster-Powell et al., 2002).

A fundamental assumption regarding LGI foods is that they produce a low glycaemic response as a result of a lower rate of digestion of carbohydrate in the intestinal lumen, and that this subsequently slows the absorption of glucose into the circulation. However, plasma glucose concentration is a function of both the rate of appearance of glucose ($R_{\text{glucose}}$) into the systemic circulation and the rate of disappearance of glucose ($R_{\text{dglucose}}$) from the systemic circulation.

A recent study by Schenk and colleagues used stable isotope methodology to investigate the underlying glucose kinetics responsible for the different glycaemic responses of a HGI breakfast cereal (Corn Flakes) and a LGI breakfast cereal (All Bran). Surprisingly, the authors reported that the lower GI of All Bran than Corn Flakes was not due to a lower rate of appearance of glucose but instead to an earlier postprandial hyperinsulinaemia and an earlier increase in the rate of disappearance of glucose, which attenuated the increase in plasma glucose concentration (Schenk et al., 2003). At present, further investigations on other LGI foods have not been carried out however, the results of this study highlight that further information on glucose kinetics is needed to supplement GI values so that the postprandial responses to carbohydrate-containing foods can be fully understood.

2.6.1 Factors which modulate the Glycaemic Index

As already described, numerous methodological factors may influence the value and/or variability of the GI obtained for a given test food. The factors which may affect the GI of a given food are described below.

2.6.1.1 Between-subject and within-subject variation

Different subjects vary in their glycaemic responses over a wide range (Wolever & Mehling 2003). Despite this, when the glycaemic response to a food is expressed relative to that of a reference food consumed by the same individual, the variability between individuals is dramatically reduced to the extent that it is no longer statistically different (Wolever et al., 1990, Wolever et al., 1992). Nevertheless, the
glycaemic responses of the same subject after consuming standard test meals under standardised conditions do, however, vary from day to day. To reduce variability and hence result in more normally distributed GI values, it is recommended that the GI be calculated using the mean of at least three tests of the standard food taken by each subject (FAO/WHO 1998)

2.6.1.2 Choice of reference food

Both glucose and white bread have been used as the reference food in GI testing. In both cases, the reference food is assigned an arbitrary GI value of 100 and all foods tested are compared to this response. The use of white bread as a standard produces GI results that are 1.4 times greater than when glucose is used because glucose has a glycaemic response that is 40% greater than that of white bread (Wolever 2003). For international standardisation, GI values are adjusted if necessary so that they are expressed on the standard glucose scale. Throughout this thesis, GI values were taken from the International table of glycaemic index and glycaemic load values: 2002 (Foster-Powell et al., 2002) which used glucose as the standard food.

2.6.1.3 Nature of the starch

Starches are polymers of glucose. The glucose may be arranged in a linear form (amylose) or, more frequently in branched chains (amylopectin). The glucose residues are joined together by mainly α 1-4 linkages with some α 1-6 linkages that cause the branching of the glucose chain in amylopectin (Webb 1995). It has been reported that starch with a high amylopectin content could induce higher glycaemic responses compared to amylose (Behall et al., 1988, Kabir et al., 1998). A high ratio of amylose/amylopectin in starch may therefore attenuate glycaemic responses. In addition, resistant starch, which is an indigestible form of starch, results in reductions in postprandial glycaemia and insulinaemia due to a slower rate of digestion and absorption of the CHO food (Raben et al., 1994).

2.6.1.4 Dietary fibre

The extent to which fibre in a particular food is responsible for its GI is a subject of much debate (Pi-Sunyer 2002). A study carried out by Wolever and colleagues
investigated this relationship in 25 foods. It was reported that total dietary fibre was related to the GI although only weakly ($r=0.461, p<0.05$). When broken down into soluble and insoluble fibre, it was found that there was no significant relationship between soluble fibre and GI; however, there was a stronger relation between insoluble fibre and GI ($r=0.584, p<0.001$) (Wolever 1990). In contrast to this, other studies in which fibre has been added to a carbohydrate meal suggest that only soluble fibre has an effect on postprandial glucose concentrations and not the insoluble component (Nuttall 1993). Other studies in this area have produced equivocal results nevertheless a high intake of dietary fibre has been consistently associated with lower glycaemic responses and a lower risk of diabetes (Meycr et al., 2000, Stevens et al., 2002).

2.6.1.5 Food processing and cooking

The method of processing of a single food can greatly change its GI. Grinding, rolling, mashing or even chewing can disrupt the starch granules in a food (Brand et al., 1985). Disrupting these granules allows amylose or amylopectin to become available for hydrolysis which can therefore affect the GI as explained above. The application of heat and moisture during cooking also affects the starch granules in a food. Disorganisation of the crystalline structure of the starch occurs as it encounters greater heat and moisture for a longer period of time (Pt-Sunyer 2002). Gelatinisation occurs first, with disruption of the crystalline structure followed by a disruption of the granules. A fully gelatinised starch is easy to digest as the swollen granules have a greater surface area to contact the hydrolytic enzymes in the intestine. On cooling, gelatinised starch recrystallises, a change known as retrogradation (Cummings & Englyst 1995). Retrograded starch, particularly amylose, is more resistant to hydrolysis and therefore results in reduced glycaemic responses (Cummings & Englyst 1995). It is therefore clear that even simple food processing such as heating and cooling will affect the glycaemic response to some carbohydrate foods.

2.6.1.6 Fat and protein

Large amounts of fat and protein added to CHO have frequently been demonstrated to affect postprandial responses. Several studies have shown that protein added to CHO increases the insulin response to this food; however, the glucose response does not change much or actually decreases (Spiller et al., 1987; van Loon et al., 2000b).
Adding protein to CHO that is ingested post-exercise has been shown to significantly increase the insulin responses compared to when CHO alone is ingested and this therefore may facilitate greater muscle glycogen resynthesis (Van Hall et al., 2000, Ivy et al., 2002). The addition of fat to CHO is generally considered to reduce glucose and insulin responses because of reduced upper gastrointestinal motility (Welch et al., 1987). However, fat also potentiates gastric inhibitory polypeptide (GIP) secretion which may have an acute effect in increasing insulin secretion (Collier et al., 1984). Nevertheless, it is believed that the range of fat and protein contents in normal meals is not large enough to have a detectable effect on postprandial glucose and insulin responses in normal subjects (Wolever 2000). A study carried out by Wolever & Bolognesi fed five unmatched meals to normal subjects. The meals varied in energy, protein, fat and carbohydrate content and also in GI. It was reported that 90% of the variance of postprandial insulin responses was explained by the amount and GI of the meal carbohydrate (Wolever & Bolognesi 1996a).

2.6.1.7 The addition of organic acids

An increase in the acidity of a meal can greatly lower its GI. The addition of vinegar to a meal, for instance will affect the glucose response following its ingestion. It has been established that the addition of certain organic acids to foods, such as bread, may reduce postprandial glycaemia in normal subjects (Laljeberg et al., 1995). The physiological mechanisms for the acute effects appear to vary; whereas lactic acid lowers the rate of starch digestion in bread, acetic acid and propionic acids appear instead to prolong the gastric emptying rates (Ostman et al., 2002).

2.6.1.8 Time of day

The time of day glycaemic response tests are carried out may affect both the absolute and relative glycaemic responses of the foods (Wolever 2003). A study carried out by Wolever and colleagues compared the glycaemic responses of two different breakfast cereals in healthy subjects consumed either in the morning after a 13-hour fast, or at midday 4 hours after they had consumed a standard breakfast. Absolute glycaemic responses (AUC) at lunchtime were significantly less than those at breakfast despite consuming exactly the same meal (Wolever & Bolognesi 1996b). The FAO/WHO protocol therefore recommends that GI testing takes place in the morning following an overnight fast (FAO/WHO 1998).
2.6.1.9 Nature of the monosaccharide absorbed

The digestible carbohydrate in starch is absorbed entirely as glucose. However, only approximately half the carbohydrate in sucrose, fruits and dairy products is absorbed as glucose, the other half being fructose or galactose. Fructose produces a lower glycaemic response than most other carbohydrates (Wolever 2003). Human studies have demonstrated that fructose may play an active catalytic role in the augmentation of hepatic glucose uptake and subsequent lowering of the glycaemic response to additional dietary carbohydrate (Moore et al., 2000). However, large amounts of fructose have been associated with raised serum triglycerides and may cause insulin resistance therefore intake of more than approximately 10% of energy as added sugar is generally not recommended (Wolever 2003).

2.6.1.10 Gorging and nibbling

The beneficial effect of the manipulation of feeding patterns on glycaemic responses has been investigated. In a study carried out by Jenkins and colleagues, subjects were fed with 50g of glucose in 700ml of water on two occasions: over 5-10 min (bolus) and at a constant rate over 3.5h (sipping). Despite similar 4-h blood glucose responses, large reductions were seen in serum insulin and C-peptide responses after sipping. There was also a prolonged suppression of plasma glucagon, growth hormone and free fatty acid concentrations after sipping. An intravenous glucose tolerance test at 4h demonstrated a 48 ± 10% more rapid decline in blood glucose after sipping than after the bolus (Jenkins et al., 1990). The results of this study indicate that prolonging the rate of glucose absorption enhances insulin economy and glucose disposal.

2.6.2 Application of the Glycaemic Index to mixed meals

It has been reported that the GI concept lacks clinical utility because the differences in glycaemic indexes between foods are lost once these foods are consumed in a mixed meal (Coulston et al., 1987; Hollenbeck et al., 1988). A method of calculating the GI of a mixed meal was reported by Wolever and Jenkins (Wolever & Jenkins 1986) in which the GI of the total meal is calculated from the weighted means of the GI values for the component foods as illustrated below,
Total grams (g) of carbohydrate in meal = TC
Carbohydrate content of each individual food (g) = Ga, Gb, Gc
GI of each individual food = Gla, Glb, Glc
Total GI of the meal = (Ga/TC)*Gla + (Gb/TC)*Glb + (Gc/TC)*Glc

Wolever and colleagues (1986) calculated the GI values of four different mixed meals using the method described above and compared them with the actual incremental blood glucose responses using regression analysis. The correlation coefficient for the GI of each meal was positively correlated with the actual plasma glucose response (r = 0.987; p < 0.02). In a later study, Chew and colleagues investigated the plasma glucose and insulin responses to six different mixed meals of varying ethnic backgrounds in healthy individuals. It was reported that the glycaemic and insulinaemic responses to the meals could successfully be predicted from the glycaemic indices of the component carbohydrate foods (Chew et al., 1988). Despite these results, the clinical relevance of the glycaemic index is still vigorously debated (Ludwig 2002) and many believe that the concept may be too complicated to be practical (Coulston et al., 1987). Further research on the use of mixed meals having a nutrient composition that is clearly within the normal range of habitual diets is needed (Ludwig & Jenkins 2004).

The glycaemic load (GL) is an alternative way to assess the impact of carbohydrate consumption on the diet. In contrast to the GI of a food (which is calculated using a portion that provides 50g of CHO), the GL provides a measure of the total glycaemic response to a food or a meal. The GL is calculated using the following equation:

Glycaemic Load = GI * grams of carbohydrate per serving.

It is important to acknowledge however that the GL of a diet can be lowered by reducing the GI of the carbohydrates within the diet or by reducing the total CHO content of the diet and therefore may be misleading.

2.6.3 Glycaemic Index and the “second-meal effect”
The augmented insulin secretion which follows a HGI meal may increase peripheral glucose uptake to such an extent that blood glucose concentrations fall lower than
fasting concentrations (Liljeberg et al., 1999) On the contrary, when carbohydrate absorption is prolonged, as occurs after a LGI meal, there is less of a tendency for blood glucose concentrations to fall below basal values (Wolever et al., 1988). As a result of this, there may be a smaller counter regulatory response and improved glucose disposal following the next meal i.e. the ‘second meal effect’ (Wolever et al., 1988)

Jenkins and colleagues were one of the first to report an improvement in glucose tolerance at lunchtime when lentils (a LGI food) were consumed for breakfast compared to bread (a HGI food) The authors reported that the slower rate of absorption of the lentils was responsible for the improved glucose tolerance at the subsequent meal (Jenkins et al., 1982). Evidence against malabsorption of the lentil meal was provided from breath hydrogen data. A later study by Wolever and colleagues reported that low glycaemic index foods eaten at dinner improved the subsequent breakfast glycaemic response (Wolever et al., 1988) More recently, Liljeberg and colleagues investigated the effect of the GI and the indigestible carbohydrate (resistant starch and dietary fibre) content of cereal-based breakfasts on glucose tolerance at a second meal in healthy subjects. It was reported that the slow absorption and digestion of starch from the breakfast meal, and not the content of indigestible carbohydrates in the breakfast meal, improved glucose tolerance at the second meal (Liljeberg et al., 1999)

The exact mechanism behind the so called ‘second meal effect’ is still unclear. It has been suggested that the prolonged absorptive phase following a LGI meal will favour a more efficient suppression of FFA, thus improving insulin sensitivity at the time of the next meal (Wolever et al., 1988) There is evidence to propose that LGI foods that contain a large amount of slowly absorbed fermentable CHO ingested the evening before a Oral Glucose Tolerance Test (OGTT), enhance the suppression of hepatic glucose production and FFA thus creating a more insulin sensitive environment (Thorburn et al., 1993)
2.7 Pre-exercise carbohydrate intake – the effect on metabolism and performance

It has been established since the 1960’s that carbohydrate intake before exercise increases muscle glycogen stores and can therefore improve performance (Bergstrom et al., 1967). Since then, considerable attention has focussed on nutritional strategies to maximise muscle and liver glycogen stores before endurance training and competition. A high carbohydrate intake alters the metabolic responses and substrate utilisation during exercise (Costill et al., 1977) therefore different feeding and exercise protocols have produced different physiological responses.

Carbohydrate feeding in the hour before exercise results in a large increase in plasma glucose and insulin concentrations. However, with the onset of exercise there is often a rapid fall in blood glucose concentrations as a consequence of the combined stimulatory effects of hyperinsulinaemia and contractile activity on muscle glucose uptake (Hargreaves et al., 2004). The increase in plasma FFA concentrations with exercise is attenuated following pre-exercise carbohydrate ingestion, as a consequence of insulin-mediated inhibition of lipolysis (Costill et al., 1977, Horowitz et al., 1997). Therefore, fat oxidation is reduced because of lower plasma FFA availability and also because of inhibition of lipid oxidation in the muscle.

The metabolic alterations associated with the ingestion of carbohydrate in the hour before exercise has the potential to influence exercise performance (Hargreaves et al., 2004). However, it is controversial whether the ingestion of carbohydrate improves exercise performance with some (Kirwan et al., 1988; Sherman et al., 1989; Thomas et al., 1991; Goodpaster et al., 1996; Kirwan et al., 2001b) but not all studies (Hargreaves et al., 1987) showing a positive effect.

It is more realistic that athletes will consume carbohydrate 2-4 h before exercise, therefore providing enough time for digestion and absorption and preventing gastrointestinal discomfort. Despite plasma glucose and insulin concentrations returning to baseline before exercise commences, ingestion of carbohydrate in the hours before exercise may result in a transient fall in plasma glucose with the onset of exercise, increased carbohydrate oxidation and a blunting of FFA mobilisation (Coyle
et al., 1985). These metabolic perturbations appear not to be detrimental to performance, with an increased carbohydrate availability apparently compensating for the greater carbohydrate utilisation.

Ingestion of a carbohydrate meal 3-4 h before exercise has been shown to increase muscle glycogen (Coyle et al., 1985, Chryssanthopoulos et al., 2004). Although no improvements in exercise performance have been reported when carbohydrate was consumed 4 h before exercise (Okano et al., 1996; Whitley et al., 1998), the majority of studies have reported an enhanced exercise performance compared to exercise in the fasted state (Sherman et al., 1989, Chryssanthopoulos & Williams 1997; Schabort et al., 1999, Chryssanthopoulos et al., 2002). Differences in results are likely to be due to differences in exercise and feeding protocols and the type and amount of carbohydrate provided.

2.7.1 The influence of the glycaemic index of pre-exercise carbohydrate feeding
Carbohydrate availability for exercise can be optimised by manipulating the GI of the carbohydrate supplementation (Burke et al., 1998b). The ingestion of carbohydrates that do not induce hyperinsulinaemia has received considerable attention because they protect against hypoglycaemic symptoms during the exercise period and reduce the suppression of fat oxidation that usually occurs after a high carbohydrate intake.

Thomas and colleagues were the first to manipulate the GI of pre-exercise carbohydrate feedings. Subjects were provided with either water or 1g CHO kg⁻¹ BM in the form of lentils (LGI), mashed potato (HGI) or glucose (HGI) 60 min before cycling to exhaustion at 67% \( \text{VO}_2 \text{max} \). Endurance time was reported to be 20 min longer following the LGI pre-exercise carbohydrate intake compared to the HGI intake (p<0.05) (Thomas et al., 1991). This result was attributed to the lower postprandial hyperglycaemia and hyperinsulinaemia and therefore more stable plasma glucose and FFA concentrations during the exercise period. In a later study, Febbraio and Stewart examined the effect of pre-exercise carbohydrate ingestion on muscle glycogen metabolism and exercise performance (Febbraio & Stewart 1996). Subjects were provided with 1g CHO kg⁻¹ BM in the form of lentils (LGI), mashed potato (HGI) or a placebo 45 min before cycling for 120 min at 70% \( \text{VO}_2 \text{max} \) followed by a
15 min performance cycle. The authors reported that the differences in the pre-exercise glucose and insulin responses to the two meals disappeared shortly after the onset of exercise. Similar rates of muscle glycogen utilisation during the sub-maximal cycle and total work outputs during the performance cycle were found in all trials. It was concluded that the pre-exercise carbohydrate ingestion, while increasing carbohydrate oxidation irrespective of GI, does not influence the rate of muscle glycogen utilisation or exercise performance. Interestingly, in a later study, Febbraio et al. reported that the ingestion of a HGI carbohydrate 30 min before exercise resulted in hyperinsulinaemia, which increased glucose uptake and decreased FFA availability compared to when a LGI carbohydrate was consumed. This resulted in an augmented rate of carbohydrate oxidation possibly due to glycogenolysis. Despite this, there were no differences in exercise performance between the trials following the 120 min of sub-maximal exercise (Febbraio et al., 2000b).

Subsequent studies investigating the effect of the glycaemic index of pre-exercise carbohydrate feedings have yielded inconsistent results (See Table 2.1). A study carried out by DeMarco and colleagues was one of the first to attempt to provide mixed meals with different glycaemic indices before exercise instead of single foods. Subjects were provided with a HGI meal, a LGI meal or water 30 min before cycling for 2 h at 70% \( V_{\text{O}_2\text{max}} \) followed by a cycle to exhaustion at 100% \( V_{\text{O}_2\text{max}} \). Although no differences in the glycaemic response to the two meals were reported during the postprandial period, plasma glucose concentrations were higher and respiratory exchange ratio values were lower after 2 h exercise in the LGI trial compared to the HGI trial. Time to exhaustion at the end of the 2 h was 59% longer after the LGI meal than the HGI meal (DeMarco et al., 1999). It is important to note however that the mixed meals used in this study were not matched for fibre, fat, protein and total energy content. This is likely to have affected the glycaemic responses to the two meals. It is also quite unlikely that athletes would choose to consume a meal 30 min before the onset of exercise. Time to exhaustion was found to be similar in a study by Wee and colleagues who fed subjects HGI or LGI meals containing 2g CHO kg\(^{-1}\) BM 3h before running to exhaustion at 70% \( V_{\text{O}_2\text{max}} \). Although no differences in exercise capacity were reported, CHO oxidation was 12% lower and fat oxidation 118% higher during the first 80 min of exercise in the LGI
trial compared to the HGI trial (Wee et al., 1999b). Although the investigators attempted to match the total carbohydrate, protein and fat contents of the two test meals, the LGI meal consisted of only boiled lentils whereas the HGI meal consisted of a mixture of foods including roast potatoes, tuna, crumpets and honey. The apparent lack of any glucose or insulin response following the lentils meal suggests that this food may have been malabsorbed therefore explaining the significantly higher fat oxidation rates in this trial.

Differing experimental protocols may explain some of the variation of the results in the performance studies. Most importantly, the measurement of performance has not been consistent throughout the studies. Some have measured endurance capacity, in terms of time to exhaustion at a constant workload on a cycle ergometer or at a constant pace on a motorised treadmill (Thomas et al., 1991; Thomas et al., 1994; Kirwan et al., 1998; DeMarco et al., 1999; Wee et al., 1999b; Kirwan et al., 2001a, Kirwan et al., 2001b). Others have measured endurance performance, in terms of work power output during a fixed time (Febbraio & Stewart 1996; Sparks et al., 1998), or time to complete a fixed distance or workload (Burke et al., 1998a) after a bout of prolonged exercise.

The timing of the meals before the exercise bout has also varied substantially (ranging from 45 min to 3 h). The majority of the research has focussed on the provision of carbohydrate within the hour before exercise despite the fact that most athletes would usually eat a few hours before exercise. Although all studies have claimed to match the carbohydrate content of the pre-exercise feedings, it is worth noting that the other macronutrient contents have not been matched in many cases (Thomas et al., 1991; Thomas et al., 1994, Febbraio & Stewart 1996, Burke et al., 1998a, Kirwan et al., 1998; DeMarco et al., 1999, Kirwan et al., 2001a; Kirwan et al., 2001b). As a consequence, test meals have not been isoenergetic which may have a substantial effect on absorption and digestion as well as exercise performance.

The underlying mechanism behind improved endurance capacity following a LGI pre-exercise meal has frequently been suggested to be related to the lower glycaemic and insulinaemic responses during the postprandial period. The ingestion of high carbohydrate foods without inducing a high insulin secretion reduces the suppression
of fat oxidation as well as providing a sustained carbohydrate source during exercise. A recent study by Wu and colleagues examined the effect of mixed meals with different glycaemic indices on substrate utilisation during exercise 3 h later. Both meals contained 2g CHO kg\(^{-1}\) BM and were macronutrient and energy matched. The results demonstrated that the HGI meal resulted in significantly greater glycaemic and insulinaemic responses during the postprandial period compared to the LGI meal. During exercise 3 h later, the calculated amount of fat oxidation was significantly higher in the LGI trial compared to the HGI trial (Wu \textit{et al}., 2003).

Although no improvement in exercise performance has been reported following a LGI pre-exercise meal in some studies, a better outcome of metabolic and physiological consequence has been observed from the data on blood metabolites and expired gas analyses such as higher FFA concentrations and higher fat oxidation rates (Thomas \textit{et al}., 1991; Thomas \textit{et al}., 1994; Febbraio & Stewart 1996; Burke \textit{et al}., 1998a, Sparks \textit{et al}., 1998, DeMarco \textit{et al}., 1999; Wee \textit{et al}., 1999b, Stannard \textit{et al}., 2000; Wu \textit{et al}., 2003). These findings indicate that LGI foods may have a potential benefit over HGI foods when considering the intake of carbohydrate before exercise.
Table 2.1 A summary of pre-exercise glycaemic index feeding studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Type and amount of CHO</th>
<th>Time before exercise</th>
<th>Exercise protocol</th>
<th>Exercise capacity/performance</th>
<th>Blood glucose during exercise</th>
<th>substrate oxidation during exercise</th>
<th>Plasma FFA during exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thomas et al 1991</td>
<td>Lentils (LGI) Potato (HGI&lt;sub&gt;1&lt;/sub&gt;) Glucose (HGI&lt;sub&gt;2&lt;/sub&gt;) Water (W)</td>
<td>60 min</td>
<td>cycle to exhaustion @ 67% VO&lt;sub&gt;2&lt;/sub&gt;max</td>
<td>capacity LGI&gt;HGI</td>
<td>HGI&lt;sub&gt;1&lt;/sub&gt;, LGI &gt; HGI&lt;sub&gt;2&lt;/sub&gt;, W</td>
<td>↑ fat oxidation in LGI trial compared to HGI&lt;sub&gt;1&lt;/sub&gt; &amp; HGI&lt;sub&gt;2&lt;/sub&gt;</td>
<td>W&gt;LGII&gt; HGI&lt;sub&gt;1&lt;/sub&gt; &gt; HGI&lt;sub&gt;2&lt;/sub&gt;</td>
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<tr>
<td></td>
<td>1g CHO kg&lt;sup&gt;-1&lt;/sup&gt; BM</td>
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<tr>
<td>Thomas et al 1994</td>
<td>Lentils (LGI) Potato (HGI) Rice cereal (HGI&lt;sub&gt;2&lt;/sub&gt;) Bran cereal (LGI&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>60 min</td>
<td>cycle to exhaustion @ 67% VO&lt;sub&gt;2&lt;/sub&gt;max</td>
<td>No differences in capacity</td>
<td>Inverse correlation with GI after 90 min</td>
<td>positive correlation with RER and GI during exercise</td>
<td>Inverse correlation with GI during final 60 min</td>
</tr>
<tr>
<td></td>
<td>1g CHO kg&lt;sup&gt;-1&lt;/sup&gt; BM</td>
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<tr>
<td>Febbrau &amp; Stewart 1995</td>
<td>Lentils (LGI) Potato (HGI) Placebo (P)</td>
<td>45 min</td>
<td>120 min cycle @ 70% VO&lt;sub&gt;2&lt;/sub&gt;max + 15 min performance test</td>
<td>No differences in performance</td>
<td>No difference</td>
<td>No difference</td>
<td>LGI, P &gt; HGI</td>
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<tr>
<td></td>
<td>1g CHO kg&lt;sup&gt;-1&lt;/sup&gt; BM</td>
<td></td>
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<tr>
<td>Burke et al 1998</td>
<td>HGI potato meal LGI pasta meal Placebo jelly meal</td>
<td>120 min</td>
<td>120 min cycle @ 70% VO&lt;sub&gt;2&lt;/sub&gt;max + 300 kJ performance test</td>
<td>No differences in performance</td>
<td>No differences</td>
<td>No differences</td>
<td>No differences</td>
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<tr>
<td></td>
<td>2g CHO kg&lt;sup&gt;-1&lt;/sup&gt; BM (+ CHO ingestion during exercise)</td>
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<tr>
<td>Study</td>
<td>Type and amount of CHO</td>
<td>Time before exercise</td>
<td>Exercise protocol</td>
<td>Exercise capacity/ performance</td>
<td>Blood glucose during exercise</td>
<td>Substrate oxidation during exercise</td>
<td>Plasma FFA during exercise</td>
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<tr>
<td>Sparks et al</td>
<td>Lentils (LGI) Potato (HGI) Placebo (P)</td>
<td>45 min</td>
<td>50 min cycle @ 67% VO₂max + 15 min performance test</td>
<td>No differences in performance</td>
<td>HGI &lt; LGI, C until 30 min</td>
<td>↑ fat oxidation in LGI trial compared to HGI trial</td>
<td>P, LGI &gt; HGI</td>
</tr>
<tr>
<td>1998</td>
<td>1g CHO kg⁻¹ BM</td>
<td></td>
<td></td>
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<tr>
<td>Kirwan et al</td>
<td>Rolled oats (MGI) Whole-oat flour (F) Placebo (P) 75g CHO</td>
<td>45 min</td>
<td>cycle to exhaustion @ 60% VO₂max</td>
<td>Capacity</td>
<td>No differences</td>
<td>RER higher in MGI and F than in P</td>
<td>P &gt; MGI, F during first 90 min</td>
</tr>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td></td>
<td>MGI &gt; P</td>
<td></td>
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</tr>
<tr>
<td>DeMarco et al</td>
<td>HGI mixed meal LGI mixed meal</td>
<td>30 min</td>
<td>120 min cycle @ 70% VO₂max + cycle to exhaustion @ 100% VO₂max</td>
<td>time to exhaustion 59% longer in LGI trial compared to HGI trial</td>
<td>HGI, LGI &lt; C @ 20 min</td>
<td>↑ fat oxidation in LGI trial compared to HGI trial during first 100 min</td>
<td>LGI &gt; HGI</td>
</tr>
<tr>
<td>1999</td>
<td>1.5g CHO kg⁻¹ BM</td>
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<tr>
<td>NCM</td>
<td>HGI mixed meal LGI mixed meal Placebo meal &gt; 2g CHO kg⁻¹ BM</td>
<td>180 min</td>
<td>run to exhaustion @ 70% VO₂max</td>
<td>No differences</td>
<td>LGI &gt; HGI @ 20 min</td>
<td>↑ fat oxidation in LGI trial compared to HGI trial during first 80 min</td>
<td>LGI &gt; HGI</td>
</tr>
<tr>
<td>Wee et al</td>
<td>HGI mixed meal LGI mixed meal Placebo meal &gt; 2g CHO kg⁻¹ BM</td>
<td>180 min</td>
<td>run to exhaustion @ 70% VO₂max</td>
<td>No differences</td>
<td>LGI &gt; HGI @ 20 min</td>
<td>↑ fat oxidation in LGI trial compared to HGI trial during first 80 min</td>
<td>LGI &gt; HGI</td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
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<tr>
<td>Stannard et al</td>
<td>Glucose (HGI) Pasta (LGI) Placebo (P) 1g CHO kg⁻¹ BM</td>
<td>65 min</td>
<td>incremental exercise test to fatigue</td>
<td>No differences</td>
<td>LGI &gt; HGI</td>
<td>No differences from 200W to exhaustion</td>
<td>-</td>
</tr>
<tr>
<td>2000</td>
<td></td>
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<td>Study</td>
<td>Type and amount of CHO</td>
<td>Time before exercise</td>
<td>Exercise protocol</td>
<td>Exercise capacity/ performance</td>
<td>Blood glucose during exercise</td>
<td>Substrate oxidation during exercise</td>
<td>Plasma FFA during exercise</td>
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<tr>
<td>Febbraio et al 2000</td>
<td>Muesli (LGI) Potato (HGI) Placebo (P)</td>
<td>30 min</td>
<td>120 min cycle @ 70% VO₂max + 30 min performance cycle</td>
<td>No differences @ 15-30 min</td>
<td>LGI &gt; HGI</td>
<td>Tendency for ↑ fat oxidation in LGI trial compared to HGI trial</td>
<td>LGI &gt; HGI</td>
</tr>
<tr>
<td></td>
<td>1g CHO kg⁻¹ BM</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Kirwan et al 2001a</td>
<td>Rolled oats (MGI) Puffed rice (HGI) Placebo (P)</td>
<td>45 min</td>
<td>cycle to exhaustion @ 60% VO₂max</td>
<td>MGI &gt; P</td>
<td>MGI &gt; HGI, C @ 60-90 min</td>
<td>↑ CHO oxidation in MGI trial</td>
<td>MGI, HGI &lt; C @ 60-120 min</td>
</tr>
<tr>
<td></td>
<td>75g CHO</td>
<td></td>
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<tr>
<td>Kirwan et al 2001b</td>
<td>Rolled oats (MGI) Placebo (P)</td>
<td>45 min</td>
<td>cycle to exhaustion @ 60% VO₂max</td>
<td>No difference</td>
<td>No difference</td>
<td>↑ CHO oxidation in MGI trial compared to placebo</td>
<td>P &gt; MGI for first 120 min</td>
</tr>
<tr>
<td></td>
<td>75g CHO</td>
<td></td>
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<tr>
<td>Wu et al 2003</td>
<td>HGI mixed meal LGI mixed meal Placebo (P)</td>
<td>No performance or capacity measure 60 min run @ 65% VO₂max</td>
<td>No performance or capacity measure 60 min run @ 65% VO₂max</td>
<td>-</td>
<td>HGI &lt; LGI @ 15 min</td>
<td>↑ fat oxidation in LGI trial compared to HGI trial</td>
<td>LGI &gt; HGI @ 30-60 min</td>
</tr>
</tbody>
</table>
2.8 Carbohydrate intake during recovery from endurance exercise.

