INFLUENCE OF HIGH AND LOW GLYCAEMIC INDEX CARBOHYDRATE PRE-EXERCISE MEAL ON FAT METABOLISM IN MEN AND WOMEN.

by

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This thesis is dedicated to my Dad,
my husband Ian and
our daughters Melanie and Heather.

Dedicated to the memory of my Mum who died in February.

"There are souls in this world which have the gift of finding joy everywhere
and of leaving it behind them when they go."
Frederick William Faber

Abstract

Evidence supports increased fat oxidation during sub-maximal exercise, 3 h following the ingestion of a low glycaemic index (LGI) carbohydrate (CHO) meal compared with high glycaemic index (HGI) carbohydrate meal. The meals contained the equivalent of 2g/kg body mass of available carbohydrate. The aim of this thesis was to investigate whether or not the increased fat oxidation following a low glycaemic index meal is evident in recreationally active participants following the ingestion of breakfast with an available carbohydrate content of $1g.kg^{-1}BM$.

Study 1 investigated the glycaemic responses to four commonly consumed breakfasts, two HGI (toast; cornflakes) and two LGI (muesli; fruit). Although peak blood glucose concentrations were similar following all four breakfasts, the shape of the blood glucose curves were different, suggesting that some foods classified as LGI, based only on the 'area under the blood glucose curve', may have metabolic consequences that are closer to the responses expected after consuming HGI CHO foods. The second study (male participants) has extended the observations beyond the postprandial period to a 60 min exercise period. There were no statistically significant differences in the rate nor in the total fat oxidised during the 60 min exercise. Findings were similar in female participants in study 3. Using a different LGI breakfast a similar strong trend for greater fat oxidation during exercise was demonstrated in study 4. There was no statistically significant difference in the peak or total insulin IAUC between the two trials. Therefore, it is not surprising that there was no significant difference in substrate oxidation during the 60 min exercise between the HGI and LGI trials in men or women. Thus the evidence from these studies do not support the hypothesis that eating a low GI breakfast will promote a greater rate of fat oxidation during subsequent exercise when compared with consuming a high GI breakfast.

Keyword: Glycaemic Index, Glycaemic Load, Blood Glucose, Plasma Insulin, Carbohydrate, Mixed meals, sub-maximal exercise, Fat oxidation, Men, Women.

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Publications

The findings from Chapter 4 and 5 have been published as follows:

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CHAPTER 1

Introduction

Fat and carbohydrate (CHO) are the principal fuels used by skeletal muscle during rest and physical activity. While fat stores are plentiful, carbohydrate stores are limited and need to be regularly replenished. Although protein can also be used as a fuel source, it seems unlikely that it can contribute more than 10% of energy requirements even in extreme conditions.

The intake of carbohydrate results in an increase in insulin secretion which has a strong inhibition on fat metabolism (Horowitz, Mora-Rodriguez, Byerley and Coyle 1997). However due to the abundance of fat stores this is not considered a limiting factor to energy production for exercise performance (Johnson, Stannard and Thompson 2004). An increased contribution of fat to energy production will reduce carbohydrate oxidation and will spare muscle glycogen (Costill, Coyle, Dalsky, Evans, Fink and Hoopes 1977).

Carbohydrates consumed can have a significant glycaemic and insulinaemic effect. Even in well-fed individuals, after an overnight fast, the liver will be low in glycogen. Pre-exercise CHO meals will increase liver (Nilsson and Hultman 1973) and muscle glycogen concentrations (Coyle, Coggan, Hemmert, Lowe and Walters 1985) before exercise and provide a source of CHO during exercise as it empties from the stomach (Marmy-Conus, Fabris, Proietto and Hargreaves 1996).

Traditionally carbohydrates were described as simple and complex depending on their chemical structure. However ingestion of a simple or complex carbohydrate does not appear to be directly related to the glycaemic response that follows. There is a great variability in the extent to which different carbohydrates raise blood glucose concentration. Therefore , it is now

acknowledged that simple carbohydrate exchanges based on chemical analysis (food tables) do not predict their physiological response. Great differences in glycaemic response occurs not only between food groups but also within groups (i.e. cereals, vegetables, fruit). Therefore a classification of foods according to their effects on blood glucose was introduced i.e. the glycaemic index (GI).

Jenkins and colleagues (1981) defined the glycaemic index (GI) as a measure of the glycaemic response and documented it as a nutritional tool to aid the management of type 1 diabetes (Jenkins, Wolever, Taylor, Barker, Fielden, Baldwin, Bowling, Newman, Jenkins and Goff 1981). By definition, ingestion of a high glycaemic index (HGI) food results in a high and rapid peak blood glucose concentration i.e. large glycaemic response. In contrast ingestion of a low glycaemic index food (LGI) results in a small peak in blood glucose concentration i.e. a small glycaemic response. However blood glucose concentration is a function of the rate of blood glucose appearance as well as the rate of blood glucose removal from the systemic circulation. Glucose rate of appearance depends on glucose absorption from the intestines into the portal circulation and it depends on the properties of carbohydrate. The rate of glucose removal is influenced primarily by insulin (DeFronzo and Ferrannini 1982). An early rise in postprandial insulin concentration will increase glucose extraction from the blood and facilitate a faster tissue uptake, reducing the magnitude of GI (as measured using the IAUC). Hence, in principle, a food item or meal can have a low GI because of a relatively slow glucose appearance, or perhaps because of a relatively fast glucose disappearance which may affect not only its GI values but the blood glucose peaks as well (Schenk, Davidson, Zderic, Byerley and Coyle 2003).

There is a consensus view that CHO foods in the form of fruit and wholegrain products (predominantly thought to be LGI) are beneficial to health. There are also strong indications that highly processed, fibre depleted and energy dense CHO foods (predominantly HGI) can lead to over consumption and obesity-related diseases (WHO 2000). Schultze and colleagues (2004) concluded that a diet high in rapidly absorbed carbohydrates and low in cereal fibre was associated with an increased risk of type 2 diabetes (Schulze, Liu, Rimm, Manson, Willett and Hu 2004).

There are a number of epidemiological and nutritional studies which have indicated that GI can be used as a tool in the management of diabetes and weight loss. A meta-analysis of randomised controlled trials by Brand-Miller and colleagues (2003) revealed that LGI foods consumed instead of HGI food as a medium-term glycaemic control, benefited diabetic patients. The benefit was incremental and was similar to that offered by drugs targeting postprandial hyperglycaemia (Brand-Miller, Hayne, Petocz and Colagiuri 2003). In spite of this some continue to question the relevance of GI and its benefits to the population as a whole (healthy, at risk or ill) (Pi-Sunyer 2002)

Most early studies only studied the effects of single foods with differing GI values, while in everyday life most typical meals consist of a mixture of several foods, some rich in carbohydrate and others containing predominantly fat and protein. The GI provides information about the glycaemic response to a carbohydrate rich meal or to an individual food item. It merely reflects the biological impact of the available CHO in the meal, but it does not provide information on the amount of carbohydrate present in a serving of the meal. A more realistic measure of the effect of the ingested CHO quantity might be the glycaemic load (GL), as it could prove to be a better indicator of the glucose response and insulin demand of a meal. The glycaemic load of a meal

is calculated from the weighted means of the GI values of components foods. A revised GI table including GL values was published by Foster-Powell and colleagues in 2002 (Foster-Powell, Holt and Brand-Miller 2002).
Consumption of a CHO meal is followed by a rise in blood glucose concentration and increased insulin secretion. Elevated levels of insulin have a strong inhibitory effect on fat oxidation (Horowitz, *et al.*, 1997). Coyle and colleagues showed that CHO availability could directly regulate fat oxidation during exercise (Coyle, Jeukendrup, Wagenmakers and Saris 1997). Several subsequent studies have shown that the pre-exercise ingestion of LGI CHO foods result in higher circulating plasma free fatty acids (FFA) and increased fat oxidation during subsequent exercise than the ingestion of HGI CHO foods (Stevenson, Williams, Mash, Phillips and Nute 2006; Wee, Williams, Tsintzas and Boobis 2005; Wu, Nicholas, Williams, Took and Hardy 2003). In contrast ingestion of HGI meals increased carbohydrate oxidation and depressed the mobilisation of fatty acids (FFA).

Treatments for the prevention of weight gain and obesity are of considerable interest both to the general public and health-professionals (Thompson, Townsend, Boughey, Patterson and Bassett 1998). Regular exercise that increases daily energy expenditure and fat oxidation is important for the reduction of fat stores. Therefore, exercise coupled with a LGI diet may further increase fat oxidation and aid weight loss (Brand-Miller, Holt, Pawlak and McMillan 2002).

Previous studies from our laboratory were conducted on endurance athletes, hence the available CHO content of the pre-exercise meals was set at $2g.kg^{-1}$ body mass. This amount of CHO would result in a meal too large for sedentary and even recreationally active people. Therefore the studies described in this thesis were undertaken to investigate the glycaemic and later

insulinaemic responses to HGI and LGI breakfasts (with an available CHO content of 1g.kg^{-1} BM) during a 3 h postprandial period, followed by 60 min of exercise, in recreationally active men and women. The aim being to test the hypothesis that eating a low GI breakfast will promote a greater rate of fat oxidation during subsequent exercise when compared with consuming a high GI breakfast.

The thesis is presented as follows:

Chapter 2 presents a review of literature examining the effect of CHO intake on glycaemic and insulinaemic responses and substrate oxidation rates in humans, during the postprandial period and exercise. It also reviews the literature on glucose kinetics and the role of readily available glucose in the glycaemic response following HGI and LGI food intake.

Chapter 3 outlines the methods, general procedures and equipment used in the experiments, described in this thesis.