Fatigue during prolonged strenuous exercise is often associated with the depletion of muscle glycogen stores and therefore high pre-exercise muscle glycogen concentrations are believed to be essential for optimum performance. During recovery from prolonged exercise, the restoration of muscle glycogen is of high metabolic priority however carbohydrate intake is necessary to facilitate this process. Therefore, research has focussed on the amount, timing and type of carbohydrate supplementation required to optimise muscle glycogen resynthesis, especially when the recovery period is short.

2.8.1 The amount of carbohydrate required

Typically, the most important dietary factor affecting muscle glycogen storage is the amount of carbohydrate consumed (Burke et al., 2004). When no carbohydrate is ingested, muscle glycogen synthesis rates have been reported to be as low as 5-12 mmol/kg dw/h (Ivy et al., 1988; Van Hall et al., 2000). When carbohydrate is consumed, there appears to be a direct and positive relationship between the quantity of dietary carbohydrate and post-exercise glycogen storage, at least until the muscle storage capacity or threshold has been reached (Burke et al., 2004). Early research in this area failed to find differences in post-exercise muscle glycogen resynthesis when subjects were fed a carbohydrate intake of 0.35 g kg\(^{-1}\) h\(^{-1}\) and 0.7 g kg\(^{-1}\) h\(^{-1}\) (Blom et al., 1987) or between 1.5-3 g kg\(^{-1}\) h\(^{-1}\) (Ivy et al., 1988) fed at 2 h intervals. However, more recently, van Loon and colleagues reported that when the rate of carbohydrate ingestion was increased from 0.8 to 1.2 g kg\(^{-1}\) h\(^{-1}\), this resulted in higher muscle glycogen resynthesis rates (van Loon et al., 2000a). In this study, carbohydrate supplements were provided at 30 min intervals, while studies in which no differences in muscle glycogen resynthesis were found with increasing carbohydrate intake supplemented carbohydrate at 2 h intervals. It has been suggested that carbohydrate supplements provided every 2 h do not adequately increase and maintain blood glucose and insulin concentrations to maximise glycogen storage as is the case when more frequent feedings are provided (Jentjens & Jeukendrup 2003). Several studies have reported very high muscle glycogen synthesis rates (40-43 mmol/kg dw/h) when 1.0-1.85 g kg\(^{-1}\) h\(^{-1}\) carbohydrate have been consumed at regular intervals over a 3-4 h recovery period (Casey et al., 1995, Jentjens et al., 2001). However, because these studies did not make direct comparison between glycogen storage and different
amounts of carbohydrate and different feeding schedules, it is difficult to draw final conclusions on the optimal carbohydrate intake in the early recovery phase (Burke et al., 2004) Nevertheless, the results of these studies suggest that the threshold for early glycogen recovery is reached by a carbohydrate feeding schedule that provides 1.2 g kg⁻¹h⁻¹. This is based on the failure to increase muscle glycogen storage when insulin concentrations are further increased by the addition of protein to carbohydrate feedings.

Only two studies have directly investigated the relationship between feeding different amounts of carbohydrate and glycogen resynthesis over a 24 h period (Costill et al., 1981, Burke et al., 1995). The results of these studies show an increase in glycogen storage with increasing carbohydrate intake with a glycogen storage threshold at a daily carbohydrate intake of around 7-10 g kg⁻¹BM.

### 2.8.2 Timing of carbohydrate intake

Following glycogen depleting exercise, two phases of glycogen resynthesis occur: non-insulin dependent and insulin dependent (Price et al., 1994). On completion of exercise, an initial, rapid insulin-independent phase of glycogen repletion occurs followed by a more prolonged insulin-dependent phase. During the insulin-dependent phase, the muscle demonstrates a marked increase in the sensitivity and responsiveness of glucose transport and glycogen resynthesis to insulin (Kuo et al., 2004). This is made possible by an increase in GLUT-4 transporter proteins and an increase in the activation of the enzyme glycogen synthase (Wojtaszewski et al., 2002). Carbohydrate intake immediately after exercise appears to take advantage of these effects. A study by Ivy and colleagues demonstrated high muscle glycogen storage (7.7 mmol kg⁻¹ wwh⁻¹) during the first 2 h of recovery, slowing thereafter to the more typical rates of storage (4.3 mmol kg⁻¹ wwh⁻¹) (Ivy et al., 1988). Indeed, Ivy and colleagues also demonstrated in the same study that when carbohydrate ingestion post-exercise was delayed by 2 h muscle glycogen resynthesis was 45% lower compared to when carbohydrate was ingested immediately post — exercise. Nevertheless, in a study by Parkin and colleagues (Parkin et al., 1997) delayed ingestion of a high glycaemic index meal by 2 h did not affect the rate of muscle glycogen resynthesis during an 8 h post-exercise period. These results appear to be in contrast to those of Ivy and colleagues (1988) however in this study, muscle glycogen...
resynthesis rates were only determined over a 4 h period. It is also worth noting that in the study by Parkin and colleagues, subjects were provided with 0.8 g kg⁻¹ h⁻¹ during the first 4 h of recovery in the ‘immediate feeding condition’ but no carbohydrate was ingested thereafter. It cannot be ruled out that if carbohydrate feeding was continued for a second 4 h period, this might have resulted in a higher muscle glycogen concentration 8 h post-exercise. It is therefore recommended that athletes consume carbohydrate as soon as possible following exercise to increase the rate of muscle glycogen resynthesis. This is especially important when the recovery time between exercise sessions is short (1-4 h).

Whether carbohydrate is best consumed as a series of snacks or as large meals has also been investigated. Frequent eating may provide a practical strategy allowing athletes to increase energy intake while concomitantly reducing the gastric discomfort of infrequent large meals (Hawley & Burke 1997). Studies investigating feeding patterns over a 24 h recovery period have reported that muscle glycogen is the same whether a given amount of carbohydrate is provided as two large meals or seven smaller meals (Costill et al., 1981) or as four large meals or 16 one hourly snacks (Burke et al., 1996).

As already discussed, during short term recovery, carbohydrate provided at 15-30 min intervals results in very high rates of muscle glycogen resynthesis (Van Hall et al., 2000; van Loon et al., 2000a; Jentjens et al., 2001). Results from these studies have reported resynthesis rates that exceed those observed when carbohydrate is provided at 2 h intervals over a 4-6 h recovery period (Blom et al., 1987; Ivy et al., 1988).

It would appear that when recovery time is more prolonged (12-24 h), meeting the total carbohydrate requirement is more important than the pattern of the feeding. Smaller feedings may provide a benefit in reducing gastric discomfort and appear to be more beneficial to increase muscle glycogen storage when the recovery time is short.

2.8.3 The presence of other nutrients
Insulin stimulates both muscle glucose uptake and the activation of muscle glycogen synthase; therefore many studies have attempted to increase post-exercise insulin
concentrations to optimise muscle glycogen resynthesis (Zawadzki et al., 1992, Roy & Tarnopolsky 1998, Van Hall et al., 2000, van Loon et al., 2000a; Jentjens et al., 2001, Ivy et al., 2002). It is well known that the addition of protein and certain amino acids exert a synergistic effect on insulin release when combined with a carbohydrate load. The co-ingestion of protein with carbohydrate has therefore received the most attention in terms of glycogen recovery and has provided a source of some debate due to inconsistent results. Some studies have reported an increase in muscle glycogen resynthesis when protein is consumed along with carbohydrate compared to carbohydrate alone (Zawadzki et al., 1992; van Loon et al., 2000a, Ivy et al., 2002) whereas others have not seen this effect (Tarnopolsky et al., 1997; Carrathers et al., 2000, Van Hall et al., 2000, Jentjens et al., 2001). The conflicting results are likely to be due to differences in experimental design, including frequency of feedings, the amounts of carbohydrate and protein provided and the recovery time investigated.

2.8.4 The influence of the glycaemic index of carbohydrate consumed during recovery from endurance exercise

Since muscle glycogen storage is influenced by both insulin and a rapid supply of glucose substrate, it is logical that carbohydrate sources with a moderate to high glycaemic index would enhance post-exercise refuelling (Burke et al., 2004). This hypothesis was confirmed by Blom and colleagues using single nutrient feedings of mono- and disaccharides. It was reported that the intake of glucose and sucrose after prolonged exercise both produced higher rates of muscle glycogen resynthesis compared with fructose (a LGI sugar) (Blom et al., 1987).

In 1990, Kiens and colleagues compared muscle glycogen resynthesis over 44 h following a high carbohydrate diet consisting mainly of HGI or LGI carbohydrates consumed following strenuous exercise. It was reported that a greater storage of muscle glycogen was achieved in the HGI trial after 6 h of recovery. However, there were no differences in muscle glycogen content between the trials after 20, 32 and 44 h of recovery (Kiens et al., 1990). This study has been criticised however as the diets were referred to interchangeably as HGI/simple and LGI/complex carbohydrates which led to confusion in the interpretation of the results.

Burke and colleagues were the first to investigate the influence of HGI and LGI meals.
on 24 h recovery of muscle glycogen. Five cyclists were provided with 10 CHO kg\(^{-1}\) BM which was evenly distributed between meals eaten at 0, 4, 8 and 21 h following a glycogen depletion protocol. Muscle biopsy data showed that the increase in muscle glycogen content was significantly greater following the HGI recovery diet compared to the LGI diet. However, the magnitude of the increase (~30%) was substantially greater than the difference in 24 h blood glucose and insulin profiles. It was reported that the meal provided immediately after exercise produced a large glycaemic and insulinaemic response that was independent of the glycaemic index of the carbohydrate consumed (Burke et al., 1993). Other studies have reported an exaggerated glycaemic response to carbohydrates consumed following exercise compared with the same feeding consumed at rest (Rose et al., 2001), this has been explained by a greater gut glucose output and a greater hepatic glucose escape. Although a higher rate of muscle glycogen resynthesis was observed by Burke et al. (1993), performance during subsequent exercise was not assessed.

An additional mechanism to explain reduced glycogen storage following a LGI diet is malabsorption of the carbohydrate. A study carried out by Jozsi and colleagues provided eight cyclists with glucose (HGI), maltodextrin (glucose polymer) (HGI), waxy starch (100% amylopectin) (HGI) or resistant starch (100% amylose) (LGI) during 24 h recovery from glycogen depleting exercise. Glycogen storage was lower following the amylose starch mixture than after the other three treatments. The authors hypothesised that poor digestibility explained the differences and concluded that indigestible carbohydrate forms provide a poor substrate for muscle glycogen resynthesis (Jozsi et al., 1996).

As a result of these studies, athletes are recommended to choose HGI carbohydrates during recovery from exercise in order to promote a higher rate of muscle glycogen resynthesis.

### 2.9 Glycaemic Index and health and disease

Although the glycaemic index has become a useful tool in sports nutrition, it was originally developed to provide a physiological basis for carbohydrate exchange in the diets of diabetics (Jenkins et al., 1981). High glycaemic index (HGI) carbohydrates
are associated with rapid hyperglycaemia and hyperinsulinaemia which in many individuals, is followed by hypoglycaemia, elevated free fatty acid concentrations and therefore the secretion of counter regulatory hormones. The habitual consumption of HGI carbohydrates initiates a cycle of hyperinsulinaemia and insulin resistance that places the beta cell under long term increased demand (Ludwig 2002). Several studies have therefore investigated the relationship between the glycaemic index of carbohydrates and the incidence of insulin resistance and type 2 diabetes as well as investigating the benefits of LGI diets in the treatment and management of the disease. For a full review of the relationship between glycaemic index and the risk of type 2 diabetes mellitus, readers are referred to Willett and colleagues (Willett et al., 2002).

More recently, insulin resistance has been associated with the aetiology of other diseases, namely coronary heart disease (CHD) and some cancers (mainly: breast, colon and prostate). A number of large cohort studies have investigated the association between dietary glycaemic index and dietary fibre with cancer and CHD risk in different populations. Readers are referred to Leeds (Leeds 2002) for an in depth review of the research that has been carried out on the relationship between glycaemic index and heart disease. In addition, several nutritional intervention studies indicate that a LGI diet may be beneficial in maintaining normal blood lipid profiles in healthy subjects, or improving lipid profiles in hyperlipidaemic subjects, diabetes mellitus patients, obese and overweight individuals and those with CHD (Jenkins et al., 1985; Jenkins et al., 1987; Wolever et al., 1992; Jarvi et al., 1999, Bouche et al., 2002; Heilbronn et al., 2002; Wolever & Mehlng 2003; Sloth et al., 2004). Indeed, in a retrospective cross-sectional study of 2200 British adults, the glycaemic index of the diet was the only dietary variable significantly related to serum HDL-cholesterol (Frost et al., 1999). It would therefore appear that the glycaemic index of a habitual diet is an important factor to be considered in the prevention and treatment of diseases such as coronary heart disease and diet related cancers.

High glycaemic index carbohydrates are now also associated with weight gain and obesity. Reducing fat intake has been the primary focus of dietary prevention and treatment of obesity for over 20 years (Brand-Miller et al., 2002). However, the relationship between dietary fat and obesity has been questioned on several grounds.
including that both cross-sectional and longitudinal analyses have failed to show a considerable association between dietary fat and body fat (Kant et al., 1995; Larson et al., 1996) and that weight loss on low fat diets has been transient and modest (Lissner & Heitmann, 1995). In addition, the so-called “fat paradox” can be seen in several countries in which there is a poor association between dietary fat intake and percentage of the population that is overweight (Sarris, 2003).

A potential adverse consequence of the decrease observed in meal fat intake in recent years is a concomitant increase in dietary GI (Ludwig et al., 1999). The hyperinsulinaemia associated with HGI foods promotes carbohydrate oxidation at the expense of fat oxidation during the postprandial period. As a consequence, they alter fuel partitioning in a way that may be conducive to body fat gain (Brand-Miller, 2003). Long term exposure to chronic hyperglycaemia and hyperinsulinaemia results in decreased expression of the rate-limiting enzymes and alters the potential for fat oxidation (Brand-Miller et al., 2002). Research into the relationship between the GI of carbohydrates in the diet and weight status has not received a great deal of attention probably because of the small number of long term studies that have been carried out in this area (Brand-Miller, 2003). However, the results of several medium term studies have been inconclusive. For a review of the literature in this area, see Brand Miller et al. (2002).

### 2.10 Glycaemic Index and satiety

The consumption of HGI foods is also associated with reduced satiety, increased hunger and/or increased food intake. A number of short term studies (lasting for a single meal or a single day) have addressed the question of whether the consumption of LGI foods reduces hunger and/or promotes satiety relative to the consumption of HGI foods (see Anderson, 2003 for a review). The majority of studies have shown either a significant or non-significant reduction in subsequent hunger and/or increased satiety following consumption of LGI foods compared to HGI foods. These responses were obtained using analog scales or measured by subsequent energy intake (Roberts, 2003). Many of the early studies used single foods or simply compared glucose and fructose or amylose and amylopectin. Differences in test diets such as energy content and palatability also make some of the results difficult to interpret.
Overall, despite limitations to some of the existing studies, the combination of results from a wide range of studies indicates that relative to LGI carbohydrates, consumption of HGI carbohydrates promotes a more rapid return of hunger and increases subsequent energy intake (Roberts 2003). The implication of this is that HGI diets may contribute to the maintenance of excess weight in the obese and perhaps also in the etiology of weight gain in susceptible individuals (Roberts 2003). Further long term studies on the effects of LGI diets and the mechanisms of weight loss and weight control are required.
3 General Methods

3.1 Introduction

This chapter describes the methodologies and equipment used in the investigations reported in this thesis. All the protocols and procedures used in the studies were approved by the Ethical Advisory Committee of Loughborough University before commencement (See Appendix A for sample form). All experimental trials were conducted in the Exercise Physiology Laboratories in the School of Sport and Exercise Sciences at Loughborough University.

Male and female subjects (aged 18-30) were recruited mostly from the student population of Loughborough University through local advertisements. Prior to participation, all volunteers were informed both verbally and in writing of the exact nature, demands and possible risks of each investigation. Volunteers were also told that they were free to withdraw from the investigation at any time without reason. Before participation, volunteers also completed a health screen questionnaire to provide information about their medical history (Appendix B) and gave written consent.

3.2 Preliminary tests

3.2.1 Body mass

Body mass was measured using a calibrated beam balance (Avery, England). Subjects were weighed wearing only light clothing in the preliminary trials and nude before and after the main trials. The latter was used to estimate changes in body mass through sweating; therefore, subjects were asked to dry themselves prior to these measurements.
3.2.2 Determination of running economy

Prior to participation in the main trials, subjects were required to complete preliminary exercise tests to determine exercise intensity. The first of these tests was designed to determine the oxygen cost of running at various sub-maximal speeds. Subjects ran for 16 min on a motorised treadmill (Technogym, Italy) during which the running speed increased every 4 min. The initial speed was set between 9 and 11 km h\(^{-1}\) depending on the running ability of the individual and the speed increased by 1 km h\(^{-1}\) at the end of each 4 min stage. Expired air samples, heart rate, monitored by short range telemetry (Technogym, Italy) and rating of perceived exertion (RPE) (Borg 1973) were collected during the final minute of each stage.

3.2.3 Determination of maximal oxygen uptake

A continuous incremental uphill running test on a motorised treadmill was used to determine subjects' maximal oxygen uptake (\(\text{VO}_2\max\)). The treadmill speed remained constant throughout the test and the gradient was increased at 3 min intervals until the subject reached volitional exhaustion. The initial treadmill gradient was set at 3.5\% and was increased by 2.5\% at the end of each 3 min stage. Expired air samples, heart rate and rating of perceived exertion were collected during the last minute of each stage as well as at the point of volitional exhaustion (Williams et al., 1990). A linear regression equation was used to calculate the relationship between running speed and oxygen uptake (\(\text{VO}_2\)). The criteria used to determine whether a true \(\text{VO}_2\max\) had been obtained were: i) an increase in \(\text{VO}_2\) less than or equal to 5 ml kg\(^{-1}\) min\(^{-1}\) with a change of gradient, ii) a respiratory exchange ratio of greater than 1.15, iii) attainment of age predicted maximal heart rate (±10 b.min\(^{-1}\)) all at the end of the test.

3.2.4 Familiarisation run

At least a week before the first main trial in each study, subjects undertook a 45 min treadmill run in order to confirm the relative exercise intensity (% \(\text{VO}_2\max\)) and to familiarise the subjects with the treadmill running and the experimental procedures. In studies 1, 2 and 3 (Chapters 4, 5 and 6), the exercise intensity was 70\% \(\text{VO}_2\max\) and in studies 4, 5 and 6, (Chapters 7, 8 and 9) the intensity was set at 65\% \(\text{VO}_2\max\). Expired air samples were collected every 15 min during the run in order to adjust the
running speed to match the target running intensity for each study

3.3 Main experimental trials

In each study, subjects recorded their dietary intake for 2 days using the weighed food intake method before the first main trial and repeated the same diet before the second trial of that study. Analyses of the energy and nutrient content of their habitual diet were made from these food diaries (Comp-Eat 5.1, Nutrition Systems, England) (See Appendix E for pre-trial nutrient intake). All subjects were asked to refrain from any heavy exercise in the 2 days before each experimental trial. These two requirements were put in place to minimise differences in pre-testing intramuscular substrate concentrations between experimental trials. Subjects were also asked to abstain from caffeine and alcohol consumption for 24 h before all main trials. All trials were performed at the same time of day and under similar experimental and environmental conditions. The same treadmill was also used throughout the experiment (Technogym™ Run Race Treadmill, 47035, Gambettolo, Italy.)

On the day of each experimental trial, subjects arrived in the laboratory at 0800 h following a 12-13 hour overnight fast. After completion of the health questionnaire, the subjects provided a urine sample and then nude body mass was obtained. Subjects then rested on an examination couch and a cannula (Venflon 18G, Sweden) was inserted into an antecubital vein for blood sampling. Subjects remained at rest for 10 min and then a resting venous blood sample was obtained. A fasting 5 min expired air sample was collected. In Chapters 4 and 5, the basal samples were collected whilst the subject was standing on the treadmill. In Chapters 6, 7, 8, and 9, basal samples and were collected whilst the subject was seated. All postprandial samples were collected from the subject whilst seated. The collection times for further blood samples and expired air samples for each study are described in the relevant chapter.

3.4 Test meals

The test meals in all studies reported in this thesis were composed of commercially available foods that were purchased from the same supermarket. The nutritional content of each meal was calculated from information provided by the manufacturer. All glycaemic index values were obtained from The International table of glycemic index and glycemic load values: 2002 (Foster-Powell et al., 2002). Mixed meal GI
values were calculated using the method proposed by Wolever (Wolever & Jenkins 1986), as previously described (Chapter 2 Section 2.6.2). All meals provided 2g CHO·kg\(^{-1}\)·BM and the snacks provided in study 1 (Chapter 4) all provided 1g CHO·kg\(^{-1}\)·BM. Other foods were added to the diet (e.g., cheese and lettuce) to make them more palatable; however, the same quantity was used in both diets. The individual meals were matched for nutrient and energy content between the trials. The content of all the test meals used in the studies in this thesis are presented in Table 3.1.
<table>
<thead>
<tr>
<th>Meal</th>
<th>Description</th>
<th>Ingredients</th>
<th>Macronutrient Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGI breakfast</td>
<td>Corn Flakes &amp; skimmed milk Toast with low fat margarine spread and jam</td>
<td>62g Corn Flakes, 257ml skimmed milk, 80g white bread, 10g flora, 20g jam, 155ml Lucozade Original Drink</td>
<td>730 kcal</td>
</tr>
<tr>
<td></td>
<td>Lucozade Original Drink</td>
<td></td>
<td>139 g CHO</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 g fat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 g protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NSP 1.76 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GI = 77</td>
</tr>
<tr>
<td>LGI breakfast</td>
<td>Muesli with skimmed milk Peelled apple and tinned peaches (in juice) with</td>
<td>86g muesli, 257ml skimmed milk, 67g apple, 103g tinned peaches, 128g yoghurt, 257ml apple juice</td>
<td>732 kcal</td>
</tr>
<tr>
<td></td>
<td>strawberry yoghurt Served with unsweetened apple juice drink</td>
<td></td>
<td>139 g CHO</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 g fat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>23 g protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NSP 7.6 g</td>
</tr>
<tr>
<td>HGI lunch</td>
<td>Grilled fresh turkey breast, grated cheddar cheese and iceberg lettuce</td>
<td>158g white bread, 150g raw turkey breast, 50g cheese, 40g lettuce, 180g banana, 200ml Lucozade Original Drink</td>
<td>1060 kcal</td>
</tr>
<tr>
<td></td>
<td>sandwich served with banana (peeled) and Lucozade Original Drink</td>
<td></td>
<td>147 g CHO</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24 g fat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>64 g protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NSP 4.7 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GI = 44</td>
</tr>
<tr>
<td>LGI lunch</td>
<td>Boiled whole wheat pasta mixed with tomato based pasta sauce, grilled fresh</td>
<td>154g whole wheat pasta (dry weight), 150g raw turkey breast, 50g cheese, 40g lettuce, 185g pasta sauce, 150g pear, 150ml apple juice</td>
<td>1078 kcal</td>
</tr>
<tr>
<td></td>
<td>turkey breast and grated cheddar cheese Lettuce served on the side Pear</td>
<td></td>
<td>145 g CHO</td>
</tr>
<tr>
<td></td>
<td>(peeled and sliced) Served with unsweetened apple juice drink</td>
<td></td>
<td>25 g fat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>70 g protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NSP 10.4 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GI = 34</td>
</tr>
<tr>
<td>LGI dinner</td>
<td>Baked potato (in skin) served with tinned spaghetti in tomato sauce with</td>
<td>255g baked potato, 410g tinned spaghetti, 50g cheese, 40g lettuce, 67g Mars bar, 170ml Lucozade Original drink</td>
<td>1100 kcal</td>
</tr>
<tr>
<td></td>
<td>grated cheddar cheese and lettuce on the side Mars bar and Lucozade</td>
<td></td>
<td>176 g CHO</td>
</tr>
<tr>
<td></td>
<td>Original drink</td>
<td></td>
<td>31 g fat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28 g protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NSP 10.1 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GI = 76</td>
</tr>
</tbody>
</table>
### Chapter 3 General Methods

<table>
<thead>
<tr>
<th>Description</th>
<th>Ingredients</th>
<th>Energy (kcal)</th>
<th>Carbohydrates (g)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LGI dinner</strong></td>
<td>Wheat tortilla filled with tinned mixed beans in a chilli tomato sauce, grated cheddar cheese and sliced iceberg lettuce Served with fresh orange juice</td>
<td>360g chilli beans, 200g wheat tortilla, 50g cheese, 40g lettuce, 260ml orange juice</td>
<td>1100 kcal</td>
<td>176 g CHO</td>
<td>29 g fat</td>
<td>39 g protein</td>
</tr>
<tr>
<td><strong>HGI snacks</strong></td>
<td>White bread toasted with low fat margarine spread and strawberry jam</td>
<td>154g white bread, 40g jam, 20g flora</td>
<td>600 kcal</td>
<td>96 g CHO</td>
<td>17 g fat</td>
<td>15 g protein</td>
</tr>
<tr>
<td><strong>LGI snacks</strong></td>
<td>Pot of strawberry yoghurt, natural oat flapjack and apple (peeled and sliced)</td>
<td>170g yoghurt, 100g apple, 100g flapjack</td>
<td>625 kcal</td>
<td>97 g CHO</td>
<td>25 g fat</td>
<td>15 g protein</td>
</tr>
</tbody>
</table>

#### 3.5 Measurement of expired air samples

In all studies, expired air samples were collected using the Douglas Bag method. During exercise, 1 min expired air samples were collected and during rest the collections were for 5 min. Each sample was collected via a low resistance one-way respiratory valve and a lightweight Falconia tubing (Baxter, Woodhouse and Taylor, UK). The collected samples were analysed for oxygen and carbon dioxide content by passing a small fraction of the sample through a gas analyser (Servomex 1440, England). The analyser was calibrated before use against nitrogen and a mixture of gases of known concentration (16% oxygen and 4% carbon dioxide) (British Oxygen Company, UK). The remaining volume in the Douglas bag was then measured using a dry gas meter (Harvard Apparatus) and the temperature of the gas was measured at the same time using a thermometer (Edale Instruments, model C, UK). The dry gas meter was routinely calibrated using a Hans Rudolph 3 litre syringe (5530 Hans Rudolph Inc, Kansas, Massachusetts, USA). Oxygen uptake ($V_{O_2}$), carbon dioxide production ($V_{CO_2}$), ventilation rate ($VE$) and RER were calculated using the method previously described (Williams et al., 1990). The rate of carbohydrate and fat oxidation were calculated using non-protein stoichiometric equations (Frayn 1983).
CHO oxidation rate (g min\(^{-1}\)) = 4.585 * \(\dot{V}CO_2\) - 3.226 * \(\dot{V}O_2\)
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 amount of fat and CHO oxidised were calculated from the area under the carbohydrate and fat oxidation rate versus time curve during rest and during exercise for each subject.

3.6 Blood sampling

All blood samples were collected from an indwelling cannula (Venflon, 18G, BOC, Ohmeda, Sweden) inserted into the antecubital vein. The cannula was connected to a 3-way stopcock (Connecta Ltd., Sweden) with a 10 cm extension tube. Immediately after insertion and following all blood samples, the cannula was flushed with sterile saline (0.9% Sodium Chloride, Steripak Ltd., UK) to keep it patent. All resting samples were collected from the subject whilst seated.

At each sampling point, 10 ml of blood were collected in Chapters 4, 5, 7, 8 and 9 and 7ml of blood were collected in Chapter 6. Samples were drawn into syringes and immediately dispensed into blood collection tubes (Sarstedt Ltd., UK). Five ml of blood was dispensed into tubes containing the anticoagulant ethylenediaminetetraacetic (EDTA), where aliquots of blood were removed for the determination of haemoglobin, hematocrit and lactate. Tubes were subsequently centrifuged at 3000 rpm for 10 min at 4 °C (Allegra X-22R Centrifuge, Beckman Coulter, Germany) to obtain plasma that was immediately dispensed and frozen at -85 °C. The remainder of the blood sample was dispensed into a non-coated tube, left to clot for 45 min and then centrifuged at 3000 rpm for 10 min at 4 °C to obtain serum. The serum was dispensed and stored at -85 °C for further analysis.

3.7 Blood analysis

All analysis of blood samples was conducted at Loughborough University.

3.7.1 Plasma volume

Duplicate samples (20μl) were removed from EDTA treated blood for the
determination of haemoglobin by the cyanmethaemoglobin method (Boehringer Mannheim, GmbH Diagnostica, Germany) using a spectrophotometer (UV mini 1240, Shimazu, Japan) Haematocrit values were determined in triplicate with a micro-haematocrit reader (Hawksley Ltd, Lancing, UK) following micro-centrifugation for 15 min. Changes in plasma volume were calculated using the haematocrit and haemoglobin values (Dill & Costill 1974).

3.7.2 Blood lactate
Blood lactate concentrations were determined from whole blood (2x 20μl) deproteinised in 200μl of 2.5% perchloric acid and then centrifuged for 3 min at 13000 rpm (Eppendorf centrifuge 5415D, Hamburg, Germany) Blood lactate concentrations were measured using a modified fluorometric method based on that described by Maughan (Maughan 1982) (Appendix F)

3.7.3 Plasma glucose, free fatty acids and glycerol
Plasma glucose (GOD-PAP method, Randox, Ireland), free fatty acids (FFA) (ACS-ACOD method Wako NEFA C, Germany) and glycerol concentrations (GPO-PAP method, Randox, Ireland) were determined using a automatic photometric analyser (Cobas-Mira Plus, Roche Diagnostic Systems, Switzerland).

3.7.4 Serum insulin and cortisol
Serum was analysed for insulin concentrations by radio-immunoassay (Coat-A-Count Insulin ICN Ltd, Eschwege, Germany) using a gamma counter (Cobra 5000, Packard Ltd, Boston, MA, USA). The cross reactivity of the insulin assay calculated at fifty percent trace binding was 100% In Chapters 4, 5 and 6, serum was also analysed for cortisol by radio-immunoassay (Corti-Cote ICN Ltd, Eschwege, Germany) using the same gamma counter. The cross reactivity of the cortisol assay calculated at fifty percent trace binding was 100% The sensitivity of the assay is 0.07μgdl⁻¹ determined at -2 SD from B₀ (n=20).

3.7.5 Intra-assay variation
The coefficient of variation (Standard Deviation/Mean*100) of the blood, plasma and serum assays are shown in Table 3 2 Each coefficient of variation was determined using at least 20 samples
Table 3.2 Intra-assay variation

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lactate</td>
<td>1.5 mmol l⁻¹</td>
<td>1.3</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>5.56 mmol l⁻¹</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>8.40 mmol l⁻¹</td>
<td>0.7</td>
</tr>
<tr>
<td>Plasma FFA</td>
<td>0.6 mmol l⁻¹</td>
<td>0.5</td>
</tr>
<tr>
<td>Plasma glycerol</td>
<td>0.1 mmol l⁻¹</td>
<td>1.0</td>
</tr>
<tr>
<td>Serum insulin</td>
<td>17.8 µIU.ml⁻¹</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>122.4 µIU.ml⁻¹</td>
<td>4.7</td>
</tr>
<tr>
<td>Serum cortisol</td>
<td>9.5 µg dL⁻¹</td>
<td>5.2</td>
</tr>
</tbody>
</table>

3.8 Other measurements

Heart rate
Throughout all preliminary and main trials, heart rate was monitored by short range telemetry (Technogym, Gambettolo, Italy). The subject wore a chest strap and HR was electronically displayed on the treadmill control panel. Heart rate values were then recorded on a data collection sheet.

Rating of perceived exertion (RPE)
Rating of perceived exertion was measured using the Borg scale (Borg 1973) throughout all preliminary tests and main trials

Gut fullness, hunger and thirst measurements
Ratings of gut fullness and hunger were recorded during each expired air sample in all main trials using a 6-20 scale (Appendix G)

Urine collection and analysis
Urine samples were collected on the morning of each trial and analysed for osmolality
using a cryoscopic osmometer (Gonometer 030, Gonotec, Germany) to assess whether participants were euhydrated on arrival in the laboratory. Adequate hydration was assumed for osmolality values below 900 mosmol kg\(^{-1}\) (Shirreffs & Maughan 1998)

**Environmental conditions**
A whirling hygrometer (Zeal, London, UK) was used to measure dry and wet bulb temperature every 30 min during each main trial. Barometric pressure was recorded on the morning of each trial from a wall-mounted barometer (Griffin and George BHL 340X)
4 Improved recovery from prolonged exercise following the consumption of low glycaemic index carbohydrate meals

4.1 Introduction

It is well documented that the ability to perform prolonged exhaustive exercise is closely related to muscle glycogen stores (Bergstrom et al., 1967, Coyle et al., 1986). Many athletes train or compete on consecutive days and therefore the rapid restoration of muscle glycogen stores during the recovery period is essential. The intake of carbohydrate (CHO) after exercise has repeatedly been shown to increase muscle glycogen repletion (Blom et al., 1987; Ivy 1998, Tsintzas et al., 2003). Therefore the consumption of high carbohydrate food or drinks after exercise is now common practice.

Carbohydrate foods can be defined according to their postprandial glycaemic responses (Jenkins et al., 1981). The ingestion of high glycaemic index (HGI) CHO is generally associated with high blood glucose and insulin concentrations. It is logical that carbohydrate sources with a HGI would enhance post-exercise glycogen resynthesis (Burke et al., 2004). Several studies have examined the effect of HGI CHO drinks consumed during recovery from exercise (Ivy 1998; Wong & Williams 2000, Jentjens et al., 2001) however few have examined the effects of carbohydrate meals. In practice, athletes are more likely to eat a combination of foods, especially if the recovery time is longer than a few hours. Burke and colleagues have shown that consuming HGI CHO meals during a 24 h recovery period from prolonged heavy exercise resulted in higher muscle glycogen resynthesis than following the consumption of an isoenergetic low glycaemic index (LGI) diet (Burke et al., 1993). It would be reasonable to assume that endurance capacity would be greater during subsequent exercise, however this was not investigated.

Therefore, the aim of the present study is to investigate the effects of HGI and LGI CHO meals consumed during a 24 h recovery period (i.e. feeding during 12h post-exercise followed by a 12h fast) after glycogen depleting exercise on endurance capacity and the metabolic responses during exercise the following day.
4.2 Methods

Subjects
Nine recreational male athletes participated in this study. Their mean (± SD) age, height, weight and VO\textsubscript{2} max were 22.4 ± 1.5 years, 180.0 ± 1.0 cm, 79.4 ± 10.9 kg and 61.0 ± 5.7 ml. kg\textsuperscript{-1} min\textsuperscript{-1} respectively. A criterion for inclusion in the study was that participants ran regularly and were able to run for at least one hour continuously at about 70% VO\textsubscript{2} max. The protocol was approved by Loughborough University Ethical Advisory Committee and all subjects gave their written informed consent.

Experimental design
Each subject participated in two experimental trials separated by at least 7 days. Preliminary tests and main trial procedures are described in Chapter 3. The experimental testing protocol was completed over a 2-day period. A schematic representation of the experimental protocol is shown in Figure 4.1. On Day 1 of the experimental trial, each subject completed a glycogen reduction protocol, which consisted of a 90 min constant pace treadmill run at 70% VO\textsubscript{2} max (R1). Venous blood samples and 1 min expired air samples were collected every 15 min. Thereafter, subjects were provided with a recovery diet that provided at least 8g CHO kg\textsuperscript{-1} body mass (BM) over the next 22 h and was composed of either HGI or LGI carbohydrates, as described in Table 3.1. The total energy intake for each subject was the sum of the calculated energy expenditure during the 90 min run (average energy cost of 1454 kcal) and an estimation of average daily energy intake from weighed food intake diaries completed before the first trial. Subjects ate their prescribed breakfast 30 min after the cessation of R1. Subjects were then free to leave the laboratory but returned for lunch and were given their evening meal and snacks to eat at home. Subjects were instructed not to eat anything other than the food provided for them. No extra drinks, apart from water, were permitted.

On Day 2, subjects were required to run to exhaustion at 70% VO\textsubscript{2} max (R2). As in R1, venous blood and expired air samples were collected every 15 min for 90 min. No further measurements were taken after this time until the last minute of running when...
an expired air sample was collected. The last venous blood sample was taken immediately after termination of the run while the subjects were standing on the treadmill. Exhaustion was defined as the time at which the subjects were no longer able to maintain the prescribed running speed. Near the end of the run, when subjects signalled that they were unable to run for much longer, subjects had the option of slowing down the treadmill twice for 2 min each time to a walking speed of 4 kph. This was to ensure that subjects were truly fatigued. Each subject repeated the same procedure for the subsequent trial. A balanced randomisation of the trials was applied for the subjects and they were not informed of their running times or the hypothesis being tested.

Ambient temperature and relative humidity were recorded every 30 min using a hygrometer (Zeal, London, UK) during the main trials. Temperature was maintained between 22-25°C and humidity was between 54-59%. Fans were used to cool the subjects and wet sponges were available *ad libitum* throughout all runs.

**Statistical analysis**

Analysis of variance (ANOVA) for repeated measures on two factors (experimental treatment and time) was used to analyse differences in the physiological and metabolic responses in both trials. If a significant interaction was obtained, a Holm-Bonferroni step-wise post-hoc test was utilised to determine the location of the variance. Statistical analysis was carried out on data for the first 90 min of R2. The point of exhaustion was analysed separately due to the large variation in the time that this occurred. A Student’s paired t-test was used to analyse differences at this point and for non-time dependent variables.

Differences were considered significant at *p*<0.05. All results are presented as mean ± SEM.
Figure 4.1 Schematic representation of the experimental protocol
4.3 Results

Running time and subjective observations
All subjects completed the 90 min glycogen reduction run on Day 1 of both trials. Seven out of the eight subjects who completed both experimental trials ran longer following the LGI carbohydrate recovery diet compared to the HGI recovery diet. The average running time in the LGI trial (108.9 ± 7.5 min) was significantly longer than the HGI trial (96.9 ± 4.8 min) \(p<0.05\).

All subjects verbally reported feeling hungry before the start of the run to exhaustion following the consumption of the HGI recovery diet. This observation was not reported following the LGI diet despite the fact that both diets were matched for energy and nutrient content.

Heart rate and RPE
There was a consistent tendency for heart rate to be 2-3 beats \(\text{min}^{-1}\) lower during R2 in the LGI trial compared to the HGI trial however no statistically significant differences were reported. There was a trend for RPE to be higher in the HGI trial and subjects verbally reported that the LGI trial ‘felt easier’ (Table 4.1).

Substrate utilisation
There were no significant differences in substrate utilisation during R1. During R2, there was a main effect of trial for the estimated rate of fat oxidation to be higher in the LGI trial compared to the HGI trial \(p<0.05\) (Figure 4.2). Carbohydrate oxidation rates were higher in the HGI trial compared to the LGI trial however this difference did not reach statistical significance (Figure 4.3). At the point of exhaustion, fat oxidation rates were significantly higher in the LGI trial compared to the HGI trial \(p<0.05\). However, there were no significant differences in carbohydrate oxidation rates.

\(\dot{V}O_2, \dot{V}CO_2, \text{ and Respiratory Exchange Ratio (RER)}\)

The indirect calorimetry data indicated a stable \(\dot{V}O_2\) during both experimental trials \((40.9 - 43.7 \text{ ml kg}^{-1} \text{ min}^{-1})\) and elevated ~71 ± 2 \% \(\dot{V}O_2\) max (Table 4.2). Average
RER values were higher in the HGI trial compared to the LGI trial during the first 90 min of R2 ($p<0.05$) and at the point of exhaustion (Table 4 2).

Plasma glucose, FFA and glycerol

Plasma glucose concentrations were equally well maintained between 4 and 5 mmol·l$^{-1}$ over both runs in both trials. At the point of exhaustion in R2, plasma glucose concentrations were higher in the HGI trial however there were no significant differences between the trials (Figure 4.4). In both trials, plasma concentrations of FFA and glycerol rose progressively throughout exercise. During the first 30 min of recovery after R1, FFA concentrations continued to rise and increased from $0.84 \pm 0.16$ mmol·l$^{-1}$ at the end of exercise to $1.19 \pm 0.15$ mmol·l$^{-1}$ in the HGI trial and from $0.75 \pm 0.08$ mmol·l$^{-1}$ to $1.16 \pm 0.16$ mmol·l$^{-1}$ in the LGI trial. During the first 90 min of R2, there was a main trial effect for FFA concentrations to be higher in the LGI compared to the HGI trial ($p<0.05$). At the point of exhaustion, FFA concentrations were also significantly higher in the LGI trial ($p<0.05$) (Figure 4.5). There were no significant differences in glycerol concentrations during R2 but a consistent trend for higher concentrations in the LGI trial (Figure 4.6).

Serum insulin and cortisol

Serum insulin concentrations were similar between trials (Figure 4.7). In both trials, serum cortisol concentrations fell from pre-exercise values during the first 30 min of running and then increased gradually throughout the remainder of the run. At the point of exhaustion, cortisol concentrations were slightly higher in the LGI trial but this is probably due to the fact that most subjects ran for longer in this trial. There were however no significant differences between trials throughout the experimental protocol (Figure 4.8).

Blood lactate

During R1 there were no differences in blood lactate concentrations between trials (average concentrations were $2.17 \pm 0.18$ mmol·l$^{-1}$ in the HGI trial and $2.01 \pm 0.12$ mmol·l$^{-1}$ in the LGI trial). Throughout R2, there was a main effect of trial for blood lactate concentrations to be higher in the HGI trial compared to the LGI trial ($p<0.05$) (Figure 4.9). Pre-exercise values were similar between trials however in the HGI trial,
blood lactate concentrations rose to approximately 1.9 mmol·l\(^{-1}\) and were maintained around this concentration throughout the run compared to the LGI trial in which concentrations were maintained around 1.7 mmol·l\(^{-1}\). There were no differences between trials at the point of exhaustion.

*Body mass and hydration status*

At the end of R1 subjects had lost 0.3 ± 0.4% and 0.3 ± 0.3% of their pre-exercise body mass in the HGI and LGI trials respectively (NS). At the end of R2, subjects had lost 0.3 ± 0.4% of their pre-exercise body mass in the HGI trial and 0.2 ± 0.7% in the LGI trial (NS). Pre-exercise body mass was not different between trials. There were no significant differences in urine osmolality before or after R1 and R2 between trials. Only one subject started the run to exhaustion with a urine osmolality that suggested dehydration however, this was the same subject who failed to complete the experimental protocol due to injury.
4.4 Discussion

The main finding of the present study was that the ingestion of a LGI carbohydrate recovery diet consumed in the 24 h period following prolonged heavy exercise resulted in a greater endurance capacity during steady state exercise in the post-absorptive state the following day compared with a HGI carbohydrate diet.

During recovery from exercise, high glycaemic index foods are generally recommended to athletes because the large glycaemic and insulinaemic response following their ingestion favours muscle glycogen resynthesis. Nevertheless, research on the effects of the glycaemic index of carbohydrate feedings during 24 h recovery is limited and the results of studies investigating muscle glycogen resynthesis are equivocal. Burke and colleagues reported that a HGI CHO diet resulted in greater muscle glycogen resynthesis than an isoenergetic LGI CHO diet. Muscle biopsies were performed 24 h after exercise however no measure of performance was carried out at the end of the recovery period (Burke et al., 1993) Køns and colleagues attempted to study carbohydrate foods and muscle glycogen storage on the basis of actual glycaemic responses to the foods (Køns et al., 1990) They reported that at 20 h of recovery there were no differences in muscle glycogen storage between the LGI and HGI diets. However, the diets are described interchangeably as simple / HGI CHO and complex / LGI CHO therefore the results cannot be directly compared to the results reported by Burke and colleagues (Burke et al., 1993).

The higher rate of fat oxidation during the run to exhaustion in the LGI trial may explain why subjects ran longer. Plasma FFA concentrations were also significantly higher in the LGI trial compared to the HGI trial during R2. An improvement in endurance performance has been reported when plasma FFA concentrations have been elevated (Lambert et al., 1997; Pitsiladis et al., 1999) and investigators have suggested glycogen sparing as the main reason for this improvement (Jeukendrup et al., 1998) Despite this, the methods used to increase FFA concentrations have not involved normal dietary practice. Furthermore, although fat supplementation both before and during exercise has been shown to increase fat oxidation, this is usually
regarded as undesirable as endogenous fat stores are adequate and make additional fat supplementation inappropriate (Jeukendrup et al., 1998)

It is unclear exactly why fat oxidation rates were higher during run 2 in the LGI trial compared to the HGI trial. Pre-exercise substrate availability has emerged as an important regulator of the patterns of fuel oxidation during exercise (Arkinstall et al., 2004) therefore this may provide a possible explanation. A recent study carried out by Arkinstall and co-workers reported that there was a greater utilisation of muscle glycogen during a 60 min run at 70% VO$_2$ max when pre-exercise muscle glycogen concentrations were high (Arkinstall et al., 2004) The authors also reported that there was a greater contribution from carbohydrate oxidation to total energy expenditure when commencing exercise with high glycogen concentrations. In the present study, the higher rate of fat oxidation and consequently lower carbohydrate oxidation rate in the LGI trial may therefore be a result of lower pre-exercise muscle glycogen concentrations compared to the HGI trial.

It is widely accepted that muscle glycogen is the primary fuel source during prolonged exercise (Romijn et al., 1993) There is now accumulating evidence to suggest that intramuscular triacylglycerol (IMTG) also functions as an important substrate source during prolonged exercise in healthy subjects (van Loon 2004). Several studies have reported a reduction of IMTG stores during prolonged exercise (Krassak et al., 2000; Brechtel et al., 2001, Johnson et al., 2003, van Loon et al., 2003a) therefore, the repletion of both muscle glycogen and muscle fat stores during the recovery period needs to be taken into consideration. Recent research has reported that a high CHO diet consumed after exercise inhibits the post-exercise resynthesis of IMTG because increased FFA concentrations are required for the replenishment or elevation of IMTG content (Johnson et al., 2003) Decombez and colleagues reported that a high CHO, low fat diet (70% CHO, 14% fat and 14% protein of total energy) fed during the 30h preceding a 2h run at 50% VO$_2$ max resulted in IMTG stores 5-17% lower than pre-exercise values (Decombez et al., 2000) Similarly, Starling and colleagues reported that a high CHO diet fed during the 24 h period following 120 min cycling at 65% VO$_2$ max resulted in significantly lower IMTG concentrations than when a high fat diet was consumed (Starling et al., 1997)
Interestingly, Kiens and Richter (Kiens & Richter 1998) and more recently, Kimber and colleagues (Kimber et al., 2003) both reported a decrease in IMTG concentrations during recovery from glycogen depleting exercise despite a large intake of CHO (8-10g CHO kg⁻¹BM·day⁻¹). Both studies reported that muscle glycogen repletion has such high metabolic priority during recovery that utilisation of lipids is essential to cover the energy expenditure in muscle. To date, no studies have investigated whether changing the type of carbohydrate consumed during recovery from exercise can influence IMTG replenishment. Many studies have observed larger glycaemic and insulinaemic responses and a greater suppression of fat metabolism following a HGI CHO meal compared to a LGI CHO meal (DeMarco et al., 1999; Wee et al., 1999b; Wu et al., 2003). Therefore, it is possible that FFA concentrations were higher throughout the recovery period in the LGI trial. This may have allowed some resynthesis of the IMTG stores as well as the replenishment of muscle glycogen as a result of the carbohydrate intake. This may help to explain the increased fat oxidation and endurance capacity in the LGI trial.

The effects of a number of other variables that may have influenced running performance in the HGI trial can be ruled out. For example, there were no significant differences in the physiological and metabolic responses to R1 between trials; therefore the glycogen reduction runs were similar. Furthermore, there were no differences in hydration status either at the end of R1 or at the start of R2 between trials. Plasma glucose, serum insulin and cortisol concentrations were also similar during R2 in both trials. Blood lactate concentrations were significantly higher during R2 in the HGI trial compared to the LGI trial. This would be expected as HGI CHO induce a higher rate of glycolysis than LGI CHO foods (Stannard et al., 2000). Blood lactate concentration did not, however, exceed 2.3mmol L⁻¹ in the HGI trial and so it is unlikely that this was a limiting factor to exercise performance.

In conclusion, the results of the present study show that the consumption of a LGI CHO diet in the 24h following prolonged running increased endurance capacity the next day beyond that which was achieved following the consumption of a HGI CHO recovery diet. A higher rate of fat oxidation throughout the run to exhaustion in the LGI trial is a possible explanation for this increase in endurance capacity. The results
of the present study also suggest that maximal muscle glycogen resynthesis should not be the only aim of post-exercise nutrition following prolonged exercise.
Table 4.1 Heart rate (HR) and rate of perceived exertion (RPE) during the high glycaemic index (HGI) and the low glycaemic index (LGI) CHO trials (Mean ± SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>HR (beats min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGI</td>
<td>156 ± 3</td>
<td>161 ± 5</td>
<td>164 ± 4</td>
</tr>
<tr>
<td>LGI</td>
<td>154 ± 2</td>
<td>161 ± 2</td>
<td>156 ± 3</td>
</tr>
<tr>
<td>RPE</td>
<td>13 ± 0</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>HGI</td>
<td>13 ± 0</td>
<td>14 ± 0</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>LGI</td>
<td>13 ± 0</td>
<td>14 ± 0</td>
<td>14 ± 1</td>
</tr>
</tbody>
</table>
Table 4.2 Oxygen Uptake ($\text{VO}_2$) carbon dioxide expired ($\text{VCO}_2$) and the respiratory exchange ratio during the high glyceamic index (HGI) and the low glyceamic index (LGI) CHO trials (Mean ± SEM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>Resting Period Day 1</th>
<th>Exercise Period Day 1</th>
<th>Resting Period Day 2</th>
<th>Exercise Period Day 2</th>
<th>Point of Fatigue Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{VO}_2)</td>
<td>HGI</td>
<td>0.38 ± 0.03</td>
<td>3.35 ± 0.09</td>
<td>0.40 ± 0.03</td>
<td>3.33 ± 0.08</td>
<td>3.31 ± 0.11</td>
</tr>
<tr>
<td>(l min(^{-1}))</td>
<td>LGI</td>
<td>0.42 ± 0.04</td>
<td>3.32 ± 0.09</td>
<td>0.41 ± 0.03</td>
<td>3.36 ± 0.08</td>
<td>3.34 ± 0.11</td>
</tr>
<tr>
<td>(\text{VCO}_2)</td>
<td>HGI</td>
<td>0.35 ± 0.03</td>
<td>3.06 ± 0.11</td>
<td>0.36 ± 0.03</td>
<td>3.28 ± 0.11</td>
<td>3.28 ± 0.11</td>
</tr>
<tr>
<td>(l min(^{-1}))</td>
<td>LGI</td>
<td>0.38 ± 0.05</td>
<td>2.98 ± 0.10</td>
<td>0.36 ± 0.03</td>
<td>2.97 ± 0.10</td>
<td>3.26 ± 0.12</td>
</tr>
<tr>
<td>RER</td>
<td>HGI</td>
<td>0.90 ± 0.04</td>
<td>0.91 ± 0.02</td>
<td>0.88 ± 0.04</td>
<td>0.91* ± 0.01</td>
<td>0.90* ± 0.01</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.87 ± 0.03</td>
<td>0.90 ± 0.01</td>
<td>0.86 ± 0.02</td>
<td>0.88 ± 0.01</td>
<td>0.87 ± 0.01</td>
</tr>
</tbody>
</table>

*values significantly higher in the HGI trial than in the LGI trial (p<0.05)
Figure 4.2 The rate of fat oxidation (g min⁻¹) during run 2 in the HGI and LGI trials (Mean ± SEM). Main trial effect for fat oxidation to be higher during the first 90 min in the LGI trial than in the HGI trial. *LGI trial significantly higher than HGI trial (p<0.05). (φ Point of exhaustion)
Figure 4.3. The rate of CHO oxidation (g mm$^{-1}$) during run 2 in the HGI and LGI trials (Mean ± SEM). (ø Point of exhaustion)
Figure 4.4 Plasma glucose concentrations (mmol l⁻¹) during run 2 in the HGI and LGI trials (Mean ± SEM). (¶ Point of exhaustion)
Figure 4.5 Plasma free fatty acid (FFA) concentrations (mmol·l⁻¹) during run 2 in the HGI and LGI trials (Mean ± SEM). Main trial effect for FFA concentrations to be higher during the first 90 min in the LGI trial than in the HGI trial. *LGI significantly higher than HGI (p<0.05). (φ Point of exhaustion)
Figure 4.6. Plasma glycerol concentrations (mmol l\(^{-1}\)) during run 2 in the HGI and LGI trials (Mean ± SEM) (ϕ Point of exhaustion)
Figure 4.7 Serum insulin concentrations (µU ml⁻¹) during run 2 in the HGI and LGI trials (Mean ± SEM). (φ Point of exhaustion)
Figure 4.8. Serum cortisol concentrations (µg dl⁻¹) during run 2 in the HGI and LGI trials (Mean ± SEM). (φ Point of exhaustion)
Figure 4.9 Blood lactate concentrations (mmol L⁻¹) during run 2 in the HGI and LGI trials (Mean ± SEM). Main trial effect for blood lactate concentrations to be higher in the HGI than in the LGI trial (p<0.05) (φ Point of exhaustion).
5 The metabolic responses to high carbohydrate meals with different glycaemic indices consumed during recovery from prolonged strenuous exercise

5.1 Introduction

It is well established that the onset of fatigue during prolonged strenuous exercise is associated with muscle glycogen depletion (Tsintzas & Williams 1998). During the post-exercise recovery period, muscle glycogen repletion has high metabolic priority (Kiens & Richter 1998). Nutritional interventions during this time therefore focus on methods of enhancing this process so that performance in a subsequent exercise bout is not impaired.

When no carbohydrate (CHO) is consumed during recovery from prolonged exercise, very little muscle glycogen resynthesis will occur (Ivy et al., 1988). Research has therefore focussed on the amount, type and timing of carbohydrate supplementation required to optimise muscle glycogen resynthesis. Athletes are usually encouraged to ingest carbohydrates immediately after exercise rather than hours later. A major reason for this is so that they take advantage of the acute effects of exercise per se to stimulate both glucose transport and glycogen synthase activity, promoting faster muscle glycogen resynthesis (Wojtaszewski et al., 2003a). High glycaemic index (HGI) carbohydrates are recommended due to the high insulinaemic and glycaemic responses that occur following their consumption. The early research in this area focused on single foods or drinks only. In reality, athletes are more likely to consume mixed meals, especially if the recovery time is greater than a few hours. Few studies have investigated the effects of carbohydrate meals with different glycaemic indices on recovery from prolonged exercise. Burke and colleagues reported that the ingestion of HGI CHO meals resulted in higher muscle glycogen resynthesis compared to the resynthesis following the ingestion of LGI meals during a 24h recovery period (Burke et al., 1993). Interestingly, the author suggested that the differences in the glycaemic and insulinaemic responses to the meals were not sufficient to explain the differences in muscle glycogen resynthesis in the two trials. It was later hypothesised that the LGI CHO may have been mal-absorbed thus providing a lower net amount of CHO for muscle glycogen resynthesis (Burke et al., 1996) however
this hypothesis was not directly tested. It would be expected that endurance capacity during subsequent exercise would be greater following the HGI diet but no assessment of endurance capacity was carried out in this study. In contrast, the results of the study presented in Chapter 4 showed that endurance capacity was increased following a LGI recovery diet in comparison to a HGI recovery diet.

The purpose of the present study was therefore to further investigate the metabolic responses to HGI and LGI CHO meals consumed during the early period of recovery from prolonged strenuous exercise.

5.2 Methods

Subjects
Eight male, well-trained recreational athletes participated in this study. Their mean (± SD) age, height, weight and VO\textsubscript{2} max were 22.5 ± 2.3 years, 181.0 ± 1.0 cm, 72.4 ± 9.7 kg and 64.5 ± 9.3 ml. kg\textsuperscript{-1} min\textsuperscript{-1} respectively. A criterion for inclusion in the study was that participants ran regularly and were able to run for at least one hour continuously at about 70% VO\textsubscript{2} max. Loughborough University Ethical Advisory Committee approved the protocol and all subjects gave their written informed consent.

Experimental design
Preliminary tests and main trial procedures are described in Chapter 3. The experimental protocol can be seen in Figure 5.1. Briefly, each subject participated in two experimental trials separated by at least 7 days. On each occasion, subjects completed a glycogen reduction protocol, which consisted of a 90 min constant pace treadmill run at 70% VO\textsubscript{2} max (R1). This was followed by a 5 h recovery period in which subjects were provided with two recovery meals consisting of either HGI or LGI carbohydrates as described in Table 3.1. The first meal was provided 30 min after the cessation of R1 and the second meal was provided 2 h later. A balanced randomisation of the trials was applied for the subjects.
One-minute expired air samples and venous blood samples were collected every 30 min throughout the 90 min run. Heart rate was closely monitored and RPE was also recorded every 30 min. A final venous blood sample was collected 30 min after the cessation of R1 and then subjects ate their prescribed breakfast. During the recovery period, subjects remained in the laboratory at rest. Ten ml venous blood samples and 5 ml expired air samples were taken at 15, 30, 60, 90 and 120 min after each meal. During each collection of expired air, ratings of gut fullness and hunger were recorded using 6-20 scales. Subjects were instructed not to eat anything other than the food provided for them. No extra drinks, apart from water, were permitted.

Ambient temperature and relative humidity were recorded every 30 min using a hygrometer (Zeal, London, UK) during the main trials. Temperature was maintained between 21-23°C and humidity was between 54-59%.

The incremental area under the curve for plasma glucose and serum insulin was estimated using the methodology described by Wolever and colleagues (Wolever & Jenkins 1986). The blood sample collected at 30 min post-exercise was used as the baseline concentration.

Statistical analysis
Analysis of variance (ANOVA) for repeated measures on two factors (experimental treatment and time) was used to analyse differences in the physiological and metabolic responses in both trials. If a significant interaction was obtained, a Holm-Bonferroni step-wise post-hoc test was utilised to determine the location of the variance. Differences were considered significant at \( p<0.05 \). All results are presented as mean ± SEM.
Figure 5.1 Schematic representation of the experimental protocol

Key:
- C: Cannulation
- W: Weight
- UR: Urine Sample
- VS: Venous Sample
- EA: Expired Air Sample
- Crossed lines: Warm up
- Plain lines: Run
5.3 Results

Heart rate and rating of perceived exertion
There were no significant differences in heart rate (HR) or rating of perceived exertion (RPE) during RI between trials (Table 5.1).

Body Mass and Hydration Status
There were no significant differences in urine osmolality before RI and none of the values suggested that any of the participants began exercise in a dehydrated state (632 ± 116 mosmol.kg\(^{-1}\) and 598 ± 113 mosmol kg\(^{-1}\) in the HGI and LGI trials respectively) At the end of RI subjects had lost 0.13 ± 0.38 and 0.18 ± 0.59 % of their pre-exercise body mass in the HGI and LGI trials respectively (NS).

Plasma volume
There was a significant decrease in plasma volume by the end of the exercise bout in both trials (4.8 ± 1.9% and 5.1 ± 2.2% in the HGI and LGI trials respectively) (p<0.05) however there were no differences between the trials During the postprandial periods, plasma volume was restored to pre-exercise levels.

Plasma glucose and serum insulin
Following ingestion of the HGI and LGI breakfasts, plasma glucose concentrations increased rapidly and peaked at 15 min during the postprandial period in both conditions. The peak concentration following the HGI breakfast was greater than following the LGI breakfast (7.8 ± 0.3 mmol.l\(^{-1}\) and 6.0 ± 0.4 mmol l\(^{-1}\) respectively) (p<0.01) (Figure 5.2). Thereafter, plasma glucose concentrations decreased in both trials until lunch. Nevertheless, values remained higher in the HGI trial than the LGI trial until 90 min into the postprandial period (p<0.05) Plasma glucose concentrations did not fall below pre-exercise fasting values in either trial Following lunch, the increase in plasma glucose concentrations was considerably less than that seen following breakfast. Again, the peak plasma glucose concentrations occurred at 15 min after the meal in both trials and was larger in the HGI trial compared to the LGI trial (5.9 ± 0.3 mmol l\(^{-1}\) and 5.1 ± 0.2 mmol.l\(^{-1}\)
respectively) \((p<0.05)\). Following this peak, plasma glucose concentrations remained stable in the LGI trial however in the HGI trial, a decline was again seen with concentrations falling below fasting concentrations by 120 min after the meal.

In both trials, serum insulin concentrations peaked 30 min after breakfast \((66.9 \pm 7 \mu\text{IU ml}^{-1} \text{ and } 55.1 \pm 5.9 \mu\text{IU ml}^{-1} \text{ in the HGI and LGI trials respectively})\) (NS) and then declined for the rest of the postprandial period (Figure 5.3). Throughout the last hour of the postprandial period following breakfast, serum insulin concentrations were higher in the HGI trial compared to the LGI trial \((p<0.01)\). In comparison to the response to breakfast, the serum insulin response to the lunch was considerably greater in the HGI trial and considerably lower in the LGI trial. Peak insulin concentrations occurred 15 min after the meal in both trials, however the peak was considerably greater in the HGI trial than the LGI trial \((113.8 \pm 13.7 \mu\text{IU ml}^{-1} \text{ and } 38.4 \pm 2.9 \mu\text{IU ml}^{-1} \text{ respectively})\) \((p<0.005)\). Following the peak, serum insulin concentrations declined rapidly in the HGI trial but remained fairly constant in the LGI trial. Despite this, concentrations remained significantly higher throughout the postprandial period following lunch in the HGI trial compared to the LGI trial \((p<0.01)\).

The incremental area under the curve (IAUC) for both glucose \((235.9 \text{ mmol l}^{-1} \times 120 \text{ min vs. } 115.6 \text{ mmol l}^{-1} \times 120 \text{ min})\) and insulin \((5435 \mu\text{IU ml}^{-1} \times 120 \text{ min vs. } \ 3440 \mu\text{IU ml}^{-1} \times 120 \text{ min})\) over the postprandial period following breakfast was significantly greater in the HGI trial compared to the LGI trial \((p<0.005)\). Following lunch, the IAUC for insulin was significantly greater in the HGI trial compared to the LGI trial \((3483 \mu\text{IU ml}^{-1} \times 120 \text{ min vs. } \ 1250 \mu\text{IU.ml}^{-1} \times 120 \text{ min})\) \((p<0.005)\) however no differences in the IAUC for plasma glucose were seen \((29.42 \text{ mmol l}^{-1} \times 120 \text{ min vs. } \ 23.01 \text{ mmol l}^{-1} \times 120 \text{ min})\) (NS).

**Serum Cortisol**

Throughout the postprandial period following breakfast there were no differences in serum cortisol concentrations between trials however there was a trend for concentrations to be higher in the HGI trial. Following lunch, serum cortisol concentrations were
significantly higher at 15, 30 and 60 min during the postprandial period in the LGI trial (p<0.05) (Figure 5.4).

**Plasma free fatty acids (FFA) and glycerol**

In both trials, plasma FFA concentrations peaked 30 min after exercise (i.e. just before breakfast was consumed). Following both the HGI and LGI meals, FFA concentrations were reduced however there was a trend for concentrations to be higher in the LGI trial compared to the HGI trial (p=0.06) This trend was observed throughout the whole recovery period (Figure 5.5). Plasma glycerol concentrations peaked at 90 min of exercise and again were suppressed following consumption of both the HGI and LGI meals. Once more, there was a trend for plasma glycerol concentrations to be higher throughout the recovery period in the LGI trial compared to the HGI trial (p=0.08) (Figure 5.6).

**Blood Lactate**

At the end of R1, blood lactate concentrations were 1.6 ± 0.2 mmol.l⁻¹ and 1.5 ± 0.2 mmol.l⁻¹ in the HGI and LGI trials respectively (NS). At 15 min and 30 min during the postprandial period following breakfast, blood lactate concentrations were significantly higher in the LGI trial compared to the HGI trial (p<0.05). Following lunch, blood lactate concentrations increased slightly however there were no differences between trials (Figure 5.7).

**Estimated carbohydrate and fat oxidation rates**

Throughout the recovery period there were no significant differences in the estimated rate of fat (Figure 5.8) and carbohydrate (Figure 5.9) oxidation between trials. During the first 30 min of the postprandial period following breakfast, there was a trend for fat oxidation to be higher and carbohydrate oxidation to be lower in the HGI trial than the LGI trial but this did not persist throughout the remainder of the postprandial period.

Following lunch, there was a trend for fat oxidation to be higher and carbohydrate oxidation to be lower in the LGI trial. This is reflected in the slightly lower respiratory
exchange ratio (RER) values throughout this time (0.90 ± 0.10 and 0.87 ± 0.10 in the HGI trial and LGI trial respectively) (NS) (Table 5.2)

**Gut fullness and hunger scales**

Subjects reported significantly higher ratings of gut fullness ($p<0.05$) and significantly lower ratings of hunger ($p<0.05$) throughout the recovery period in the LGI trial compared to the HGI trial (Table 5.1).

### 5.4 Discussion

The recovery of skeletal muscle from exercise is critical so that performance during subsequent exercise is not impaired. Central to this recovery process is the resynthesis of muscle glycogen stores. To facilitate muscle glycogen resynthesis, it is recommended that HGI carbohydrates are consumed immediately after exercise as the ingestion of HGI foods is associated with high blood glucose and insulin concentrations (Burke *et al.*, 2004). In the present study, participants were fed a high carbohydrate HGI or LGI breakfast 30 min after the cessation of prolonged strenuous exercise. Following ingestion of the two meals there were no differences in the insulinaemic responses during the first hour of the postprandial period. This finding is in agreement with Burke and colleagues who also reported that a HGI and a LGI meal consumed immediately post exercise produced a insulin response that was independent of the GI of the foods (Burke *et al.*, 1993).

When muscle glycogen is severely reduced following prolonged strenuous exercise, resynthesis becomes a metabolic priority in the recovery period. Two phases of muscle glycogen resynthesis have been observed during the post-exercise period (Price *et al.*, 1994). On completion of exercise an initial, rapid insulin-independent phase of glycogen repletion occurs followed by a more prolonged insulin-dependent phase (Kuo *et al.*, 2004). During the insulin-dependent phase, the muscle demonstrates a marked increase in the sensitivity and responsiveness of glucose transport and glycogen resynthesis to insulin (Kuo *et al.*, 2004). This is made possible by an increased availability of GLUT-4 transporter proteins and an increase in the activation of the enzyme glycogen synthase.
Chapter 5

(Wojtaszewski et al., 2003b). The similar insulin responses to the HGI and LGI
breakfasts observed in this study may therefore be explained by an increase in insulin
sensitivity following the exercise bout. Acute exercise has previously been shown to
decrease the insulin response to an oral glucose tolerance test suggesting that peripheral
insulin sensitivity is increased (Young et al., 1989). Indeed, the insulin concentrations
observed in this study were considerably lower than concentrations observed when the
same meals were consumed without previous exercise (See Chapter 6)

In the present study, plasma glucose concentrations were significantly higher 15 min after
breakfast in the HGI trial compared to the LGI trial. This is in contrast to the results from
the study by Burke and colleagues (1993), who reported that the meal provided
immediately after exercise produced a large glycaemic response that was independent of
the GI of the foods eaten. The author suggested that this effect be due to selective hepatic
insulin insensitivity

The second meal consumed during the recovery period resulted in very different plasma
glucose and serum insulin responses compared to those following breakfast. The
incremental area under the curve (IAUC) for serum insulin following the HGI lunch was
significantly greater than the response to the LGI lunch however there were no
differences in the IAUC for plasma glucose. The exercise-induced changes in insulin
sensitivity of muscle glucose transport are linked to carbohydrate availability in the post-
exercise period (Wojtaszewski et al., 2002). Muscle glycogen itself is thought to be an
important regulator of enhanced insulin action on glucose metabolism following exercise
(Wojtaszewski et al., 2003b). Therefore a high carbohydrate intake early in the post-
exercise recovery period increases muscle glycogen resynthesis and may reduce insulin
sensitivity. Despite this, it has been reported that muscle glycogen concentrations alone
cannot entirely explain the changes in insulin sensitivity after exercise (Cartee et al.,
1989). This has been clearly demonstrated by the fact that rodents continue to show
enhanced insulin sensitivity beyond the point of full glycogen resynthesis (Cartee et al.,
1989). Although the insulin response to the HGI lunch was significantly greater than the
LGI lunch, the responses to both meals were still lower than the responses to the same

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meals observed when no exercise was carried out (See Chapter 6) This suggests that despite a large intake of carbohydrate during the recovery period, enhanced insulin sensitivity may persist but to a lesser extent than when no carbohydrate has been consumed.

The frequency of carbohydrate feedings during the first 4-6 h following prolonged exercise has been shown to influence the rate of muscle glycogen resynthesis. Several studies have reported that carbohydrate feedings provided at 15-30 min intervals result in higher rates of muscle glycogen resynthesis compared to less frequent feedings (Van Hall et al., 2000, van Loon et al., 2000a). This has been attributed to the higher sustained insulin and glucose profiles achieved by such a feeding protocol. In the present study, the meals were provided 2 h apart and a reduction in plasma glucose and serum insulin concentrations were observed between the meals. As discussed however, the second HGI meal resulted in a larger insulin response than the LGI meal. The GI of the post-exercise feedings may therefore not be so important if the carbohydrate is given in small frequent feedings over a short recovery period. However when meals are provided at longer time intervals, the GI of the carbohydrates consumed greatly affects the metabolic responses to the meals and perhaps therefore muscle glycogen resynthesis.

Following run 1, FFA and glycerol concentrations increased during the first 30 min of recovery however the ingestion of breakfast suppressed these metabolites in both trials. High insulin concentrations suppress FFA mobilisation (Horowitz et al., 1997) and inhibit the rate of long-chain fatty acid transport into mitochondria for β-oxidation (Sidossis & Wolfe 1996). In the present study, the reduction in the concentration of FFA was not as marked as may be expected This is probably due to the fact that the approximate energy expenditure of the 90 min run was 1300 kcal and the test breakfasts provided only approximately 730 kcal (70kg person). Participants were therefore in a negative energy balance throughout the first part of the recovery period. Other studies have reported high FFA concentrations throughout the post-exercise recovery period despite a large intake of carbohydrate (Kiens & Richter 1998; Kimber et al., 2003). The depletion of muscle glycogen from the previous exercise creates a fuel deficit in the
muscle. It is hypothesised by the authors that the exogenous glucose is directed to the muscle for glycogen synthesis and FFA are therefore required for oxidative muscle metabolism (Kiens & Richter 1998). Following lunch, there was a trend for free fatty acid and glycerol concentrations to be higher in the LGI trial than the HGI trial. The higher insulin concentrations in the HGI trial may have suppressed free fatty acid and glycerol concentrations to a greater extent than in the LGI trial. There was also a trend for the estimated fat oxidation rate to be higher and estimated carbohydrate oxidation rate to be lower in the LGI trial. These results provide support for the possible mechanisms described in Chapter 4 explaining the increased endurance capacity following the LGI recovery diet.

Throughout the postprandial period following the ingestion of the HGI and LGI breakfasts, blood lactate concentrations were elevated but were significantly higher in the LGI trial. Several studies have reported elevated blood lactate concentrations following the ingestion of fructose (Koivisto et al., 1981, Moore et al., 2000). Following the ingestion of fructose, about two thirds is converted to glucose and the rest of the metabolised fructose is released from the liver as lactate (Henry et al., 1991). In the present study, the LGI breakfast contained more fructose (25g/70kg body mass) than the HGI breakfast (11g/70kg body mass) and therefore this may explain the significantly higher blood lactate concentrations in the postprandial period. No differences in blood lactate concentrations were seen following the ingestion of lunch however very little fructose was present in these meals.

In both trials, serum cortisol concentrations were highest at the end of the exercise period and remained high throughout the first two hours of recovery. As previously mentioned, the energy content of the first meal was insufficient to replace the energy expended during the exercise bout. All the subjects were therefore in a negative energy balance throughout the first part of the recovery period and therefore this is likely to have caused high cortisol concentrations.
Throughout the postprandial period following both meals, the sensation of gut fullness was reported to be higher in the LGI trial and ratings of hunger were lower. This was despite the fact that the meals were isoenergetic and contained the same nutrient composition. Several studies have reported higher satiety ratings following the ingestion of LGI foods compared to HGI foods (Brand-Miller et al., 2002; Ball et al., 2003). This is of importance to those who want to lose weight as maintaining a sense of satiety for a longer period may help to reduce voluntary food intake.

In conclusion, the results of the present study provide further evidence that insulin sensitivity is increased following an acute bout of exercise. Additionally, the results suggest that the glycaemic index of the carbohydrates consumed immediately after exercise may not be important as long as sufficient carbohydrate is consumed and perhaps provided as small frequent feedings. However, the GI of the carbohydrates consumed later in the post-exercise period may be important due to their influence on substrate oxidation. The results of this study suggest that a LGI diet may be more beneficial for continued utilisation of fat during the recovery period. Although muscle glycogen was not measured in the current study, it would be reasonable to speculate that the high insulin concentrations following a HGI meal may facilitate further muscle glycogen resynthesis later in the recovery period.
Table 5.1 Heart Rate (HR), Rate of Perceived Exertion (RPE) Gut fullness (GF) and hunger ratings during the HGI and LGI trials
(Mean ± SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>Exercise</th>
<th>Postprandial Period Meal 1</th>
<th>Postprandial Period Meal 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>HR (beat. min⁻¹)</td>
<td>HGI</td>
<td>158 ± 4</td>
<td>164 ± 4</td>
<td>167 ± 4</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>159 ± 4</td>
<td>161 ± 4</td>
<td>164 ± 5</td>
</tr>
<tr>
<td>RPE</td>
<td>HGI</td>
<td>12 ± 0</td>
<td>13 ± 0</td>
<td>14 ± 0</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>12 ± 0</td>
<td>13 ± 0</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>Gut Fullness</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>Hunger</td>
<td>HGI</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 5.2 Oxygen uptake (VO$_2$), carbon dioxide production (VCO$_2$) and respiratory exchange ratio (RER) during the HGI and LGI trials (Mean ± SEM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>Resting</th>
<th>Exercise Period</th>
<th>Postprandial Period</th>
<th>Postprandial Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meal 1</td>
<td>Meal 2</td>
</tr>
<tr>
<td>VO$_2$ (l.min$^{-1}$)</td>
<td>HGI</td>
<td>0.36 ± 0.02</td>
<td>3.26 ± 0.10</td>
<td>0.39 ± 0.01</td>
<td>0.40 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.31 ± 0.02</td>
<td>3.25 ± 0.10</td>
<td>0.40 ± 0.01</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>VCO$_2$ (l.min$^{-1}$)</td>
<td>HGI</td>
<td>0.32 ± 0.01</td>
<td>2.91 ± 0.09</td>
<td>0.33 ± 0.01</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.39 ± 0.01</td>
<td>2.89 ± 0.11</td>
<td>0.35 ± 0.01</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>RER</td>
<td>HGI</td>
<td>0.86 ± 0.02</td>
<td>0.89 ± 0.01</td>
<td>0.85 ± 0.01</td>
<td>0.90 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.82 ± 0.01</td>
<td>0.89 ± 0.01</td>
<td>0.87 ± 0.01</td>
<td>0.87 ± 0.01</td>
</tr>
</tbody>
</table>
Figure 5.2 Plasma glucose concentrations (mmol l⁻¹) in the HGI and LGI trials (Mean ± SEM). * HGI trial significantly higher than LGI trial ($p<0.05$).
Figure 5.3 Serum insulin concentrations (µU ml⁻¹) in the HGI and LGI trials (Mean ± SEM). †HGI trial significantly higher than LGI trial (p<0.01).
Figure 5.4 Serum cortisol concentrations (µg dL⁻¹) in the HGI and LGI trials (Mean ± SEM) *LGI trial significantly higher than HGI trial (p<0.05)
Figure 5.5. Plasma FFA concentrations (mmol l⁻¹) during the recovery period in the HGI and LGI trials (Mean ± SEM).
Figure 5.6 Plasma glycerol concentrations (μmol·l⁻¹) during the recovery period in the HGI and LGI trials (Mean ± SEM)
Figure 5.7 Blood lactate concentrations (mmol l⁻¹) in the HGI and LGI trials (Mean ± SEM). *LGI trial significantly higher than HGI trial (p<0.05).
Figure 5.8 Estimated rate of fat oxidation (g.min⁻¹) during the recovery period in the HGI and LGI trials (Mean ± SEM).
Figure 5.9. Estimated rate of carbohydrate oxidation (g min⁻¹) during the recovery period in the HGI and LGI trials (Mean ± SEM)
Chapter 6

6 The influence of the glycaemic index of breakfast and lunch on substrate utilisation during the postprandial periods and subsequent exercise.

6.1 Introduction

The ingestion of carbohydrate (CHO) before exercise is beneficial as it increases both muscle and liver glycogen stores (Nilsson & Hultman 1973). Nevertheless, a high CHO intake also alters the metabolic responses and substrate utilisation during exercise (Coyle et al., 1997). A number of studies have observed a depression in the rate of fat oxidation following CHO ingestion due to hyperinsulinaemia in the postprandial period (Horowitz et al., 1997, Wee et al., 1999b, Wu et al., 2003). However, altering the type of CHO consumed has been shown to have an effect on the magnitude of hyperinsulinaemia and therefore depression of fat oxidation (Wee et al., 1999b; Wu et al., 2003).

It has repeatedly been shown that the consumption of a low glycaemic index (LGI) CHO results in lower insulinaemic and glycaemic responses during rest in the postprandial period compared to when a high glycaemic index (HGI) CHO is consumed. Several studies have therefore manipulated the GI of pre-exercise feedings and have reported a higher rate of fat oxidation and a better maintenance of plasma glucose concentrations during subsequent exercise after ingesting a single LGI CHO food compared to a high HGI CHO (Thomas et al., 1991; Thomas et al., 1994; Febbraio & Stewart 1996; Sparks et al., 1998, Wee et al., 1999b).

The metabolic responses to single foods with a HGI or LGI are clear; however it is not common practice to eat single foods at meal times in daily life. Research into the metabolic responses to mixed meals containing foods with different GI values is fairly limited despite its obvious applicability to real-life situations. It has previously been reported that the GI concept lacks clinical utility because the differences in glycaemic indexes between foods are lost once these foods are consumed in a mixed meal (Coulston et al., 1987). A
study carried out by DeMarco and colleagues compared the postprandial glycaemic, insulinaemic and physiological responses to pre-exercise mixed meals composed of either HGI or LGI CHO. No differences in the glycaemic responses were reported between the meals however the total energy, fat and protein content of the two test meals were not matched (DeMarco et al., 1999). The addition of fat to a carbohydrate meal enhances insulin secretion but also decreases the plasma glucose response (Collier et al., 1984). Similarly, adding protein to a carbohydrate meal increases the insulin secretion without augmenting glucose concentrations (Pt-Sunyer 2002). Therefore to compare mixed meals accurately, it is important that the non-carbohydrate sources in the meals are matched.

The effect of consuming mixed breakfasts containing either HGI or LGI CHO on substrate utilisation during exercise performed 3h later was investigated by Wu and colleagues. Both breakfasts were energy and nutrient matched. In agreement with studies carried out on single foods, the HGI meal resulted in a significantly greater glycaemic and insulinaemic response during the postprandial period. It was also reported that the calculated amount of fat oxidation was significantly higher during exercise commencing 3 h after consuming the LGI mixed meal compared to when the HGI mixed meal was consumed (Wu et al., 2003).

To date, no studies have been carried out to investigate the effects of two HGI or LGI mixed CHO meals on resting postprandial metabolism and substrate utilisation during subsequent exercise. Many individuals exercise in the afternoon after work and will therefore consume breakfast and lunch before the exercise session. Based on previous research already described, it would be reasonable to expect consistently higher glycaemic and insulinaemic responses to HGI mixed meals compared to LGI mixed meals. It is also hypothesised that the rate of fat oxidation would be higher during exercise following two LGI meals compared to two HGI meals. Therefore the aim of the present study was to investigate the effects of changing the GI of the CHO within two mixed meals (breakfast and lunch) on the postprandial metabolic responses and substrate utilisation during rest and during a subsequent 60-min run at 70% V0₂ max.
6.2 Methods

Subjects
Nine male recreational athletes participated in this study. Their mean (± SD) age, height, weight and VO\textsubscript{2}max were 23.7 ± 2.1 years, 177.0 ± 10 cm, 74.3 ± 7.0 kg and 64.6 ± 5.6 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} respectively. Loughborough University Ethical Advisory Committee approved the protocol and all subjects gave their written informed consent.

Experimental Design
Preliminary tests and main trial procedures are described in Chapter 3. A schematic representation of the experimental protocol can be seen in Figure 6.1. Each subject participated in two experimental trials separated by a week. On each occasion, subjects were provided with two meals (breakfast and lunch) which were both followed by 3 h rest. Following the 3 h postprandial period after lunch, subjects ran for 60 min on a motorised treadmill at 70% VO\textsubscript{2}max. The test meals were composed mainly of either HGI or LGI CHO (see Table 3.1) and the order of the trials was randomised. All blood samples taken during the postprandial period were obtained from the subject whilst seated. In addition to the basal blood sample, further samples were taken at 15, 30, 60, 90, 120, and 180 min after each meal and at 15 min intervals during exercise. Samples of expired gas were collected pre-meal and at 15, 30, 60, 90, 120, and 180 min throughout the postprandial periods. Expired gas samples were also collected at 15 min intervals throughout the run. Running samples were collected for 1 min and resting samples for 5 min.

Ambient temperature and relative humidity were recorded at 30 min intervals during the postprandial period and at 15 min intervals throughout the run using a hygrometer (Zeal, London, UK). Temperature was maintained between 16-22 °C and humidity was 50-60% in all trials.
Total carbohydrate and fat oxidation was estimated from the area under the rate of oxidation v. time curve for each subject.

**Statistical analysis**

Analysis of variance (ANOVA) for repeated measures on two factors (experimental treatment and time) was used to analyse differences in the physiological and metabolic responses in both trials. If a significant interaction was obtained, a Holm-Bonferroni step-wise post-hoc test was applied to determine the location of the variance. Differences were considered significant at $p<0.05$. All results are presented as mean ± SEM.
Figure 6.1 Schematic representation of the experimental protocol
6.3 Results

**Plasma glucose and serum insulin**

Following ingestion of the HGI and LGI breakfasts, plasma glucose concentrations increased and peaked at 15 min during the postprandial period (6.89 ± 0.31 mmol·l⁻¹ and 5.1 ± 0.4 mmol·l⁻¹ respectively) \((p<0.01)\) (Figure 6.2). Thereafter, plasma glucose concentrations decreased below fasting values by 30 min in the LGI trial and 60 min in the HGI trial and continued to decrease throughout the postprandial period. Following the ingestion of the HGI and LGI lunch, plasma glucose concentrations again peaked at 15 min during the postprandial period (6.96 ± 0.39 mmol·l⁻¹ and 5.42 ± 0.28 mmol·l⁻¹ respectively) \((p<0.05)\). Following this peak, a sharp decline in plasma glucose concentrations was observed in both trials however, during the 2 h before the start of exercise, plasma glucose concentrations were equally maintained at approximately 5 mmol·l⁻¹. During the first 30 min of exercise, there were no differences in plasma glucose concentrations between trials. At 45 min, plasma glucose concentrations were higher in the LGI trial compared to the HGI trial (5.47 ± 0.13 and 4.91 ± 0.18 mmol·l⁻¹ respectively) \((p<0.01)\). At the end of the 60-min run, plasma glucose concentrations were still higher in the LGI trial compared to the HGI trial (5.69 ± 0.09 and 4.92 ± 0.16 mmol·l⁻¹ respectively) \((p<0.001)\).

In both trials, serum insulin concentrations peaked at 15 min during the postprandial period following breakfast (HGI 133.3 ± 19.3 and LGI 92.7 ± 10.0 μU·ml⁻¹) \((p<0.05)\) (Figure 6.3). Serum insulin concentrations then declined throughout the postprandial period but were higher in the HGI trial than in the LGI trial at 120 min \((p<0.05)\). Following the ingestion of lunch, serum insulin concentrations again peaked at 15 min during the postprandial period. Once again, the peak was greater in the HGI trial compared to the LGI trial (125.7 ± 10.9 and 72.2 ± 9.0 μU·ml⁻¹ respectively) \((p<0.05)\). Throughout the rest of the postprandial period following lunch, serum insulin concentrations decreased but remained higher in the HGI trial compared to the LGI trial \((p<0.05)\). No differences were observed between trials once exercise commenced.
**Plasma free fatty acids and glycerol**

Plasma free fatty acid (FFA) concentrations were reduced following the consumption of both the HGI and LGI breakfasts and remained reduced throughout both postprandial periods (Figure 6.4). Throughout the exercise period, plasma FFA increased gradually in both trials however no differences between the trials were seen. Plasma glycerol concentrations showed a similar response to that of plasma FFA. Once again, there were no significant differences between the trials (Figure 6.5).

**Blood lactate**

Following ingestion of breakfast, blood lactate concentrations were significantly higher in the LGI trial compared to the HGI trial for the first 90 min of the postprandial period (p<0.01) (Figure 6.6). No differences were observed between trials following lunch or throughout the 60-min run.

**Estimated carbohydrate and fat oxidation rates**

There were no differences in the estimated total amount of CHO or fat oxidised throughout the postprandial period following breakfast. In contrast, following lunch, the calculated total amount of CHO oxidised was higher in the HGI trial than in the LGI trial (HGI 71.7 ± 3.4, LGI 58.9 ± 3.3 g 3h⁻¹) (p<0.005) (Figure 6.7) and the estimated total amount of fat oxidised was higher in the LGI than in the HGI trial (HGI, 9.0 ± 1.1, LGI 12.5 ± 1.1 g 3h⁻¹ (p<0.01) (Figure 6.8).

During the exercise period, there were no differences in the total amount of CHO oxidised (HGI, 215.7 ± 8.0, LGI, 214.4 ± 12.6 g h⁻¹) or total amount of fat oxidised (HGI, 11.5 ± 2.3, LGI, 10.9 ± 2.5 g h⁻¹).

**Heart rate and rate of perceived exertion**

There were no significant differences between heart rate and rate of perceived exertion between the trials (Table 6.3).

**Hydration status and plasma volume**

There were no significant differences in urine osmolality at the start of each trial (592 ± 118 mosmol kg⁻¹ and 631 ± 113 mosmol kg⁻¹ in the HGI and LGI trials).
respecively) Using a cut off point of 900 mosmol kg\(^{-1}\) (Shirreffs & Maughan 1998), none of the values suggested that any of the participants began the trials in a dehydrated state. There were no significant differences in plasma volume between trials.

_Gut fullness and hunger scales_

Ratings of perceived hunger were significantly lower during the postprandial period following the LGI lunch compared to the HGI lunch \((p<0.05)\). There were no significant differences in gut fullness between trials (Table 6.2).

### 6.4 Discussion

The main aim of the present study was to investigate the metabolic responses to HGI and LGI mixed meals. The metabolic responses to single foods with different GI values are now well understood, however there is a lack of information on the responses to mixed meals with nutrient compositions that are clearly within prevailing norms (Ludwig & Jenkins 2004). During the postprandial periods following both meals, plasma glucose concentrations and serum insulin concentrations were significantly higher in the HGI trial compared to the LGI trial. The validity of the GI values of mixed meals has been questioned in several studies (Coulston et al., 1984, Hollenbeck et al., 1988). The results from the present study show that significant differences in hyperglycaemia and hyperinsulinaemia can repeatedly be achieved by changing the GI of the CHO in a mixed meal. This therefore offers support for calculation of the GI values for mixed meals suggested by Wolever and Jenkins (Wolever & Jenkins 1986).

The postprandial metabolic responses to carbohydrate-dense HGI foods such as those described above, have been used to provide a possible explanation as to why low fat diets have not lived up to their potential to inhibit weight gain when consumed ad libitum (Brand-Miller et al., 2002). Postprandial hyperglycaemia and hyperinsulinaemia promotes postprandial CHO oxidation at the expense of fat oxidation, thus altering fuel partitioning that may be conducive to body fat gain. Low
GI diets have therefore been promoted as an effective weight control method as they minimize postprandial insulin secretion therefore promoting fat oxidation.

In the present study, the amount of fat oxidised during the postprandial period following lunch was significantly higher in the LGI trial compared to the HGI trial. This is in contrast to previous studies investigating pre-exercise feeding and GI which reported no differences in substrate oxidation at rest during the postprandial period following a single LGI food or meal (Wee et al., 1999b; Wu et al., 2003). It is important to highlight however that previous studies in this area have only investigated the metabolic responses to a single meal or one portion of a single food and the differences in the present study were only apparent after the second meal. It is not possible to speculate on the chronic effects of a LGI diet from this data however the results of this study provide evidence that changes in fuel partitioning and substrate oxidation can occur even over a single day when consuming LGI CHO instead of HGI CHO.

Low glycaemic index foods are also reported to be beneficial for weight loss because their consumption has been reported to result in prolonged feelings of satiety and therefore reduced hunger and food intake (Ludwig et al., 1999, Warren et al., 2003). In the present study, ratings of gut fullness and hunger were recorded at regular intervals following breakfast and lunch in both trials. Hunger ratings were significantly lower in the LGI trial compared to the HGI trial despite the meals being matched for energy and nutrient content.

As reported in Chapter 5, blood lactate concentrations were significantly elevated following the ingestion of the LGI breakfast whereas the increase was minimal following the HGI breakfast. Several studies have reported that CHO that have high fructose concentrations result in higher blood lactate concentrations (Koivistio et al., 1981; Moore et al., 2000). Once fructose enters the cell, it is rapidly converted to fructose-1-phosphate (F1P). The high concentrations of F1P inhibit the degradation of glycogen and facilitate the production of lactate (Henry et al., 1991). About two thirds of fructose is converted to glucose and the rest of the metabolised fructose is released from the liver as lactate (Henry et al., 1991). In the present study, the LGI breakfast contained more fructose (25g/70kg body mass) than the HGI breakfast (11g/70kg body mass).
body mass) This may therefore explain the higher blood lactate concentrations in the LGI trial during the postprandial period. Similar blood lactate results were also reported by Wu et al who used similar breakfasts to those used in the present study (Wu et al., 2003).

The second aim of the present study was to investigate the effects of the glycaemic index of breakfast and lunch on substrate utilisation during a subsequent 60-min run at 70% $\dot{V}O_2$ max. A previous study from our laboratory reported that the calculated rate of fat oxidation was significantly higher during 60 min exercise commencing 3 h after consuming a LGI meal compared to the fat oxidation following a HGI meal (Wu et al., 2003). In the present study, two LGI meals were provided in the 6 h before the exercise bout however, the calculated rate of fat oxidation during exercise was not different compared to when two HGI meals were provided. It is important to note that the exercise intensity in the present study was higher than in the study carried out by Wu et al (70% and 65% $\dot{V}O_2$ max respectively). The exercise intensity that elicits the maximal rate of fat oxidation has recently been reported to be between approximately 50% and 64% $\dot{V}O_2$ max (Achten et al., 2002). Therefore, it is possible that the exercise intensity in the present study was too high to highlight any differences in substrate oxidation.

At 45 min into the run and on completion of the 60 min, plasma glucose concentrations were significantly higher in the LGI trial compared to the HGI trial. Other studies investigating the effect of the GI of pre-exercise feedings have reported higher plasma glucose concentrations towards the end of an exercise session following a LGI meal (Thomas et al., 1991, Thomas et al., 1994, DeMarco et al., 1999). Low GI CHO theoretically release glucose from the gut at a slower rate and therefore for an extended period (DeMarco et al., 1999). Hence, this would allow maintenance of blood glucose for a longer period of time compared to HGI CHO.

The clinical relevance of the glycaemic index has been vigorously debated in recent years (Ludwig 2002) and some believe that the concept may be too complicated to be practical. The results of the present study show that the GI concept can be successfully applied to mixed meals that would be consumed in a real-life setting.
Although no differences in substrate oxidation were seen during exercise at the end of the day, the results of the present study show that the GI of the meals consumed at breakfast and lunch can alter substrate oxidation during the postprandial periods whilst at rest.
Table 6.1 Oxygen uptake (\( \text{VO}_2 \)), carbon dioxide production (\( \text{VCO}_2 \)) and respiratory exchange ratio (RER) during the HGI and LGI trials (Mean ± SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>Postprandial Period</th>
<th>Postprandial Period</th>
<th>Exercise Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Period 1</td>
<td>Period 2</td>
<td>1</td>
</tr>
<tr>
<td>( \text{VO}_2 ) (l.min(^{-1}))</td>
<td>HGI</td>
<td>0.31 ± 0.02</td>
<td>0.35 ± 0.01</td>
<td>0.40* ± 0.01</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.32 ± 0.02</td>
<td>0.36 ± 0.01</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>( \text{VCO}_2 ) (l.min(^{-1}))</td>
<td>HGI</td>
<td>0.26 ± 0.02</td>
<td>0.32 ± 0.01</td>
<td>0.37* ± 0.01</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.27 ± 0.02</td>
<td>0.33 ± 0.01</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>RER</td>
<td>HGI</td>
<td>0.84 ± 0.02</td>
<td>0.91 ± 0.01</td>
<td>0.92* ± 0.01</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.84 ± 0.02</td>
<td>0.90 ± 0.01</td>
<td>0.89 ± 0.01</td>
</tr>
</tbody>
</table>

*Mean values were significantly different from those in the LGI trial (\( p<0.05 \))

Table 6.2 Gut fullness and hunger during the HGI and LGI trials (Mean ± SEM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>Postprandial Period</th>
<th>Postprandial Period</th>
<th>Exercise Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Period 1</td>
<td>Period 2</td>
<td>1</td>
</tr>
<tr>
<td>Gut fullness</td>
<td>HGI</td>
<td>8 ± 0</td>
<td>10 ± 0</td>
<td>11 ± 1</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>8 ± 1</td>
<td>10 ± 0</td>
<td>11 ± 0</td>
</tr>
<tr>
<td>Hunger</td>
<td>HGI</td>
<td>13 ± 1</td>
<td>11 ± 1</td>
<td>11* ± 1</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>14 ± 1</td>
<td>10 ± 1</td>
<td>9 ± 1</td>
</tr>
</tbody>
</table>

*Mean values were significantly different from those in the LGI trial (\( p<0.05 \))
Table 6.3 Heart rate (HR) and rating of perceived exertion (RPE) throughout the exercise period in the HGI and LGI trials (Mean ± SEM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats min⁻¹)</td>
<td>HGI</td>
<td>158 ± 4</td>
<td>162 ± 4</td>
<td>168 ± 4</td>
<td>164 ± 4</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>162 ± 4</td>
<td>165 ± 4</td>
<td>167 ± 5</td>
<td>168 ± 5</td>
</tr>
<tr>
<td>RPE</td>
<td>HGI</td>
<td>12 ± 1</td>
<td>13 ± 0</td>
<td>13 ± 0</td>
<td>14 ± 1</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>12 ± 0</td>
<td>13 ± 0</td>
<td>13 ± 0</td>
<td>13 ± 0</td>
</tr>
</tbody>
</table>
Figure 6.2 Plasma glucose concentrations (mmol l⁻¹) in the HGI and LGI trials

(Mean ± SEM) * HGI trial significantly higher than LGI trial (p<0.05) † LGI trial significantly higher than HGI trial (p<0.01)
Figure 6.3 Serum insulin concentrations (µIU.ml\(^{-1}\)) in the HGI and LGI trials (Mean ± SEM) * HGI trial significantly higher than LGI trial (p<0.05)
Figure 6.4 Plasma FFA concentrations (mmol l⁻¹) in the HGI and LGI trials (Mean ± SEM).
Figure 6.5 Plasma glycerol concentrations (μmol·l⁻¹) in the HGI and LGI trials

(Mean ± SEM)
Figure 6.6 Blood lactate concentrations (mmol l⁻¹) during the HGI and LGI trials

(Mean ± SEM) †LGI trial significantly higher than HGI trial (p<0.01).
Figure 6.7 Estimated rate of carbohydrate oxidation (g min\(^{-1}\)) during the HGI and LGI trials (Mean ± SEM).
Figure 6.8. Estimated rate of fat oxidation (g min\(^{-1}\)) during the HGI and LGI trials
(Mean ± SEM)
7 The influence of high-carbohydrate mixed meals with different glycaemic indices on substrate utilisation during subsequent exercise in women

7.1 Introduction
Although the glycaemic index (GI) was originally devised to aid diabetics control the glycaemic impact of their diet (Jenkins et al., 1981), the concept now has a wider utility. The GI has been extensively used in sports nutrition to aid athletes in the selection of appropriate carbohydrates to consume before, during and after exercise. More recently, diets composed of low glycaemic index (LGI) carbohydrates have been promoted as an effective weight loss strategy.

Several studies have reported that the pre-exercise ingestion of LGI carbohydrates results in an increased rate of fat oxidation during the exercise bout compared to when HGI carbohydrates are consumed (Thomas et al., 1991; Thomas et al., 1994, Febbraio & Stewart 1996, Sparks et al., 1998; DeMarco et al., 1999; Wee et al., 1999b; Febbraio et al., 2000a, Febbraio et al., 2000b; Wu et al., 2003). The large glycaemic and insulinaemic perturbations accompanying the consumption of HGI foods increases carbohydrate oxidation and blunts the mobilisation and oxidation of free fatty acids (Sidossis & Wolfe 1996, Coyle et al., 1997). Increasing the oxidation of fat at the expense of carbohydrate oxidation has important implications both for endurance trained athletes and for those who are aiming to reduce body fat mass for health purposes. However, many of the previous studies in this area have provided single foods in the hour before exercise (Thomas et al., 1991; Febbraio & Stewart 1996; Sparks et al., 1998, Febbraio et al., 2000b; Stannard et al., 2000) which is unlikely to reflect normal dietary behaviour. Athletes are recommended to consume a high carbohydrate meal 3-4 h before exercise (Hargreaves et al., 2004) and many recreational athletes will exercise at lunchtime 3-4 h after consuming breakfast.

In a recent study from our laboratory, subjects were provided with a HGI or a LGI high carbohydrate breakfast 3 h before exercise (Wu et al., 2003). Although plasma
glucose and serum insulin concentrations had returned to baseline by the start of exercise, the calculated amount of fat oxidised was significantly higher during exercise following the LGI breakfast compared with the fat oxidation following the HGI breakfast.

Interestingly, the majority of the research carried out in this area has used male subjects. This is despite the fact many females exercise to reduce body fat mass or may use the GI to select appropriate carbohydrates to consume before training and competition. Several studies have reported that gender differences exist in the relative contribution from carbohydrate and fat to oxidative metabolism during exercise (Tarnopolsky et al., 1990, Tarnopolsky et al., 1995, Fredlander et al., 1998, Horton et al., 1998; Knechtle et al., 2004, Venables et al., 2005). In contrast, others have reported that there are no differences in the total amount of fat and carbohydrate oxidised by men and women but that different sources of lipid are utilised (Mittendorfer et al., 2002; Roepstorff et al., 2002). Nevertheless, gender differences in the metabolic responses to HGI and LGI meals during rest and subsequent exercise may exist. Therefore, the aim of the present study is to investigate the metabolic responses to HGI and LGI meals during rest and subsequent exercise in women.

7.2 Methods

Subjects

Eight healthy, recreationally active eumenorrheic females participated in this study. Their mean (± SD) age, height, weight and VO₂ max were 18.6 ± 0.9 years, 166.0 ± 3.0 cm, 59.9 ± 7.1 kg and 48.7 ± 1.1 ml kg⁻¹ min⁻¹ respectively. None of the subjects had used oral contraceptives for more than 6 months before the study. All main experimental trials were carried out during the early follicular phase of the menstrual cycle (days 3-7). A criterion for inclusion in the study was that participants ran regularly and were able to run for one hour continuously at about 65% VO₂ max. Loughborough University Ethical Advisory Committee approved the protocol and all subjects gave their written informed consent.
Experimental Design

Preliminary tests and main trial procedures are described in Chapter 3. A schematic representation of the experimental protocol can be seen in Figure 7.1. All subjects completed two experimental trials in a randomised crossover design. Subjects consumed the test meals described 3h before a 60 min run at 65% VO$_{2}$max on a motorised treadmill. The test meals were composed mainly of either HGI or LGI carbohydrates (see Table 3.1) and the order of the trials was randomised. All blood samples taken during the postprandial period were obtained from the subject whilst seated. In addition to the basal blood sample, further samples were taken at 15, 30, 60, 90, 120, and 180 min after each meal and at 15 min intervals during exercise. Samples of expired gas were collected for 5 min pre-meal and at 15, 30, 60, 90, 120, and 180 min throughout the postprandial period. Expired gas samples were also collected for 1 min at 15 min intervals throughout the run.

Ambient temperature and relative humidity were recorded every 30 min using a hygrometer (Zeal, London, UK) during the main trials. Temperature was maintained between 17-21°C and humidity was between 48-60%.

Statistical analysis

Analysis of variance (ANOVA) for repeated measures on two factors (experimental treatment and time) was used to analyse differences in the physiological and metabolic responses in both trials. If a significant interaction was obtained, a Holm-Bonferroni step-wise post-hoc test was utilised to determine the location of the variance. Differences were considered significant at $p<0.05$. All results are presented as mean ± SEM.
Figure 7.1 Schematic representation of the experimental protocol

- Breakfast
- %VO_{2max}

- Overnight
- Fast
- 8am
- 8:30am
- 9am
- 9:15am
- 9:45am
- 10am
- 10:15am
- 10:45am
- 11am
- 11:45am
- 12pm
- 12:15pm
- 12:30pm
- 12:45pm
- 1pm

- C cannulation
- W weight
- UR urine sample
- VS venous blood sample
- EA expired air sample

- 60min run at 65% VO_{2max}
- 5min warm up at 55% VO_{2max}
7.3 Results

**Plasma glucose and serum insulin**

Following the ingestion of both breakfasts, plasma glucose concentrations rapidly increased and peaked at 15 min during the postprandial period. The peak concentration was higher in the HGI trial than in the LGI trial (7.51 ± 0.71 and 5.88 ± 0.19 mmol l⁻¹ in the HGI and LGI trials respectively) \((p<0.05)\) (Figure 7.2). Following this peak, plasma glucose concentrations declined in both trials and fell below fasting values after the first hour of the postprandial period. However, plasma glucose concentrations remained higher in the HGI trial than in the LGI trial throughout the postprandial period and were significantly higher at 90 min and 120 min \((p<0.05)\). Throughout exercise, there were no differences in plasma glucose concentrations between trials and were maintained at ~ 4.5 mmol l⁻¹.

Serum insulin concentrations peaked 15 min after breakfast in both trials however the peak concentration was higher in the HGI trial than in the LGI trial (169.6 ± 16.2 and 125.6 ± 10.8 μIU ml⁻¹ in the HGI and LGI trials respectively) \((p<0.05)\) (Figure 7.3). Serum insulin concentrations then declined throughout the rest of the postprandial period in both trials but remained higher in the HGI trial than in the LGI trial at all time points \((p<0.05)\). During exercise, serum insulin concentrations fell below fasting values in both trials. No differences between the trials were observed.

**Plasma free fatty acid (FFA) and glycerol concentrations**

Following both breakfasts, there was a decline in plasma FFA (Figure 7.4) and glycerol concentrations (Figure 7.5) from fasting values \((p<0.05)\). They remained suppressed throughout the first two hours of the postprandial period. By the third hour of the postprandial period, concentrations of both metabolites had began to increase and concentrations were higher in the LGI trial compared to the HGI trial \((p<0.05)\). Throughout exercise, there was a progressive increase in both plasma FFA and glycerol concentrations with time in both trials. However FFA concentrations were higher throughout the LGI trial compared to the HGI trial at all time points \((p<0.05)\) and glycerol concentrations were higher at 30 min and 45 min in the LGI trial \((p<0.05)\)
**Blood lactate concentrations**

Following ingestion of the HGI and LGI breakfasts, blood lactate concentrations increased significantly and peaked at 30 min during the postprandial period \( (p<0.05) \) (Figure 7.6) Throughout the first 2 h of the postprandial period, blood lactate concentrations were higher in the LGI trial than in the HGI trial \( (p<0.05) \) At the start of exercise, blood lactate concentrations had not returned to fasting values in either trial During exercise, there was a trend for blood lactate concentrations to be higher in the HGI trial than in the LGI trial but this difference did not reach statistical significance at any time point.

**Estimated rate of carbohydrate and fat oxidation**

The ingestion of both breakfasts resulted in a slight increase in carbohydrate oxidation and a concomitant decrease in fat oxidation \( (NS) \). There were no differences in the rate of carbohydrate (Figure 7.7) or fat (Figure 7.8) oxidation during the postprandial period between the trials. During exercise, the estimated rate of fat oxidation was significantly higher in the LGI trial than in the HGI trial \( (p<0.05) \) and carbohydrate oxidation was higher in the HGI trial than in the LGI trial \( (p<0.05) \). The total amount of fat oxidised throughout the run was \( 8.3 \pm 2.2 \, \text{g hr}^{-1} \) in the HGI trial and \( 18.7 \pm 4.5 \, \text{g hr}^{-1} \) in the LGI trial \( (p<0.05) \) The total amount of carbohydrate oxidised throughout the 60 min run was \( 101.5 \pm 12.0 \, \text{g hr}^{-1} \) in the HGI trial and \( 70.5 \pm 10.2 \, \text{g hr}^{-1} \) in the LGI trial \( (p<0.05) \) The respiratory exchange ratio values were lower during exercise in the LGI trial compared to the HGI trial \( (p<0.05) \) (Table 7.1).

**Feelings of Gut Fullness**

Following consumption of both breakfasts, ratings of gut fullness increased however, higher ratings were recorded in the LGI trial \( (p<0.05) \). Throughout the postprandial period, ratings of gut fullness remained higher in the LGI trial compared to the HGI trial \( (p<0.05) \) No differences in perceived gut fullness were reported during exercise (Table 7.2)

**Plasma volume changes and hydration status**

There were no significant differences in urine osmolality at the start of each trial. Using a cut off point of 900 mosmol kg\(^{-1}\) none of the values suggested that any of the
participants began the trials in a dehydrated state (672 ± 78 mosmol kg\(^{-1}\) and 594 ± 68 mosmol kg\(^{-1}\) in the HGI and LGI trials respectively). There was a significant decrease in plasma volume during exercise in both trials (10.9 ± 2.2% and 12.5 ± 2.5% in the HGI trial and LGI trial respectively) \((p<0.01)\), however, there were no significant differences between trials.

*Heart rate and rate of perceived exertion*

There were no differences in heart rate or in RPE during the 60 min run between trials (Table 7.2)

### 7.4 Discussion

The main finding of the present study was that the glycaemic index of a pre-exercise carbohydrate meal significantly altered substrate oxidation during subsequent exercise in females. The total amount of fat oxidised during exercise was greater when subjects consumed a LGI breakfast 3 h before exercise compared to a HGI breakfast. The results of this study therefore extend the findings of numerous studies that have reported a higher fat oxidation rate during exercise following a LGI pre-exercise meal or food using male subjects to women (Thomas *et al.*, 1994; Febbraio & Stewart 1996; DeMarco *et al.*, 1999; Wee *et al.*, 1999a; Wu *et al.*, 2003).

It has previously been reported that when a LGI breakfast is consumed 3 h before exercise, less carbohydrate is stored as muscle glycogen compared to when a HGI breakfast is consumed (Wee *et al.*, 1999a). A 15% increase in muscle glycogen concentration was reported at the end of a 3 h postprandial period following the HGI breakfast however, only a small non-significant increase in muscle glycogen was reported following the LGI breakfast. This was accounted for by the low glycaemic and insulinaemic responses to the LGI meal (secondary to slow digestion and absorption of the ingested foods). During exercise, fat oxidation was higher in the LGI trial and a greater rate of carbohydrate oxidation was accompanied by a greater degradation of muscle glycogen during the HGI trial (Wee *et al.*, 1999a). Therefore, the lower CHO oxidation during the LGI trial could be entirely explained by a lower rate of muscle glycogen utilisation.
The GI concept is based on the incremental area under the blood glucose curve following the ingestion of a CHO-rich food compared to glucose (Jenkins et al., 1981). In the present study, the breakfasts were composed of a variety of HGI and LGI foods and so the GI of the meal was calculated using a method described by Wolever et al. (1986). The estimated GI ratio of the two test breakfasts was 1.75 (77/44) and the actual measured ratio of the incremental area under the blood glucose curve 1.82 (71 mmol.l⁻¹ x 180 min / 39 mmol.l⁻¹ x 180 min). The glycaemic responses to mixed meals with different GI values are therefore similar in females and males and the results of this study provide further support for the calculations proposed by Wolever and Jenkins (1986).

Although female subjects have been used in some glycaemic index intervention trials (Liu et al., 2000, Schulze et al., 2004; Sloth et al., 2004), no studies have specifically investigated the metabolic responses to HGI and LGI meals in female subjects whilst controlling for menstrual cycle phase and the use of oral contraceptives. Synthetic steroids used as oral contraceptives have been reported to alter glucose metabolism and insulin sensitivity in women at rest (Jensen & Levine, 1998; Bosseau et al., 2001) and during exercise (Suh et al., 2003). Therefore, oral contraceptive use could have a significant effect on the metabolic responses to foods with differing GI values. In the present study, none of the subjects were taking oral contraceptives or had used oral contraceptives in the last 6 months. However, due to their extensive use, research is necessary to investigate whether oral contraceptives do alter the metabolic responses to HGI and LGI foods.

The menstrual cycle is associated with several metabolic and hormonal variations (Lariviere et al., 1994). Although no differences have been observed across the menstrual cycle in resting substrate oxidation and glucose turnover (Pierson et al., 1995), it has been reported that insulin sensitivity is reduced during the luteal phase of the menstrual cycle (Pulido & Salazar, 1999). Therefore testing female subjects during different phases of the menstrual cycle may have a confounding effect on the insulinaemic responses to HGI and LGI foods.

The effect of the menstrual cycle on fuel metabolism appears to be more pronounced under conditions of metabolic stress, for example during exercise. Some studies have
suggested that there may be greater lipid oxidation and lower carbohydrate oxidation during mild - to moderately high intensity exercise (<75% VO₂ max) performed in the luteal vs. follicular phase of the menstrual cycle (Hackney et al., 1994, Campbell et al., 2001; Zderic et al., 2001), although this has not always been observed (Bailey et al., 2000, Horton et al., 2002). Therefore, although research is not conclusive, it is possible that GI testing in different phases of the cycle could result in differences in substrate oxidation during exercise that is not a consequence of changing the carbohydrates provided before, during or after exercise.

The subjects in this study were all tested during the early follicular phase (3-7 days after the onset of menstruation) of the menstrual cycle to avoid any affect of menstrual cycle phase on the results. Both trials were carried out on the same day of the cycle for individual subjects unless this fell on a weekend. The early follicular phase was chosen as concentrations of both oestrogen and progesterone are at their lowest at this time and therefore are least likely to have an affect on substrate oxidation. For a complete understanding of the metabolic responses to HGI and LGI foods in female subjects, further research is required to investigate whether menstrual cycle phase does alter the glycaemic and insulinaemic responses to foods. This is important to establish the necessity of controlling for sex steroid hormone status when women are included in metabolic studies (Horton et al., 2002).

As previously mentioned, a higher rate of fat oxidation was observed during the LGI trial in the present study. Increasing fat oxidation during exercise is beneficial for those who are exercising in order to lose body fat mass. Although exercise in the fasted state promotes optimal fat oxidation, many find it difficult or impractical to exercise whilst fasted. Consuming a LGI breakfast may therefore be a good compromise. The majority of the pre-exercise feeding studies have fed subjects within the hour before exercise. The time course in the present study would however be realistic for those who consume breakfast and then exercise at lunch time. Although the chronic effects of a LGI diet can not be predicted from a single day study, it would not be unreasonable to assume that a LGI diet combined with regular exercise may be an effective and healthy way to optimise the loss of body fat mass. In the present study, subjects completed a 60 min run at 65% VO₂ max. It is recognised that both the
duration and intensity of this exercise may not be suitable for many individuals however, clear differences in the rate of fat oxidation were obvious after only 15 min of exercise between the trials. Further research is required to investigate whether differences in fat oxidation still exist when exercising at a lower intensity (i.e. walking) and for different durations.

As well as promoting fat oxidation at the expense of carbohydrate oxidation, LGI foods are hypothesised to promote weight regulation by promoting feelings of satiety. In the present study, feelings of gut fullness were recorded throughout the postprandial period and during exercise. In agreement with previous studies, higher ratings of gut fullness were reported during the postprandial period in the LGI trial compared with the HGI trial. This is despite the two meals being isoenergetic and matched for carbohydrate, protein and fat content. Changes in blood glucose concentrations and their effects on satiety have been researched for many years. However, the exact relationship between glycaemic carbohydrates and satiety remains unclear as few studies have made concurrent measurements of blood glucose, appetite and food intake over extended periods and the literature contains contradictory information on the relationship (Anderson & Woodend 2003). Further studies are needed to delineate the role of glycaemic carbohydrates and their mechanisms of action in determining satiety (Anderson & Woodend 2003).

Following the LGI breakfast in the present study, blood lactate concentrations were significantly higher than when the HGI breakfast was consumed. This is a similar response to that reported for male subjects consuming HGI and LGI meals (Chapters 5 & 6). The LGI breakfast used in this study contained more fructose than the HGI breakfast. As previously explained, once fructose enters the cell, it is rapidly converted to fructose-1-phosphate (F1P). The high concentrations of F1P inhibit the degradation of glycogen and facilitate the production of lactate (Henry et al., 1991). The increased lactate production following the LGI breakfast did not however alter lactate concentrations during the exercise period.

In conclusion, the total amount of fat oxidised during exercise was significantly greater when the LGI mixed meal was consumed 3 h previously compared to when the HGI mixed meal was consumed. The metabolic responses to HGI and LGI mixed
meals reported in this study are similar to those previously reported in male subjects. Further research is required to investigate whether this phenomenon occurs during exercise at different intensities and of shorter duration.
Table 7.1 Oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($VCO_2$) and respiratory exchange ratio (RER) in the HGI and LGI trials (Mean ± SEM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</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>0.23 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>1.97* ± 0.10</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.24 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>1.91 ± 0.10</td>
</tr>
<tr>
<td>$VCO_2$ (l min$^{-1}$)</td>
<td>HGI</td>
<td>0.20 ± 0.01</td>
<td>0.25 ± 0.01</td>
<td>1.86* ± 0.09</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.20 ± 0.01</td>
<td>0.26 ± 0.02</td>
<td>1.70 ± 0.11</td>
</tr>
<tr>
<td>RER</td>
<td>HGI</td>
<td>0.86 ± 0.02</td>
<td>0.92 ± 0.01</td>
<td>0.94* ± 0.01</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.84 ± 0.04</td>
<td>0.91 ± 0.03</td>
<td>0.89 ± 0.02</td>
</tr>
</tbody>
</table>

*Values significantly higher in the HGI trial than in the LGI trial ($p<0.05$)
Table 7.2 Heart rate (HR), Rating of perceived exertion (RPE) and gut fullness in the HGI and LGI trials (Mean ± SEM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>FAST</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats min⁻¹)</td>
<td>HGI</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>154 ± 5</td>
<td>162 ± 4</td>
<td>165 ± 5</td>
<td>168 ± 5</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>156 ± 4</td>
<td>162 ± 4</td>
<td>164 ± 4</td>
<td>165 ± 3</td>
</tr>
<tr>
<td>RPE</td>
<td>HGI</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>12 ± 0</td>
<td>13 ± 0</td>
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</tr>
<tr>
<td></td>
<td>LGI</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>Gut Fullness</td>
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<td>13 ± 1</td>
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<td>12 ± 1</td>
<td>11 ± 1</td>
<td>10 ± 1</td>
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<td>10 ± 1</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>8 ± 1</td>
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<td>15* ± 1</td>
<td>14* ± 1</td>
<td>12 ± 1</td>
<td>12* ± 1</td>
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<td>10 ± 1</td>
<td>8 ± 1</td>
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</tr>
</tbody>
</table>

* Values significantly higher in the LGI trial than in the HGI trial (p<0.05)
Figure 7.2. Plasma glucose concentrations (mmol l⁻¹) in the HGI and LGI trials
(Mean ± SEM) * HGI trial significantly higher than LGI trial (p<0.05).
Figure 7.3 Serum insulin concentrations (µIU ml⁻¹) in the HGI and LGI trials (Mean ± SEM). * HGI trial significantly higher than LGI trial (p<0.05).
Figure 7.4. Plasma FFA concentrations (mmol l⁻¹) in the HGI and LGI trials (Mean ± SEM) *LGI trial significantly higher than HGI trial (p<0.05).
Figure 7.5 Plasma glycerol concentrations (µmol·l⁻¹) in the HGI and LGI trials

(Mean ± SEM) *LGI trial significantly higher than HGI trial (p<0.05)
Figure 7.6 Blood lactate concentrations (mmol l⁻¹) in the HGI and LGI trials (Mean ± SEM). *LGI trial significantly higher than HGI trial (p<0.05).
Figure 7.7 Estimated rate of carbohydrate oxidation (g min⁻¹) in the HGI and LGI trials (Mean ± SEM) * HGI trial significantly higher than LGI trial (p<0.05).
Figure 7.8. Estimated rate of fat oxidation (g min⁻¹) in the HGI and LGI trials (Mean ± SEM) *LGI trial significantly higher than HGI trial (p<0.05).
8 The effect of the glycaemic index of an evening meal on the metabolic responses to a standard high glycaemic index breakfast and subsequent exercise in men.

8.1 Introduction

A high carbohydrate meal is recommended 3-4 h before exercise to provide a sustainable source of carbohydrate during the exercise bout (Hargreaves et al., 2004). Despite this, ingestion of carbohydrate in the hours before exercise often results in a transient fall in glucose with the onset of exercise, increased carbohydrate oxidation and a blunting of free fatty acid (FFA) mobilisation (Coyle et al., 1985; Sherman et al., 1989). The glycaemic index has been identified as a useful reference guide for the selection of the ideal CHO supplementation for exercise (Siu & Wong, 2004). A number of studies have examined the effect of consuming carbohydrates with different glycaemic indices before exercise (Thomas et al., 1991; Febbraio & Stewart, 1996, Sparks et al., 1998, DeMarco et al., 1999, Wee et al., 1999b; Wu et al., 2003). Studies investigating the effects on performance have yielded inconsistent results. Thomas and colleagues first reported an increased endurance capacity following ingestion of a LGI CHO 45 min before exercise compared to when a HGI CHO food was consumed (Thomas et al., 1991). Subsequent studies have however reported no differences in endurance capacity (Febbraio & Stewart, 1996, Sparks et al., 1998, Wee et al., 1999b). Despite this discrepancy in the literature, all studies investigating the effects of the GI of a pre-exercise meal have demonstrated lower postprandial glycaemia and insulinaemia following a LGI meal or single food. This is accompanied by higher concentrations of plasma free fatty acids (FFA) and therefore higher rates of fat oxidation during exercise compared to the responses following ingestion of a HGI food (Febbraio & Stewart, 1996, Febbraio et al., 2000a; Febbraio et al., 2000b, Wu et al., 2003).

Low glycaemic index pre-exercise meals are therefore recommended to athletes to maintain plasma glucose and free fatty acid concentrations during exercise without a large stimulation of insulin release in the period before exercise (Thomas et al., 1991).
Despite this, athletes do not always adhere to the recommendations. Feelings of abdominal discomfort are often reported following the ingestion of LGI foods, which may be explained by the high fibre nature of many LGI carbohydrates. From a practical perspective, this is not desirable before a training session or competition.

Studies carried out by Wolever and colleagues (Wolever et al., 1988), Jenkins and colleagues (Jenkins et al., 1982) and more recently Liljeberg and colleagues (Liljeberg et al., 1999), have reported that a single LGI meal can improve glucose tolerance and therefore reduce hyperinsulinaemia at a second meal. The study carried out by Wolever and colleagues reported that this effect even occurred after an overnight fast. It was reported that consumption of a LGI evening meal improved glucose tolerance and therefore reduced the insulin responses to a standard HGI breakfast compared to when a HGI evening meal was consumed (Wolever et al., 1988).

None of the previous studies investigating the second meal effect have considered whether the responses to exercise following the standard HGI breakfast would be different. If a LGI meal can reduce the hyperglycaemia and hyperinsulinaemia following a standard HGI breakfast, a similar effect may be achieved when a LGI pre-exercise meal is consumed and therefore there may be a higher rate of fat oxidation during the exercise bout.

Therefore the aim of the present study was to re-investigate whether a LGI meal consumed in the evening can reduce the glycaemic and insulinaemic response to a HGI standard breakfast compared to a HGI evening meal. The study also aimed to investigate whether the substrate utilisation during a subsequent exercise bout would be affected.

### 8.2 Methods

**Subjects**

Seven male recreational athletes participated in this study. Their mean (± SD) age, height, weight and VO₂ max were 23.0 ± 2.8 years, 179.0 ± 1.0 cm, 74.9 ± 7.4 kg and 61.1 ± 5.0 ml kg⁻¹ min⁻¹ respectively. A criterion for inclusion into the study was that
Chapter 8

participants ran regularly and were able to run for one hour continuously at about 65% \( \text{VO}_2 \text{max} \). Loughborough University Ethical Advisory Committee approved the protocol and all subjects gave their written informed consent.

Experimental design

Preliminary tests and main trial procedures are described in Chapter 3. A schematic representation of the experimental protocol is shown in Figure 8.1. Briefly, each subject participated in two experimental trials separated by at least 7 days. The experimental testing protocol was completed over a 2-day period. On day 1, subjects were instructed not to eat anything after 4pm. At 7pm subjects visited the laboratory and were provided with an evening meal consisting of either HGI or LGI carbohydrates (see Table 3.1). Subjects were instructed not to eat or drink anything apart from water for the rest of the evening. On day 2, subjects arrived at the laboratory following a 13 h overnight fast and were provided with a standard HGI breakfast as described in Table 3.1. Following ingestion of breakfast, subjects remained in the lab at rest for 3 hours. At the end of this postprandial period, they completed a 60 min run at 65% \( \text{VO}_2 \text{max} \). During the postprandial period, the subjects remained in the laboratory at rest. Ten ml venous blood samples and 5 ml expired air samples were taken 15, 30, 60, 90, 120 and 180 min after breakfast. During each collection of expired air, ratings of gut fullness and hunger were recorded using 6-20 scales. No extra drinks, apart from water, were permitted throughout the trial. Water intake was monitored throughout trial one and matched in trial two. During the run 1, min expired air samples and venous blood samples were collected every 15 min.

Ambient temperature and relative humidity were recorded every 30 min using a hygrometer (Zeal, London, UK) during the main trials. Temperature was maintained between 21-23°C and humidity was between 48-56%.

Statistical analysis

Analysis of variance (ANOVA) for repeated measures on two factors (experimental treatment and time) was used to analyse differences in the physiological and
metabolic responses in both trials. If a significant interaction was obtained, a Holm-Bonferroni step-wise post-hoc test was utilised to determine the location of the variance. Differences were considered significant at $p<0.05$. All results are presented as mean ± SEM.
Figure 8.1 Schematic representation of the experimental protocol

Day 1

- Dinner
- Overnight fast

Day 2

- Breakfast
- \%VO_2\max

7pm 8am 8:30am 9am 9:15am 9:45am 10:15am 10:45am 11:45am 12pm 12:15pm 12:30pm 12:45pm 1pm

C cannulation
W weight
UR urine sample
VS venous blood sample
EA expired air sample

- 60 min run at 65% VO_2\max
- 5 min warm up at 55% VO_2\max
8.3 Results

Plasma glucose and serum insulin

Following ingestion of the standard HGI breakfast, plasma glucose concentrations increased significantly above fasting concentrations \((p<0.001)\) and peaked at 15 min during the postprandial period in both trials (Figure 8.2). The peak concentration was higher when the HGI evening meal had been consumed compared to when the LGI evening meal was consumed \((8.1 \pm 0.6 \text{ mmol} \text{l}^{-1} \text{ and } 7.0 \pm 0.4 \text{ mmol} \text{l}^{-1} \text{ respectively}) \ (p<0.05)\). Following this peak, plasma glucose concentrations decreased in both trials however concentrations remained significantly higher in the HGI trial compared to the LGI trial at 60 min and 90 min during the postprandial period \((p<0.05)\) No differences in plasma glucose concentrations were observed throughout the 60 min run.

In both trials, serum insulin concentrations increased significantly after ingestion of the standard HGI breakfast \((p<0.001)\) and again, peaked at 15 min during the postprandial period (Figure 8.3) The peak concentration was higher when the HGI evening meal had been consumed compared to when the LGI evening meal was consumed \((181.6 \pm 12.8 \mu \text{IU ml}^{-1} \text{ and } 144.4 \pm 22.6 \mu \text{IU ml}^{-1} \text{ respectively}) \ (p<0.05)\). Throughout the rest of the postprandial period, serum insulin concentrations declined but remained higher in the HGI trial compared to the LGI trial No differences in serum insulin concentrations were observed throughout the 60 min run.

The incremental area under the curve (IAUC) for plasma glucose during the postprandial period following breakfast was greater in the HGI trial than the LGI trial \((175.2 \pm 67.0 \text{ mmol} \text{l}^{-1} \text{ and } 78.8 \pm 35.0 \text{ mmol.l}^{-1} \times 180 \text{ min respectively}) \ (p<0.01)\). Similarly, the IAUC for serum insulin during the postprandial period was greater in the HGI trial than the LGI trial \((15906 \pm 1609 \mu \text{IU ml}^{-1} \times 180 \text{ min and } 11592 \pm 1234 \mu \text{IU ml}^{-1} \times 180 \text{ min respectively}) \ (p<0.05)\)
Plasma free fatty acids (FFA) and glycerol

Following ingestion of the standard HGI breakfast, plasma FFA (Figure 8.4) and glycerol (Figure 8.5) concentrations were significantly reduced in both trials ($p<0.01$) and concentrations remained below fasting concentrations throughout the postprandial period in both trials ($p<0.005$) No differences in plasma FFA or glycerol concentrations were reported between the trials during the postprandial period During the exercise bout, plasma FFA and glycerol concentrations increased with time ($p<0.05$) however, there were no differences between the trials.

Blood lactate

In both trials, blood lactate concentrations increased significantly after ingestion of the carbohydrate breakfast and remained elevated above fasting concentrations throughout the remainder of both trials ($p<0.001$) No differences between the trials were observed throughout the experimental protocol During the exercise bout, blood lactate concentrations were maintained at about 1.6 mmol l$^{-1}$ in both trials (Figure 8.6)

Respiratory Exchange Ratio (RER) and estimated carbohydrate and fat oxidation rates

Following the ingestion of breakfast, there was no significant change in fat or carbohydrate oxidation from fasting values in either trial Despite this, there was a trend for carbohydrate oxidation to increase and fat oxidation to decrease throughout the postprandial period in both trials In both trials, the rate of fat and carbohydrate oxidation were only significantly different from fasting values during the 60 min run ($p<0.01$). There were no differences in the RER values between trials (Table 8.1) and hence no differences were found for carbohydrate (Figure 8.7) and fat (Figure 8.8) oxidation rates during the postprandial period or the 60 min run.

Gut fullness and hunger scales

Before breakfast was consumed on the morning of day 2, subjects reported significantly higher ratings of gut fullness ($p<0.01$) and significantly lower ratings of hunger ($p<0.05$) in the LGI trial compared to the HGI trial Following the ingestion of breakfast, ratings of gut fullness significantly increased ($p<0.01$) and hunger significantly decreased ($p<0.05$) from fasting values in both trials Significantly
higher ratings of gut fullness were reported in the LGI trial during the postprandial period following breakfast ($p<0.01$). No differences in gut fullness were observed during the subsequent 60 min run. There were no significant differences between trials in feelings of hunger but a trend for hunger ratings to be higher in the HGI trial (Table 8.2).

**Plasma volume changes and hydration status**

There was minimal change in plasma volume throughout the postprandial period in both trials. However, plasma volume was significantly decreased by the end of the exercise bout (-3.82 ± 2.56\% and -2.48 ± 2.96\% in the HGI and LGI trials respectively) ($p<0.05$) but there were no differences between the trials. At the start of each trial, all participants had a urine osmolality that suggested that they were well hydrated (747 ± 84 mosmol.kg$^{-1}$ and 736 ± 88 mosmol.kg$^{-1}$ in the HGI and LGI trials respectively) (NS).

### 8.4 Discussion

The main finding of the present study is that consumption of a LGI evening meal resulted in lower glycaemic and insulinaemic responses to a standard HGI breakfast compared to when a HGI evening meal was consumed. The results of the present study therefore support earlier findings that a single LGI meal can improve glucose tolerance after an overnight fast in healthy males (Wolever *et al.*, 1988). Despite the reduced hyperglycaemia and hyperinsulinaemia in the postprandial period following breakfast in the LGI trial, there were no differences in substrate utilisation during the 60 min run between trials.

Jenkins and colleagues were the first to report that a single LGI CHO food (lentils) eaten at breakfast improved glucose tolerance at lunch compared to when a single HGI CHO food (wholemeal bread) was eaten (Jenkins *et al.*, 1982). The authors reported that the slower rate of absorption of the lentils was responsible for the improved glucose tolerance at the subsequent meal. Evidence against malabsorption of the lentil meal was provided from breath hydrogen data and enteroglucagon concentrations. The findings of this study were later extended by Wolever and colleagues who reported that low glycaemic index foods eaten at dinner improved the
subsequent breakfast glycaemic response (Wolever et al., 1988). Both single foods and mixed meals were investigated in this study and similar results were reported.

Although mixed meals were used in the study carried out by Wolever and colleagues (Wolever et al., 1988), they would not be realistic for an athlete’s diet. The meals used in the present study aimed to provide a normal combination of foods that athletes may choose to eat. Nevertheless, differences in the glycaemic response to breakfast were still observed. No blood samples were taken following the evening meal in the present study. The test meals used in the present study were the same as those used in Chapter 6 and ingestion of the meals resulted in significantly different glycaemic and insulinaemic responses during the postprandial period (the IAUC for plasma glucose during the postprandial period was $227.1 \pm 46.2 \text{ mmol.l}^{-1} \times 180 \text{ min}$ and $112.0 \pm 19.6 \text{ mmol.l}^{-1} \times 180 \text{ min}$ in the HGI and LGI trials respectively) ($p<0.005$) (See Chapter 6).

A LGI CHO-rich meal has been recommended as a suitable source of CHO before exercise (Burke et al., 1998b). The reduced hyperinsulinaemia during the postprandial period following the LGI meal reduces the suppression of fat oxidation compared to when a HGI meal is consumed. This allows a shift in substrate utilisation toward fat oxidation during the subsequent exercise as well as providing a sustainable source of carbohydrate (Wu et al., 2003). Nevertheless, in the present study, despite reduced hyperglycaemia and hyperinsulinaemia in the postprandial period following the standard HGI breakfast in the LGI trial, no differences in substrate utilisation during the subsequent run were found. Previous studies reported in this thesis have observed insulin concentrations of approximately $90 \mu\text{IU ml}^{-1}$ following a LGI breakfast (See Chapters 5 and 6). The insulin concentrations in the present study peaked at approximately $145 \mu\text{IU ml}^{-1}$ following breakfast in the LGI trial. Therefore it is likely that the reduction in hyperinsulinaemia observed in the present study following the LGI evening meal was insufficient to alter substrate oxidation during subsequent sub-maximal exercise.

Recently, there has been much interest in the use of LGI foods in weight management (Warren et al., 2003). It has been hypothesised that LGI foods may be beneficial in 2 ways namely, by promote feelings of satiety and promoting fat oxidation at the expense of carbohydrate oxidation (Brand-Miller et al., 2002). Several studies have
reported increased feelings of satiety in the hours after a LGI meal compared to an isoenergetic HGI meal (Ludwig et al., 1999; Warren et al., 2003, Wu et al., 2003) In the present study, higher ratings of gut fullness and lower hunger scores were reported on the morning of day 2 (whilst still in the fasted state) in the LGI trial compared to the HGI trial. Following the standard HGI breakfast, higher ratings of gut fullness continued to be reported in the LGI trial throughout the postprandial period. This is despite the fact that the breakfast was exactly the same in both trials and both evening meals were and nutrient matched. The results therefore suggest that increased satiety following the LGI evening meal may have persisted even after an overnight fast. This has important implications in weight control because if food was available ad libitum then higher ratings of gut fullness may translate into reduced food intake.

Although no differences in substrate metabolism during exercise were reported in this study, the results from the postprandial data may have important health implications. The consumption of HGI foods is increasingly associated with increased risk of type 2 diabetes mellitus, coronary heart disease, obesity and cancer (Brand-Miller 2003). High glycaemic index meals are associated with rapid hyperglycaemia and hyperinsulinaemia which in many individuals, is followed by hypoglycaemia and the secretion of counter regulatory hormones. Although more longitudinal studies are required on the long-term effects of a LGI diet, the results of the present study show that improved glucose tolerance can be achieved in the short term by consuming a single LGI meal. It may be possible that only one meal a day needs to be composed of LGI carbohydrates to see an improvement in glucose tolerance. This may increase adherence to diet programmes rather than asking individuals to consume only LGI carbohydrates in their diet.

In conclusion, the results of the present study show that ingestion of a LGI evening meal resulted in improved glucose tolerance at breakfast compared to when a HGI evening meal was consumed. Despite this, no differences in the metabolic responses to a subsequent exercise bout were observed.
Table 8.1. Oxygen uptake (VO$_2$), carbon dioxide expired (VO$_2$), and the respiratory exchange ratio (RER) during the HGI and LGI CHO trials (Mean ± SEM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>Resting</th>
<th>Postprandial Period</th>
<th>Exercise Period</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Meal 1</td>
<td></td>
</tr>
<tr>
<td>VO$_2$ (l min$^{-1}$)</td>
<td>HGI</td>
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<td>0.35 ± 0.01</td>
<td>2.98 ± 0.07</td>
</tr>
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<td>LGI</td>
<td>0.32 ± 0.01</td>
<td>0.35 ± 0.01</td>
<td>2.96 ± 0.08</td>
</tr>
<tr>
<td>VCO$_2$ (l min$^{-1}$)</td>
<td>HGI</td>
<td>0.30 ± 0.01</td>
<td>0.31 ± 0.01</td>
<td>2.86 ± 0.06</td>
</tr>
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<td>0.29 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>2.85 ± 0.08</td>
</tr>
<tr>
<td>RER</td>
<td>HGI</td>
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<td>0.90 ± 0.02</td>
<td>0.96 ± 0.00</td>
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<tr>
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<td>LGI</td>
<td>0.93 ± 0.04</td>
<td>0.90 ± 0.01</td>
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</table>
Table 8.2 Heart Rate (HR), Rate of Perceived Exertion (RPE), Gut fullness (GF) and hunger ratings during the HGI and LGI CHO trials (Mean ± SEM)

<table>
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<th>Trial</th>
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<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
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</thead>
<tbody>
<tr>
<td>HR (beats.min⁻¹)</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>152±4</td>
<td>158±5</td>
<td>159±5</td>
<td>157±4</td>
</tr>
<tr>
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<td>LGI</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>152±4</td>
<td>157±4</td>
<td>160±4</td>
<td>162±4</td>
</tr>
<tr>
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<td>HGI</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
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<td>13±1</td>
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<td>-</td>
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<td>11±1</td>
<td>13±1</td>
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<td>Gut Fullness</td>
<td>HGI</td>
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<tr>
<td>Hunger</td>
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<td>10±1</td>
<td>10±1</td>
<td>11±2</td>
</tr>
</tbody>
</table>

Gut fullness significantly higher throughout the postprandial period in the LGI trial compared to the HGI trial (p<0.05)

†values significantly higher in the LGI trial compared to the HGI trial (p<0.01)

*values significantly higher in the HGI trial compared to the LGI trial (p<0.05)

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Figure 8.2. Plasma glucose concentrations (mmol l⁻¹) in the HGI and LGI trials

(Mean ± SEM) * HGI trial significantly higher than LGI trial \(p<0.05\).
Figure 8.3. Serum insulin concentrations (μIU.ml⁻¹) in the HGI and LGI trials (Mean ± SEM). * HGI trial significantly higher than LGI trial (p<0.05).
Figure 8.4. Plasma FFA concentrations (mmol l⁻¹) in the HGI and LGI trials (Mean ± SEM).
Figure 8.5 Plasma glycerol concentrations (μmol l⁻¹) in the HGI and LGI trials

(Mean ± SEM)
Figure 8.6 Blood lactate concentrations (mmol l\(^{-1}\)) in the HGI and LGI trials (Mean ± SEM)
Figure 8.7. Estimated rate of carbohydrate oxidation (g min⁻¹) in the HGI and LGI trials (Mean ± SEM).
Figure 8.8 Estimated rate of fat oxidation (g min\(^{-1}\)) in the HGI and LGI trials (Mean ± SEM).
9 The effect of the glycaemic index of an evening meal on the metabolic responses to a standard high glycaemic index breakfast and subsequent exercise in women.

9.1 Introduction

The rate of carbohydrate absorption after a meal, as quantified by the glycaemic index, has significant effects on the postprandial hormonal and metabolic responses (Ludwig 2002). High glycaemic index (HGI) meals are associated with rapid hyperglycaemia and hyperinsulinaemia which in many individuals, is followed by hypoglycaemia and the secretion of counter regulatory hormones. Thus, there is increasing evidence suggesting that HGI carbohydrate diets are associated with increased risk of obesity, type 2 diabetes mellitus and cardiovascular disease in both sexes.

For some time, LGI diets have been recommended to improve the management of diabetes by lowering the early postprandial hyperglycaemia and decreasing the risk for postabsorptive hypoglycaemia (Ludwig 2002). More recently, there has been a great deal of interest in the potential of LGI diets in weight management. Low glycaemic index carbohydrates are associated with a longer digestive phase and therefore a lower glycaemic and insulinaemic response. Accumulating data suggests that a diet characterised by LGI carbohydrates is capable of increasing insulin sensitivity or improving glucose tolerance (Liljeberg et al., 1999) and also may promote prolonged feelings of satiety and therefore reduced food intake (Ball et al., 2003).

The acute metabolic responses to single HGI and LGI carbohydrate foods or a single HGI or LGI meal are well understood however, the responses to more than one meal are less clear. Data from early studies has shown that consumption of a HGI carbohydrate compared with a energy and nutrient matched LGI carbohydrate adversely affects glucose tolerance at a subsequent meal 4 h later (Jenkins et al.,
A study carried out by Wolever and colleagues extended these findings by reporting that this second meal effect was still apparent even if the time between meals was extended to an overnight fast (Wolever et al., 1988). The data from these early studies demonstrated that improvements in glucose tolerance can be seen following only one LGI meal. In Chapter 8, an improvement in glucose tolerance was observed following a LGI mixed evening meal in male subjects.

Surprisingly, there is very little data on the glycaemic responses to HGI and LGI meals in women despite the fact that women may be more likely to adopt a LGI diet for weight loss purposes. Data on meals that are realistic in a real-life setting is also lacking in the GI literature despite the fact that LGI diets are now being recommended to the general public as an effective weight loss method. Therefore the main purpose of the present study was to investigate whether the acute effects of a LGI meal persist overnight in women.

In the sport and exercise setting, LGI pre-exercise meals are often recommended to maintain plasma glucose concentrations and free fatty acids during exercise without stimulation of insulin release in the period before exercise (Thomas et al., 1991). Despite this, many athletes chose not to consume LGI foods due to the gastrointestinal discomfort that may occur. Therefore if a LGI meal can reduce the hyperglycaemia and hyperinsulinaemia following a standard HGI breakfast, a similar effect may be achieved to when a LGI pre-exercise meal is consumed. Therefore, this study also aimed to investigate whether the metabolic responses to a subsequent exercise bout were affected.

9.2 Methods

Subjects
Seven female recreational athletes participated in this study. Their mean (± SD) age, height, weight and VO₂ max were 24.4 ± 3.4 years, 170.0 ± 1.0 cm, 59.9 ± 7.3 kg and 52.2 ± 4.4 ml kg⁻¹ min⁻¹ respectively. All subjects were eumenorrheic and in each trial, four subjects were in the follicular phase and three in the luteal phase of the menstrual cycle. A criterion for inclusion in the study was that participants ran regularly and were able to run for one hour continuously at about 65% VO₂ max.
Loughborough University Ethical Advisory Committee approved the protocol and all subjects gave their written informed consent.

**Experimental Design**

Preliminary tests and main trial procedures are described in Chapter 3. The experimental protocol is shown in Figure 9.1. Each subject participated in two experimental trials separated by at least 7 days. The experimental testing protocol was completed over a 2-day period. On day 1, subjects were asked to record their food intake at breakfast and lunch. They were instructed not to consume any food after 4 pm and were then provided with their test meal at 7 pm which consisted of either HGI or LGI carbohydrates as described in Table 3.1. On day 2, subjects arrived at the laboratory following a 13 h overnight fast. A fasting 10 ml venous blood sample and a 5 min resting expired air sample were collected and then the participants were provided with the standard HGI breakfast (see Table 3.1). Subjects were asked to consume the breakfast within 30 min and then the 3 h postprandial period began. At the end of the 3 h postprandial period, subjects completed a 60 min run at 65% \( \text{VO}_{2} \text{max} \).

During the postprandial period, subjects remained in the laboratory at rest. Ten ml venous blood samples and 5 min expired air samples were taken 15, 30, 60, 90, 120 and 180 min after breakfast. During each collection of expired air, ratings of gut fullness and hunger were recorded using 6-20 scales. Subjects were instructed not to eat anything other than the food provided for them. No extra drinks, apart from water, were permitted.

Ambient temperature and relative humidity were recorded every 30 min using a hygrometer (Zeal, London, UK) during the main trials. Temperature was maintained between 21-23°C and humidity was between 48-61%.

**Statistical analysis**

Analysis of variance (ANOVA) for repeated measures on two factors (experimental treatment and time) was used to analyse differences in the physiological and metabolic responses in both trials. If a significant interaction was obtained, a Holm-
Bonferroni step-wise post-hoc test was utilised to determine the location of the variance. Differences were considered significant at $p<0.05$. All results are presented as mean ± SEM.
Figure 9.1 Schematic representation of the experimental protocol

Day 1

<table>
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<tr>
<th>7pm</th>
<th>8am</th>
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Day 2

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%VO₂max

- Dinner
- Warm up at 55% VO₂ max
- Run at 65% VO₂ max
- Venous blood sample
- Cannulation
- Expired air sample
- Overnight fast
- 8am weight
- 8.30am versus
- 9am versus
- 9.15am versus
- 9.45am versus
- 10am versus
- 10.15am versus
- 10.45am versus
- 11am versus
- 11.15am versus

Legend:
- C: Cannulation
- W: Weight
- VS: Venous blood sample
- EA: Expired air sample
- 60 min run at 65% VO₂ max
- 5 min warm up at 55% VO₂ max
9.3 Results

Plasma glucose and serum insulin

There was a main trial effect for higher plasma glucose concentrations throughout the postprandial period in the HGI trial compared to the LGI trial (\(p<0.05\)) (Figure 9.2). Following ingestion of the standard HGI breakfast, plasma glucose concentrations rapidly increased and peaked at 15 min during the postprandial period in both trials. Plasma glucose concentrations then declined throughout the rest of the postprandial period. The incremental area under the curve (IAUC) for plasma glucose during the postprandial period following breakfast was greater in the HGI trial than the LGI trial (\(185 \pm 41 \text{ mmol l}^{-1} \times 180 \text{ min} \) and \(110 \pm 29 \text{ mmol l}^{-1} \times 180 \text{ min} \) respectively) \((p<0.01)\) thus confirming the main trial effect. Throughout the run, plasma glucose concentrations were maintained between 4.5 – 5 mmol l\(^{-1}\) in the LGI trial. In the HGI trial, plasma glucose concentrations increased throughout the run to approximately 6 mmol l\(^{-1}\) at the end of the 60 min.

There was also a main trial effect for higher serum insulin concentrations throughout the postprandial period following the standard breakfast when the HGI evening meal was consumed compared to when the LGI evening meal was consumed \((p<0.05)\) (Figure 9.3) Following ingestion of breakfast, serum insulin concentrations increased rapidly and peaked at 15 min during the postprandial period in both trials. Serum insulin concentrations then declined throughout the rest of the postprandial period and remained at baseline levels throughout the 60 min run in both trials. The IAUC for serum insulin during the postprandial period was greater in the HGI trial than the LGI trial (\(13374 \pm 2115 \text{ \mu U ml}^{-1} \times 180 \text{ min} \) and \(10938 \pm 1484 \text{ \mu U ml}^{-1} \times 180 \text{ min} \) respectively) \((p<0.05)\) again confirming the main trial effect.

Plasma free fatty acids (FFA) and glycerol

Before the ingestion of the standard breakfast, FFA concentrations were higher in the HGI trial compared to the LGI trial \((p<0.05)\). Following ingestion of breakfast, plasma FFA concentrations were suppressed in both trials and remained suppressed throughout the postprandial period. During the sub-maximal exercise bout, FFA concentrations increased with time but again, there were no differences between trials
Plasma glycerol concentrations showed a similar response to that of plasma FFA and there were no differences between the trials (Figure 9.5).

**Blood Lactate**

In both trials, blood lactate concentrations increased slightly after ingestion of the HGI breakfast but there were no differences between the trials. During the exercise bout blood lactate concentrations increased in both trials but remained between 1 and 2 mmol.l\(^{-1}\) throughout the run (Figure 9.6).

**Respiratory Exchange Ratio (RER) and estimated carbohydrate and fat oxidation rates**

There were no significant differences between trials in the RER (Table 9.1) and therefore the estimated rate of carbohydrate (Figure 9.7) and fat oxidation (Figure 9.8) during either the postprandial period or the 60 min run.

**Gut fullness and hunger scales**

Throughout the postprandial period following the standard breakfast, ratings of gut fullness were higher in the LGI trial compared to the HGI trial \((p<0.05)\). No differences in gut fullness were observed during the subsequent 60 min run. There were no significant differences in feelings of hunger but a trend for ratings to be higher in the HGI trial (Table 9.2).

**Plasma volume changes and hydration status**

There was minimal change in plasma volume throughout the postprandial period in both trials. Plasma volume was significantly decreased by the end of the exercise bout \((p<0.05)\) but there were no differences between the trials. At the start of each trial, all participants had a urine osmolality that suggested that they were well hydrated \((628 \pm 66 \text{ mosmol kg}^{-1} \text{ and } 487 \pm 107 \text{ mosmol kg}^{-1} \text{ in the HGI and LGI trials respectively})\) (NS).

**9.4 Discussion**

The main finding of the present study is that the plasma glucose and serum insulin responses to a standard HGI breakfast were reduced when a LGI evening meal was
consumed the night before compared to when a HGI evening meal was consumed. Despite the lower glycaemic and insulinaemic responses during the postprandial period in the LGI trial, no differences in substrate utilisation were reported during the subsequent sub maximal exercise. This is consistent with the findings reported in Chapter 8 using male subjects.

Previous studies have reported improved glucose tolerance following a single LGI meal or food either 4 h later (Jenkins et al., 1982) or the next day (Wolever et al., 1988). However, neither study investigated whether this phenomenon also occurred in women. Indeed, many of the acute studies investigating the metabolic effects of the glycaemic index of pre-exercise meals have used male subjects. The lack of data on glycaemic and insulinaemic responses to carbohydrates with different GI values in women suggests that in many cases, the results from male studies are being directly applied to the female population. Several studies have shown that differences in substrate oxidation exist both at rest and during exercise between males and females (Horton et al., 1998, Mittendorfer et al., 2002, Steffensen et al., 2002). It is therefore important to investigate the metabolic effects of consuming foods with different glycaemic indices in men and women separately.

The exact mechanism behind the so called ‘second meal effect’ is still unclear. It has been suggested that the prolonged absorptive phase following a LGI meal will favour a more efficient suppression of FFA, thus improving insulin sensitivity at the time of the next meal (Wolever et al., 1988). There is evidence to propose that LGI foods that contain a large amount of slowly absorbed fermentable CHO ingested the evening before an Oral Glucose Tolerance Test (OGTT), enhance the suppression of hepatic glucose production and FFA thus creating a more insulin sensitive environment (Thorburn et al., 1993). Therefore, it is reasonable to suggest that the metabolic effects of a reduced hepatic glucose production following the LGI meal played a role in the reduced glycaemic and insulinaemic response to the standard breakfast in the present study. Fasting FFA concentrations were also higher in the HGI trial compared to the LGI trial before the standard test breakfast was consumed. This therefore also provides further support for the proposed mechanism.
Interestingly, subjective ratings of gut fullness were reported to be higher in the postprandial period following breakfast in the LGI trial than in the HGI trial. This is despite the fact that the breakfast was exactly the same in both trials. Several studies have reported that the consumption of LGI foods reduces hunger and/or promotes satiety relative to consumption of HGI foods in the hours following consumption of the meal (Ludwig et al., 1999, Ball et al., 2003; Warren et al., 2003; Wu et al., 2003). The effect of glycaemic carbohydrates on food intake appears to be related to their effects on blood glucose (Anderson & Woodend 2003). However, the release of putative satiety peptides, mediated by the intensity and length of interaction of carbohydrates in the gastrointestinal tract appears to be a crucial component of mechanisms initiating and sustaining satiety (Anderson & Woodend 2003).

A second aim of the present study was to investigate whether a LGI evening meal would reduce the postprandial hyperglycaemia and hyperinsulinaemia following the HGI breakfast sufficiently to alter the substrate oxidation during a subsequent exercise bout. Although a reduction in hyperglycaemia and hyperinsulinaemia were achieved in the LGI trial, it was not sufficient to alter substrate oxidation during exercise 3 h later. Similarly, no differences in FFA, glycerol or lactate concentrations were reported between the trials. These results are consistent with those reported in Chapter 8 using male subjects. Several studies have reported higher rates of fat oxidation during exercise when a LGI pre-exercise meal or food is consumed. Reduced hyperinsulinaemia during the postprandial period following the LGI meal reduces the suppression of fat oxidation compared to when a HGI meal is consumed. This allows a shift in substrate utilisation toward fat oxidation during the subsequent exercise as well as providing a sustainable source of carbohydrate (Wu et al., 2003). The results of the present study therefore show that a LGI meal needs to be eaten in the 2-3 hours before the exercise bout to achieve this shift in substrate utilisation.

The results of the present study indicate that LGI foods may be beneficial in improving glucose tolerance and prolonging feelings of gut fullness in the short term. The long term role of glycaemic index in appetite and body weight regulation and many other health implications are still unclear (Sloth et al., 2004). This is rather surprising considering LGI diets are increasingly being marketed to improve weight regulation in overweight but otherwise healthy women. A recent study carried out by
Sloth et al (Sloth et al, 2004) reported that there was no difference in body weight decrease between a LGI and HGI diet consumed for 10 weeks ad libitum. The author did however report that there was a significant decrease in LDL cholesterol and a tendency to a larger decrease in total cholesterol following consumption of the LGI diet compared with the HGI diet. Recent prospective studies have also reported associations between dietary glycaemic index and type 2 diabetes in younger and middle aged women (Schulze et al., 2004) and coronary heart disease in American women (Liu et al., 2000). It is clear that the dietary glycaemic index may have important implications in women's health. Further studies on the acute responses to foods with different glycaemic indices in women are therefore required to improve our understanding of the long term implications of diets composed mainly of HGI or LGI carbohydrates.

In the present study, participants did not complete both trials at the same time in the menstrual cycle. However in both trials, four participants were in the follicular phase and three in the luteal phase of the menstrual cycle. Although traditionally known for their role in reproduction, there is increasing consensus that the ovarian hormones have important roles in metabolism (Campbell et al., 2001). Indeed, it appears that the normal cyclical variations in oestrogen and progesterone could affect a number of aspects of lipid and carbohydrate metabolism (Horton et al., 2002). Several studies have investigated the effect of the menstrual cycle phase on substrate oxidation during both rest and exercise but have produced conflicting results. A number of studies have reported no effect of the menstrual cycle on resting whole body substrate oxidation (Lariviere et al., 1994, Bailey et al., 2000, Horton et al., 2002) and resting concentrations of glucose, FFA, glycerol and insulin (Bailey et al., 2000, Zderic et al., 2001; Horton et al., 2002). However, decreases in insulin sensitivity have been reported in the luteal phase of the menstrual cycle when levels of progesterone and oestrogen are higher (Pulido & Salazar, 1999). Therefore, in the present study, it is possible that the responses to the HGI and LGI diets may have been affected by the menstrual cycle phase. Further research into the responses to carbohydrate feeding at different phases in the menstrual cycle is required to develop a comprehensive understanding of this area.
It has been reported that substrate utilisation during exercise is affected by menstrual phase because carbohydrate oxidation is lower and fat oxidation is elevated during the luteal phase when oestrogen and progesterone concentrations are higher (Campbell et al., 2001, Zdenc et al., 2001). Conversely, other studies have concluded that there are no differences in fat and carbohydrate oxidation between the menstrual phases (Bailey et al., 2000, Horton et al., 2002). A study carried out by Campbell and colleagues reported that the variations in the ovarian hormone levels throughout the menstrual cycle only alter exercise metabolism when carbohydrate stores became depleted (Campbell et al., 2001). In the present study, subjects consumed 2g CHO kg⁻¹BM 3 h before a 60 min run at 65% \( \dot{V}O_2 \text{max} \). It is therefore unlikely that glycogen stores would have become depleted in this time, consequently minimising the effect of the menstrual cycle on changes in substrate oxidation.

In conclusion, the consumption of a LGI evening meal can improve glucose tolerance at a second meal following an overnight fast in women. Despite reduced hyperglycaemia and hyperinsulinaemia in the postprandial period following the standard breakfast in the LGI trial compared to the HGI trial, no differences in substrate utilisation were reported during subsequent exercise. Further research is necessary to understand the metabolic responses to foods with different GI's in women so that accurate dietary advice can be provided.
Table 9.1. Oxygen uptake (\( \text{VO}_2 \)), carbon dioxide expired (\( \text{VCO}_2 \)), and the respiratory exchange ratio (RER) during the high glycaemic index (HGI) and low glycaemic index (LGI) CHO trials (Mean ± SEM).

<table>
<thead>
<tr>
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<th>Trial</th>
<th>Resting</th>
<th>Postprandial Period</th>
<th>Exercise Period</th>
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<td></td>
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<td>Meal 1</td>
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<tr>
<td>( \text{VO}_2 ) (1 min(^{-1}))</td>
<td>HGI</td>
<td>0.24 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>2.01 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.24 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>2.01 ± 0.11</td>
</tr>
<tr>
<td>( \text{VCO}_2 ) (1 min(^{-1}))</td>
<td>HGI</td>
<td>0.20 ± 0.02</td>
<td>0.24 ± 0.01</td>
<td>1.86 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>LGI</td>
<td>0.19 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>1.84 ± 0.11</td>
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<tr>
<td>RER</td>
<td>HGI</td>
<td>0.82 ± 0.04</td>
<td>0.89 ± 0.02</td>
<td>0.92 ± 0.02</td>
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<td>LGI</td>
<td>0.78 ± 0.04</td>
<td>0.87 ± 0.02</td>
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Table 9.2 Heart Rate (HR), Rating of Perceived Exertion (RPE), Gut fullness (GF) and hunger ratings during the HGI and LGI CHO trials (Mean ± SEM)

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<td>Gut Fullness</td>
<td>HGI</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
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<td>LGI</td>
<td>14 ± 0</td>
<td>14 ± 1</td>
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<tr>
<td>Hunger</td>
<td>HGI</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
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<tr>
<td></td>
<td>LGI</td>
<td>8 ± 1</td>
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Gut Fullness significantly higher throughout the postprandial period in the LGI trial compared to the HGI trial (p<0.05)
Figure 9.2 Plasma glucose concentrations (mmol l⁻¹) in the HGI and LGI trials

(Mean ± SEM) Main trial effect for plasma glucose to be higher in the HGI trial than in the LGI trial HGI trial (p<0.05)
Figure 9.3 Serum insulin concentrations (µIU.ml\(^{-1}\)) in the HGI and LGI trials (Mean ± SEM). Main trial effect for serum insulin concentrations to be higher during the postprandial period in the HGI trial than in the LGI trial (\(p < 0.05\)).
Figure 9.4 Plasma FFA concentrations (mmol·l⁻¹) in the HGI and LGI trials (Mean ± SEM) *Plasma FFA concentrations significantly higher in the HGI trial than in the LGI trial (p<0.05)
Figure 9.5 Plasma glycerol concentrations (μmol l⁻¹) in the HGI and LGI trials (Mean ± SEM).
Figure 9.6 Blood lactate concentrations (mmol.l\(^{-1}\)) in the HGI and LGI trials (Mean ± SEM).
Figure 9.7 Estimated rate of carbohydrate oxidation \((g\ min^{-1})\) in the HGI and LGI trials (Mean ± SEM).
Figure 9.8. Estimated rate of fat oxidation (g min⁻¹) in the HGI and LGI trials (Mean ± SEM)
10 General Discussion

This chapter aims to integrate and reflect on the major findings of the investigations in this thesis in order to develop a better understanding of the glycaemic index and its utility within sport and exercise nutrition.

10.1 The glycaemic index and its application to mixed meals

Since the introduction of the concept in 1981, the glycaemic index has had a pivotal role in highlighting the variation in metabolic responses associated with different carbohydrate containing foods (Jenkins et al., 1981). According to this system, individual foods are assigned a numeric value by comparing the area under the postprandial plasma glucose curve of the test food with that of the reference food which is given a value of 100. However, because foods are rarely eaten individually, an area of much discussion has been the ability to predict glycaemic responses to mixed meals from the GI values of the constituent foods. Throughout this thesis, mixed meals have been used and the GI of the meal was calculated from the weighted means of the carbohydrate containing foods as described in Chapter 2 (Wolever & Jenkins, 1986). The meals provided in the studies reported in this thesis contained foods that could be part of a habitual diet and contained nutrients in proportions that would be realistic to the dietary practices of many people, including athletes. Significant differences in the glycaemic and insulinemic responses to HGI and LGI meals were consistently reported, both before and after exercise and in male and female subjects. This was achieved without changing the overall composition of the diet in terms of fat, protein, carbohydrate and energy content. The studies in this thesis are also the first to investigate the provision of two or more HGI or LGI meals either before or following prolonged exercise and therefore have greater relevance to normal dietary practices.

Using the integrated area under the curve (IAUC) for glucose as a reflection of the glycaemic response to meals, the ratio of the HGI/LGI could be calculated. For
example, the ratio of the glycaemic responses for the test breakfasts used in this thesis was 2.0:1 (HGI:LGI) for male subjects and 1.82:1 (HGI:LGI) for female subjects. The ratio of the calculated GI values of the meals was 1.75:1 (77/44) which is close to what was actually determined. The ratio of the glycaemic responses to the test lunches used in this thesis was 2.0:1 compared to the ratio of the calculated GI values for the meals of 1.92.1 (73/38). Although GI tests were not actually performed for the test meals, the results of the investigations within this thesis support the calculation of the GI values for mixed meals suggested by Wolever and Jenkins (Wolever & Jenkins 1986).

Previous research that has applied the glycaemic index to sports nutrition has mainly focussed on the provision of single foods with differing GI values before exercise. Although the findings of these studies have significantly enhanced our understanding of the glycaemic index and its application within sports nutrition, the postprandial responses to LGI foods presented in some of the studies may have led to unrealistic views on the metabolic responses to LGI foods. As reported in this thesis, when LGI foods are consumed as part of a mixed meal, significant increases in glucose and insulin concentrations do occur, but importantly, the magnitude of the increase is considerably less than when a HGI mixed meal is consumed. The graphs presented in Figure 10.1 emphasise the differences in the postprandial glycaemic and insulinemic responses to HGI and LGI foods when consumed as part of a mixed meal or consumed as single foods (all providing 2g CHO kg⁻¹BM). The substantial differences shown in Figure 10.1 underline the importance of research investigating the effects of mixed meals containing HGI or LGI carbohydrates that would realistically reflect normal dietary behaviour.
Figure 10.1 Typical plasma glucose and serum insulin concentrations following a
HGI and LGI food (A & B) (adapted from Wee et al. 1999) and following a HGI and
LGI mixed meal (C & D) (adapted from the results of Chapter 7).
10.2 Postprandial hyperglycaemia, hyperinsulinaemia and substrate metabolism

The degree of hyperglycaemia and hyperinsulinaemia following a meal has a major impact on subsequent substrate oxidation. Low glycaemic index carbohydrates are characterised by the slow release of carbohydrate into the circulation and a subsequent reduced insulinaemic response compared to HGI carbohydrates. Indeed, in Chapters 5, 6 and 7 of this thesis, reduced hyperglycaemia and hyperinsulinaemia were reported following a LGI meal compared to a nutrient and energy matched HGI meal. Many of the benefits of LGI carbohydrates over HGI carbohydrates are explained in terms of this slow appearance of glucose. However, it is more likely that the differences in insulin secretion following a HGI or LGI meal can explain many of the differences in substrate oxidation and metabolism described in this thesis.

Insulin regulates metabolism by producing activity-altering modifications of pre-existing enzyme molecules or by altering the synthesis, stability or translation of specific mRNAs (O’Brien & Granner 1996). The major metabolic effects of hyperinsulinaemia following a HGI meal are summarised in Figure 10.2a and the possible metabolic effects of a reduced insulin response following a LGI meal are summarised in Figure 10.2b.
Figure 10.2a Metabolic effects of a HGI meal
Figure 10.2b Metabolic effects of a LGI meal
In Chapter 4, the provision of a LGI recovery diet improved endurance performance the following day compared to when a HGI recovery diet was provided. An increased rate of fat oxidation during exercise following the LGI diet was provided as the most probable explanation for this improvement. In Chapter 5, the metabolic responses to HGI and LGI meals during recovery from prolonged exercise were investigated and it was reported that although there was an increase in glucose and insulin concentrations following the LGI meals, this was not sufficient to completely suppress FFA concentrations (in comparison to the HGI trial).

It is well acknowledged that prolonged endurance exercise depletes muscle glycogen stores however, only recently has it been confirmed that intramuscular tracylglycerol (IMTG) concentrations are also substantially reduced during prolonged moderate intensity exercise (van Loon et al., 2003a). The habitual high glycaemic index, high carbohydrate diet consumed by most athletes during recovery from prolonged exercise successfully repletes muscle glycogen stores (Burke et al., 1993). However, research has shown that IMTG concentrations remain low on such a diet (Decombaz et al., 2001; Johnson et al., 2003) due to the suppression of FFA concentrations by insulin following the high carbohydrate intake. As shown in Figure 10.2b, the incomplete suppression of circulating FFA concentrations following the LGI diet may have resulted in a partial increase in muscle IMTG however, the small increase in insulin and glucose concentrations (in comparison to the HGI trial) may have also resulted in a simultaneous resynthesis of muscle glycogen. Therefore the ingestion of a LGI diet during recovery from exercise may allow the provision of substrates for both glycogen and tracylglycerol stores.

To test this hypothesis, we are currently undertaking a research project at the University of Sydney, to measure IMTG concentrations following a HGI or LGI recovery diet. The experimental protocol is the same as that described in Chapter 4 although subjects only complete a 90 min run on Day 2. The recovery diets are also the same as those described in this thesis. Concentrations of IMTG are measured before and after exercise on Day 2 using $^1$H magnetic resonance spectroscopy (MRS). Preliminary results from this study show that IMTG resynthesis is higher following a LGI recovery diet compared to a HGI diet and therefore IMTG utilisation was higher.
in the LGI trial (Figure 10.3). This provides a possible explanation for the higher fat oxidation rate and improved endurance capacity in the LGI trial in Chapter 4

Figure 10.3 IMTG.CR ratio pre and post exercise following a HGI or LGI recovery diet during the previous 24 h.

Following a high carbohydrate meal, fatty acid oxidation decreases partly due to the insulin-induced inhibition of lipolysis resulting in decreased plasma FFA availability. Hyperglycaemia and therefore hyperinsulinaemia have also been shown to directly inhibit fatty acid oxidation through the inhibition of the rate of long-chain fatty acid uptake into the mitochondria for β-oxidation (Sidossis & Wolfe 1996). Hyperinsulinaemia increases the concentration of malonyl-CoA in human skeletal muscle (Bavenholm et al., 2000, Rasmussen et al., 2002). Malonyl-CoA is an inhibitor of carnitine palmitoyl transferase –1 (CPT-1), the enzyme that regulates the transfer of long-chain fatty acids (LCFA) into the mitochondria in muscle for oxidation. The regulation of malonyl-CoA is thought to involve the cytosolic concentration of citrate. In rodents and humans, high glucose availability and therefore high insulin concentrations at rest have been shown to elevate the cytosolic citrate concentration and consequently the muscle malonyl-CoA concentration.
This is the likely mechanism whereby fat oxidation is inhibited by high glucose availability in resting humans. In the study carried out by Bavenholm (2000), subjects were infused with insulin at two different rates for 150 min. Interestingly, during the low-dose insulin infusion, no increase in citrate or malonyl-CoA were observed. During the high-dose infusion, a small but significant increase in citrate and malonyl-CoA were seen. It was concluded that these increases in citrate and malonyl-CoA during infusion of insulin at a high rate led to a decrease in whole-body fat oxidation and presumably fat oxidation in skeletal muscle.

The differences in fat oxidation rates described in this thesis could therefore be partly due to differences in malonyl-CoA and citrate concentrations in the muscle caused by differences in insulin concentrations during the postprandial period. However, the average plasma insulin concentration during the low infusion dose in the study carried out by Bavenholm et al. (2000) was 35 μU ml⁻¹ compared to a high dose of 104 μU ml⁻¹. Although the insulin response to the LGI meals in this thesis were significantly less than following the HGI meals, the concentrations observed in both trials in Chapters 5, 6 and 7 in this thesis were considerably higher than the high dose infusion in the study by Bavenholm (2000). It is unknown whether a dose-response relationship exists between insulin concentrations and malonyl-CoA therefore it is only possible to speculate on the differences between the HGI and LGI trials in this thesis.

A recent study has reported a decline in muscle malonyl-CoA concentrations from rest to moderate intensity exercise in humans, which may contribute to the increase in absolute fat oxidation during exercise (Roepstorff et al., 2005). In the same study, subjects exercised with either high or low pre-exercise muscle glycogen concentrations. Fat oxidation was significantly higher in the low muscle glycogen trial however, no differences in malonyl-CoA were reported between the trials suggesting that it does not play a major role in the “fine tuning” of fat oxidation during exercise. However, an increase in carnitine (a substrate for CPT-1) was reported in the low glycogen trial. Therefore during prolonged moderate intensity exercise, the regulation of fat oxidation in response to carbohydrate availability during exercise is partly mediated by the availability of free carnitine to CPT-1. Although muscle glycogen concentrations were not measured in the studies in this thesis, muscle glycogen
concentrations have been shown to be higher following a HGI diet (Burke et al., 1993, Wee et al., 1999a). Therefore, the higher fat oxidation rate reported during exercise following a LGI meal or diet may simply be due to differences in carbohydrate availability and therefore differences in the availability of free carnitine to CPT-1.

In the present thesis, meals were provided both before and following exercise. Exercise induces a marked increase in GLUT 4 translocation and glucose transport into the muscle and an increase in muscle insulin sensitivity (Holloszy 2003). The metabolic responses to a carbohydrate load are therefore likely to be altered by prior exercise. Although the effect of exercise on the metabolic responses to HGI and LGI meals was not directly investigated in this thesis, a comparison of the results of Chapter 5 and 6 can be made. In both studies, similar subjects were used and the same breakfast was provided. However, in Chapter 5, subjects had exercised for 90 min before consuming breakfast and in Chapter 6 no exercise had been carried out before breakfast. The insulinemic responses to the meal in both Chapters are shown in Figure 10.4.

![Figure 10.4](image)

**Figure 10.4** The insulinemic response to a HGI breakfast consumed following exercise or no exercise (Data from Chapters 5 and 6)
This data clearly highlights the profound effect that exercise can have on insulin sensitivity. The substantial research that has been carried out on the regulation of glucose transport into muscle has recently been described by Holloszy in an in depth memoir of the research area (Holloszy 2003). Although many of the phenomena relevant to glucose transport are still unexplained, the interaction between the effects of insulin and exercise and the increases in the muscle insulin sensitivity and responsiveness induced by exercise are discussed.

10.3 Glycaemic index and second meal effect

In humans, the ingestion of HGI carbohydrates compared to nutrient and energy matched LGI carbohydrates has been shown to adversely affect glucose tolerance at a subsequent meal (Jenkins et al., 1982; Wolever et al., 1988; Liljeberg et al., 1999). In support of previous findings, the results from two of the studies in this thesis showed improved glucose tolerance at breakfast when a LGI evening meal was consumed compared to when a HGI meal was consumed (Chapter 8 and 9) This was despite the fact that mixed meals were used and not single foods with differing GI values. In the studies described in Chapters 5, 6 and 7, a HGI or LGI meal was proceeded by another meal or exercise therefore the prolonged metabolic effects of the meals were not observed. The glycaemic index of the last meal of the day is likely to have a significant effect on metabolism throughout the evening. The prolonged absorptive phase that often occurs following a LGI meal will cause a continuing albeit small increase in insulin concentrations compared with the HGI meal that is absorbed rapidly. There is evidence to suggest that the ingestion of LGI foods the evening before a Oral Glucose Tolerance Test (OGTT) enhance the suppression of hepatic glucose production thus creating a more insulin sensitive environment (Thorburn et al., 1993). It is reasonable to suggest therefore that the metabolic effects of a reduced hepatic glucose production following the LGI meal had a major role to play in the reduced glycaemic and insulinaemic response to the standard breakfast in Chapters 8 and 9.
10.4 Hyperglycaemia, hyperinsulinaemia and satiety

Throughout the studies in this thesis, higher ratings of gut fullness were consistently reported following a LGI meal compared to a HGI meal. Several studies have reported increased satiety following a LGI carbohydrate or meal compared to a HGI carbohydrate or meal. However, the exact relationship between glycaemic responses to carbohydrates and satiety remains unclear. Transient declines in blood glucose concentrations in the postabsorptive state have been found to closely correspond with request for meals in time-blinded human subjects (Melanson et al., 1999). In the same study, Melanson et al. (1999) also reported that meal requests were associated with so-called dynamic declines in blood glucose concentrations immediately after the peak blood glucose concentration induced by carbohydrate consumption. However, in the studies in this thesis, blood glucose concentrations peaked following both the HGI and LGI meals (although the peak was higher in the HGI trials) and concentrations declined in both trials following the peak. Therefore differences in blood glucose concentrations are unlikely to be the explanation for increased satiety in the LGI trials.

Insulin has also been implicated in the regulation of energy balance and appetite. A study carried out by Holt and colleagues (Holt & Miller 1995) reported that increased insulin responses were associated with reduced satiety. This could be a possible explanation for the differences reported in the studies in this thesis but studies have also reported that increased insulin concentrations are positively correlated with satiety (Verdich et al., 2001, Blom et al., 2005). It seems improbable that insulin can act as a marker of satiety because the relationship is confounded or moderated by many other metabolic processes (de Graaf et al., 2004).

The amount of unavailable and slowly digestible carbohydrate in a diet has been shown to affect feelings of hunger in humans (Sparti et al., 2000). Low glycaemic index carbohydrates tend to contain more fibre than HGI carbohydrates and this was the case in the meals provided throughout this thesis. In a study by Sparti et al. (2000), substrate oxidation and feelings of hunger were investigated over a 24 h period following a diet high (H) or low (L) in unavailable and slowly digested carbohydrates. The H diet elicited a lower and delayed rise in postprandial carbohydrate oxidation.
and was associated with lower hunger feelings than was the L diet. Less carbohydrate and more fat was oxidised in the post-lunch and post-dinner period with the H diet than in the L diet. These differences were partially compensated for by an inverse effect during the night. These findings are in agreement with results from the studies in this thesis and may also help to explain the higher gut fullness scores reported the morning after the LGI evening meal in Chapters 8 and 9. It therefore appears that the pattern of carbohydrate utilisation was involved in the modulation of hunger feelings.

10.5 Glycaemic responses in female subjects

Two of the studies in this thesis investigated metabolic responses to HGI and LGI meals in female subjects. None of the previous GI pre-exercise feeding studies have used female participants despite the fact that gender differences in substrate oxidation during exercise exist. In Chapter 7, females were tested at the same time in the menstrual cycle (early follicular phase) and none of the subjects were taking any form of contraceptive pill. In Chapter 9 however, the trials were not carried out in the same phase of the cycle and three of the participants were taking a contraceptive pill. These extra controls were not carried out in Chapter 9 due to time constraints on the study however, significant differences were still reported between the trials and the responses observed for those who were taking exogenous ovarian hormones appeared to be no different to those who were not. Several studies have investigated whether substrate oxidation is affected by menstrual cycle phase and by the use of contraceptive pills however results remain inconclusive (See Chapter 2). To ensure tight experimental control, it may be beneficial to only use subjects who are not taking exogenous ovarian hormones. However, oral contraceptives are extensively used by both sedentary and athletic women and so it is important that this population is included in experimental trials. Overall, the metabolic responses to HGI and LGI foods in females appeared to be similar to responses previously reported in male subjects.

10.6 Considerations and conclusions

It is important to acknowledge some of the limitations of the studies reported in this thesis. As previously mentioned, venous blood taken from the antecubital vein was used to determine concentrations of blood metabolites throughout all the studies in
this thesis. Venous blood metabolites reflect the metabolism in the particular organ drained in addition to that in the body as a whole, and thus may yield a distorted picture of total body kinetics (McGuire et al., 1976). Arterial glucose concentrations are consistently higher than those obtained from venous blood due to differences in the transit times of substances throughout the circulatory paths between the two sampling sites and also due to the uptake of substances by the intervening tissue (McGuire et al., 1976). It was beyond the scope of the studies in this thesis to sample arterial blood and capillary samples were not taken as multiple sampling would have caused discomfort for the subjects. However, the same sampling site was consistently used in the studies in this thesis and therefore should not affect comparison of data between trials.

It is also important to acknowledge that throughout the studies in this thesis, plasma glucose concentrations were used to assess glycaemic responses to meals with differing GI values. Plasma glucose concentrations are a function of both the rate of appearance of glucose into the systemic circulation ($R_{\text{glucose}}$) and the rate of disappearance of glucose from the systemic circulation ($R_{\text{dglucose}}$). The lower glycaemic responses reported following the LGI meals in this thesis were assumed to be due to a slower rate of absorption of the carbohydrate however, this could only be inferred by directly measuring the plasma glucose kinetics. One of the few studies that have examined glucose kinetics following ingestion of LGI carbohydrates reported contrary to this (Schenk et al., 2003). In their study, Schenk and colleagues fed subjects an amount of corn flakes or bran cereal containing 50 g of available carbohydrate. Plasma insulin and plasma glucose kinetics (by constant rate infusion of [6,6-2H$_2$] glucose) were measured for 180 min following their ingestion. Surprisingly, it was reported that the LGI of the bran cereal was not due to a slow $R_{\text{glucose}}$ of glucose but was due to an early increase in the $R_{\text{dglucose}}$ due to postprandial hyperinsulinaemia.

However, it is important to note that this phenomenon has not been reported for other typically LGI foods and significantly lower insulin responses were consistently reported following ingestion of the LGI meals compared to the HGI meals in this thesis. Further investigations on glucose kinetics following the ingestion of foods with
Different GI values is certainly required to assess which LGI foods do cause hyperinsulinaemia in the postprandial period.

In conclusion, the glycaemic index concept can successfully be applied to mixed meals that would be realistic in normal dietary practices in both men and women. The results of the studies presented in this thesis emphasise the major impact postprandial glycaemic and in particular postprandial insulinaemic responses have on metabolism and how they can be significantly altered by simply changing the carbohydrate source within a meal. Altering substrate oxidation by changing the degree of hyperglycaemia and hyperinsulinaemia has significant utility both within sports nutrition and in the prevention and treatment of metabolic diseases.
11 References


Jensen, M. D. and J. Levine (1998) Effects of oral contraceptives on free fatty acid metabolism in women Metabolism 47,(3) 280-4


Wee, S L, C Williams, S. Gray and J. Horabin (1999a). Influence of high and low glycaemic index pre-exercise meals on muscle glycogen and exercise metabolism. 4th Annual Congress of the European College of Sports Sciences, University Institute of Motor Sciences, Rome, Italy.


Appendix A

ETHICAL ADVISORY COMMITTEE

Loughborough University

RESEARCH PROPOSAL FOR HUMAN BIOLOGICAL OR PSYCHOLOGICAL AND SOCIOLOGICAL INVESTIGATIONS

This application should be completed after reading the University Code of Practice (found at http://www.lboro.ac.uk/admin/committees/ethical/one.html) paying particular attention to the advice given in Section 6 for Human Biological Investigations and Section 7 for Psychological and Sociological Investigations.

1. Project Title
   The influence of high carbohydrate meals with different glycaemic indices on substrate utilisation during subsequent exercise in women.

2. Brief Lay Summary of the Proposal for the Benefit of Non-Expert Members of the Committee

   The ingestion of carbohydrate (CHO) before exercise has been demonstrated to improve performance (Hargreaves et al. 1987, Neufer et al. 1987) and alter the metabolic response and substrate utilisation during the exercise bout (Coyle 1997, Wee et al. 1999) However, it is well known that the ingestion of CHO depresses fat oxidation due to the increase in insulin secretion in the postprandial period (Horowitz et al. 1997). An increased rate of fat oxidation during exercise is beneficial as it spares muscle glycogen and also may aid weight management Therefore it is important to chose carbohydrates that do not induce a high insulin response and so may cause a shift in substrate utilization towards fat oxidation during subsequent sub maximal exercise.

   Carbohydrates foods can be classified in terms of their glycaemic index (GI). The GI was introduced over 20 year ago as a means of physiologically classifying carbohydrate-containing foods according to the postprandial glycaemic responses they produce (Jenkins et al. 1981). Carbohydrates that breakdown quickly during digestion have the highest glycaemic indexes. The blood glucose response is fast and high. Carbohydrates that breakdown slowly, releasing glucose gradually into the blood stream, have low glycaemic indexes.

   Several studies have considered the effects of the ingestion of foods with different GI's before exercise. Improvements in exercise performance have been found when LGI foods were consumed before exercise (DeMarco et al. 1999, Thomas et al. 1991) however, others have found no differences in exercise performance (Febbraio and Stewart 1996, Sparks et al. 1998, Stannard et al. 2000, Thomas et al. 1994, Wee et al. 1999).

   Despite the discrepancy in the literature over performance benefits, all studies investigating the effects of the GI of a pre-exercise meal have demonstrated lower postprandial glycaemia and insulinemia following a LGI meal or single food. This is accompanied by higher concentrations of plasma free fatty acids (FFA) and therefore higher rates of fat oxidation during exercise compared to the responses following ingestion of a HGI food (Febbraio and Stewart 1996, Wee et al. 1999, Febbraio et al. 2000, Wu et al. 2003).
The majority of the research carried out on glycaemic index and fat oxidation has been carried out on male subjects. Very little is known about women's responses to HGI and LGI foods however, women are often more likely to embark on weight loss programs than men. Therefore the present study will investigate the metabolic responses to HGI and LGI meals and subsequent exercise in women.

3 Details of responsible investigator (supervisor in case of student projects)
Title Miss Surname Stevenson Forename Emma
Department School of Sport and Exercise Sciences
Email address E.J Stevenson@lboro.ac.uk
Personal experience of proposed procedures and/or methodologies
Trained in the collection and analysis of expired air samples and heart rate data.
Trained in the extraction and analysis of blood from a cannula.
4 Names, experience, department and email addresses of additional investigators
Professor Clyde Williams (School of Sport and Exercise Sciences C.Williams@lboro.ac.uk) has over 30 years experience of conducting similar studies and will perform the cannulations on the participants.

Mrs Maria Nute (School of Sport and Exercise Sciences M.Nute@lboro.ac.uk) has over 20 years experience of conducting similar studies.

Miss Beth Phillips (School of Sport and Exercise Sciences B.Phillips-02@student.lboro.ac.uk), trained in the analysis of expired air collections.

Miss Laura Mash (School of Sport and Exercise Sciences L.E.Mash-02@student.lboro.ac.uk), trained in the analysis of expired air collections.

5 Proposed start and finish date and duration of project
Start date October 2004 Finish date December 2004 Duration 3 months
6 Location(s) of project
The work will be conducted in the Sports Science Laboratories of the School of Sport and Exercise Sciences at Loughborough University.

7. Reasons for undertaking the study (eg contract, student research)
This study will be undertaken as part of a PhD project and a BSc final year dissertation.

8 Do any of the investigators stand to gain from a particular conclusion of the research project?
No

9a. Is the project being sponsored?
Yes ☐ No ☒
If yes, please state source of funds including contact name and address

9b. Is the project covered by the sponsors insurance?
Yes ☐ No ☒
If no, please confirm details of alternative cover (e.g., University cover).

University Cover

10. Aims and objectives of project

The aim of the proposed study is to investigate the influence of the glycaemic index of breakfast on the metabolic responses to a subsequent exercise bout in women.

11. Brief outline of project

Following preliminary visits to the laboratory, participants will take part in two trials. On the day of each trial, participants will arrive in the lab at 0800 hr and will be provided with a breakfast consisting of high glycaemic index or low glycaemic index carbohydrates. Following consumption of the breakfast, participants will remain seated in the lab and rest for 3 h. After this 3 h postprandial period, participants will run for 60 min at 65% VO$_2$max on a motorised treadmill.

A) STUDY DESIGN

Participants will complete preliminary tests in the week preceding the first main trial. They will complete two main trials separated by at least a week in a randomised cross-over design. On the morning of each main trial, participants will arrive in the lab at 0800 hr following an overnight fast and a cannula will be inserted into the antecubital vein for blood sampling. Before breakfast, a blood sample, expired air sample, and urine sample will be taken and the participant will be weighed. Following this, the participant will be provided with their high carbohydrate breakfast. On one occasion the carbohydrates will be high glycaemic index and on the other, low glycaemic index. Once the breakfast has been consumed, the participant will be asked to remain in the lab at rest for 3 h and blood and expired air samples will be taken at regular intervals. At the end of the 3 h postprandial period, participants will complete a warm up of 5 min at 60% VO$_2$max and then will run for 60 min at 65% VO$_2$max on a motorised treadmill. At the end of the run, a final blood sample will be collected and the participants’ body mass will be obtained. After removal of the cannula, the participant will be free to leave the lab.

B) MEASUREMENTS TO BE TAKEN

PRELIMINARY TRIALS

VO$_2$max Test – An incremental treadmill test for the determination of VO$_2$max (Generic Protocol)

VO$_2$ speed test – A 16 minute sub-maximal test during which expired air will be collected using the Douglas Bag Method (Generic Protocol).

MAIN TRIAL

Participants will weigh themselves nude in the strictest of privacy before and after each run.

Heart rate will be monitored throughout the run by short range telemetry (Polar). Participants will be required to wear a chest strap throughout the protocol. The monitor will be attached to the treadmill. Heart rate will be noted every 15 minutes.

Expired air samples will be collected before breakfast, at 15, 30, 60, 90, 120 and 180
minutes after breakfast and every 15 minutes throughout the exercise period using the Douglas Bag technique.

Venous blood samples will be taken from an indwelling cannula at the same time points as expired air samples.

The participants' fullness and hunger ratings will be recorded at 15, 30, 60, 90, 120 and 180 minutes after breakfast and every 15 minutes throughout the exercise period.

The participants' Rating of Perceived Exertion (RPE) will be recorded every 15 minutes throughout exercise.

12 Please indicate whether the proposed study:

<table>
<thead>
<tr>
<th>Involves taking bodily samples</th>
<th>Yes x No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Involves procedures which are physically invasive (including the collection of body secretions by physically invasive methods)</td>
<td>Yes x No</td>
</tr>
<tr>
<td>Is designed to be challenging (physically or psychologically in any way), or involves procedures which are likely to cause physical, psychological, social or emotional distress to participants</td>
<td>Yes x No</td>
</tr>
<tr>
<td>Involves intake of compounds additional to daily diet, or other dietary supplementation</td>
<td>Yes x No</td>
</tr>
<tr>
<td>Involves pharmaceutical drugs (please refer to published guidelines)</td>
<td>Yes No x</td>
</tr>
<tr>
<td>Involves testing new equipment</td>
<td>Yes No x</td>
</tr>
<tr>
<td>Involves procedures which may cause embarrassment to participants</td>
<td>Yes No x</td>
</tr>
<tr>
<td>Involves collection of personal and/or potentially sensitive data</td>
<td>Yes No x</td>
</tr>
<tr>
<td>Involves use of radiation (Please refer to published guidelines. Investigators should contact the University’s Radiological Protection Officer before commencing any research which exposes participants to ionising radiation – e.g. X-rays)</td>
<td>Yes No x</td>
</tr>
<tr>
<td>Involves use of hazardous materials (please refer to published guidelines)</td>
<td>Yes No x</td>
</tr>
<tr>
<td>Assists/alters the process of conception in any way</td>
<td>Yes No x</td>
</tr>
<tr>
<td>Involves methods of contraception</td>
<td>Yes No x</td>
</tr>
<tr>
<td>Involves genetic engineering</td>
<td>Yes No x</td>
</tr>
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</table>

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

Bodily Samples – Participants will be required to collect a urine sample in a sample bottle before the exercise protocol.

Invasive Procedures – Blood samples will be taken by means of an indwelling cannula inserted into the antecubital forearm vein and connected to a three way tap via catheter tubing. The cannula will be kept patent by frequent flushing with a sterile saline solution. All samples will be withdrawn and dispensed by an investigator trained and experienced in this procedure. Blood samples will be stored at -80°C.

All procedures will be carried out in accordance with the Code of Practice for Workers having contact with Body Fluids.

Physical Challenge – The VO₂max test will challenge the runner but is to volitional fatigue therefore the participant will stop running when he finds the challenge.
intolerable The fatigue experienced will be similar to that familiar to the participant as part of routine training and competition

Dietary Manipulation – participants will be required to consume prescribed meals in the lab. The meals prescribed are breakfast on the day of the trial.

13. Participant Information
Details of participants (gender, age, special interests etc)
Participants will be recreationally active eumenorrheic women who include running as part of their training programmes. Ages will range from 18-35 years.

Number of participants to be recruited 10 women
How will participants be selected? Please outline inclusion/exclusion criteria to be used
Participants will be volunteers from the student population that satisfy the above criteria and will be screened via the generic health questionnaire with those with asthma, diabetes mellitus, heart problems and osteoarthritic problems being excluded from the study

How will participants be recruited and approached?
By general notice (posters, email and personal contacts)

Please state demand on participants’ time
The preliminary trials will last approximately 2 hours. Each main trial will last for approximately 4-5 hours

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 be continually monitored by an investigator throughout all testing procedures and will measure their own nude weight in the strictest privacy. At least two female investigators will be present at all times during testing

16. Possible risks, discomforts and/or distress to participants
The determination of VO2max 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 for 60 minutes or
volitional fatigue, whichever occurs first. The discomfort is, therefore, by definition tolerable and when it becomes intolerable, the runner 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 performers under the prescribed experimental conditions and are ready to abort the test should the subject report being (or appear to be) unduly stressed.

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 minimises the risk.

Details of any payments to be made to the participants: none

18. Is written consent to be obtained from participants? Yes [x] No [ ]
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?
   - Children under 18 years of age: Yes [x] No [ ]
   - People over 65 years of age: Yes [ ] No [x]
   - People with mental illness: Yes [ ] No [x]
   - Prisoners/other detained persons: Yes [ ] No [x]
   - Other vulnerable groups: Yes [ ] No [x]

If you have selected yes to any of the above, please answer the following questions:
   what special arrangements have been made to deal with the issues of consent?

   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?
   Participants will be advised, both verbally and in writing, that they may withdraw from the study at any time without reason.

21. Will the investigation include the use of any of the following?
   - Audio / video recording (delete as appropriate): Yes [ ] No [x]
   - Observation of participants: Yes [x] 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?
   All data will be coded so that the participants name does not appear on any data. Only one researcher will have access to the code.
23. What steps have been taken to ensure that the collection and storage of data complies with the Data Protection Act 1998? Please see University guidance on Data Collection and Storage and Compliance with the Data Protection Act.

Data storage will adhere to the data protection act, so that no participants’ confidentiality will be breached. All data will be coded so that the participants name does not appear on any data. Only one researcher will have access to the code. Blood samples will be anonymous and stored at -80°C for no longer than 6 months. Numerical data will be stored in raw data format for no longer than 6 years.

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  [x]

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 [x]  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*
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/cp.eilepsy. 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) An allergic reaction, eg., swelling or breathing difficulties. Yes [ ] No [ ]

4. **Is there a family history of Diabetes?** Yes [ ] No [ ]

5. **Has any, otherwise healthy, member of your family under the age**
Appendix B  Health Screen Questionnaire

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.) ........................................

Additional questions for female participants
(a) are your periods normal/regular? ...... Yes ☐ No ☐
(b) are you on “the pill”? ...... Yes ☐ No ☐
(c) could you be pregnant? ...... Yes ☐ No ☐
(d) are you taking hormone replacement therapy (HRT)? Yes ☐ No ☐

Thank you for your cooperation!

Signature ___________________________ Date ____________

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Appendix C

STATEMENT OF INFORMED CONSENT

I have read the subject information sheet, detailing the procedure and requirements which are involved with this study and I fully understand what is required of me. I have had an opportunity to ask for further information and clarification of the demands of each of the procedures. I am aware that I have the right to withdraw at any time with no obligation to give reasons for my decision.

I agree to take part in the study.

Name ___________ Phone No

Age ___________ Date of Birth

Contact Address

Signed ___________ Witnessed by

Date
Appendix D

Health Questionnaire

Please complete the following brief questions to confirm your fitness to participate:

At present do you have any health problems for which you are

1) On medication, prescribed or otherwise

YES ☐ NO ☐

2) Attending your general practitioner

YES ☐ NO ☐

Have you any symptoms of ill health, such as those associated with a cold or other common infection?

YES ☐ NO ☐

If you have answered yes to any of the above questions please give more details below:

Do you want to take part in today’s experiments? YES ☐ NO ☐

Signature ______________ Date:

........................................................................................................................................

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Appendix E

Pre-trial nutrient intake determined from two day weighed food intake diaries

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Pre-trial nutrient intake

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Appendix F

Blood lactate assay

Fluorometric method based upon Maughan (Maughan, 1982).

Principle:

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

Pyruvate + Hydrazine \[\rightarrow\] 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
Appendix F
Blood lactate assay

NAD$^+$: Free acid, grade II, ~98%, MW=663.4, Boehringer Mannheim
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 25% perchloric acid (PCA) to make 0.5, 1, 2, 4, 6, 8 mM standards.

5. Samples collection
Blood lactate concentrations were determined from a 20 μl whole blood sample deprotoinised in 200 μl 25% PCA and then centrifuged for 3 min at 13000rpm.
Samples were then stored at -85°C until analysis was carried out.

Procedures
1. Remove samples and standards from the freezer and allow to thaw at room temperature for at least one hour.
2. Mix samples thoroughly using Whirlmix, 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 (Whirlmix) and incubate for 30 min at room temperature.
   Cover tubes 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).
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