Chapter 4 (Study 1) describes the glycaemic responses during the postprandial period to four carbohydrate breakfasts : 2 of high glycaemic index and 2 of low glycaemic index. The HGI breakfasts consisted of: 1) toast and jam and 2) cornflakes and milk. The LGI breakfasts consisted of: 1) muesli and milk and 2) fruit and yoghurt. In order to use the similar ingredients as in the previous studies we split the large HGI and LGI breakfasts (2g.kg^{-1}BM available CHO) into four: two based on breakfast cereals (cornflakes; muesli), one based on bread (toast) and one based on fruit (mixed fruit). The aim was to test the glycaemic responses to all four breakfasts to identify the HGI and a LGI breakfasts with the largest difference in GI for the following studies.

As the study was designed to use participants from the general public we decided to use finger tip blood sampling instead of venous blood collected via an indwelling canula. Insulin was only measured in later studies when we worked out the methodology to use with very small finger tip blood samples.

Chapter 5 (Study 2) describes the physiological and metabolic responses, during a 3 h postprandial period and subsequent 60 min of sub-maximal exercise in recreationally active male participants, after consumption of either a HGI (toast and jam) or a LGI (fruit and yoghurt) breakfasts. Blood samples were collected for insulin measurements but were lost due to an assaying error.

Chapter 6 (Study 3) describes the physiological and metabolic responses, during a 3 h postprandial period and a subsequent 60 min of sub-maximal exercise in recreationally active female participants, after consumption of either a HGI (toast and jam) or a LGI (fruit and yoghurt) breakfasts. Blood samples were collected for insulin measurements but were lost, together with samples from the previous study due to an assaying error.

Chapter 7 (Study 4) describes the physiological and metabolic responses, during a 3 h postprandial period and a subsequent 60 min of sub-maximal exercise in recreationally active female participants, after consumption of either a HGI (toast and jam) or a LGI (muesli and milk) breakfasts.

Finally **Chapter 8** draws together the findings from the experimental work and discusses possible implications for the recreationally active population, as well as offering suggestions about areas that require further research.

CHAPTER 2

Review of Literature

2.1 Introduction

The aim of this chapter is to provide a brief overview of the current literature regarding the effect of fat and carbohydrate intake on metabolism during rest and exercise. The first part of this chapter (section 2.2) introduces fat and carbohydrate (CHO) as the main two fuels for physical activity. Fuel storage and availability as well as the relationship between exercise intensity and the contribution of these two fuels to energy demand are covered. A special focus is given to the glycaemic and insulinaemic responses to CHO food and their effect on fat oxidation during exercise. Sex differences on fat and CHO oxidation will also be discussed.

2.2 Carbohydrates and their Glycaemic effect

Glucose, the primary substrate for the brain and central nervous system, occurs naturally in food. It can also be produced by gluconeogenesis in the liver from amino acids, glycerol, pyruvate and lactate. Its regulation is very tightly controlled by the hormones insulin and glucagon. These hormones not only control the level of circulating glucose in blood, but they also play an important role in regulating liver and muscle glycogen stores.

Insulin is a hormone that regulates body glucose metabolism in tissues with the exception of the brain. It does this by increasing the rate of glucose transport across the cell membrane of muscle and adipose tissue. This is done in combination with extracellular glucose and a glucose carrier contained within the cell membrane (GLUT 4). Absence of insulin causes decrease in glucose uptake by tissues and a dangerous increase in blood glucose in the systemic circulation. Disturbance in the availability or regulation of insulin results in diabetes. Type 1 or insulin-dependent diabetes, generally occurs in

younger individuals, is characterised by total absence of insulin causes severe abnormality in glucose homeostasis. The diabetic person will be on insulin therapy to normalise daily blood glucose fluctuations. Exercise have to be carefully controlled. Hypoglycaemia can occur during prolonged sub-maximal exercise. The hepatic glucose production cannot keep up with the increased demands of glucose by the exercising muscle so blood glucose level can drop to a dangerously low level.

Type 2 diabetes, or non-insulin dependent diabetes, is caused by a decrease in insulin sensitivity. Glucose clearance is slow, and glucose accumulates in the circulation. The excess glucose is passed through the kidneys causing an increased osmotic pressure that also diminishes the reabsorbtion of water. Hence the diabetic person will loose glucose as well as extra water. With a decreased glucose uptake by the cells the diabetic person relies heavily on fat metabolism for energy. This produces an excess of ketoacids and results in acidosis which could lead to a diabetic coma.

Glucagon is the antagonist to insulin, and it is this hormone that is activated when blood glucose falls below normal levels. The alpha cells of the pancreas will secrete glucagon to normalise blood glucose. Glucagon stimulates liver to break down of liver glycogen into glucose thus raising circulating blood glucose levels. Glucagon also stimulates gluconeogenesis in the liver by promoting amino acid uptake by the liver. Similar to insulin, glucagon is also controlled by the circulating levels of glucose. When blood glucose concentration fall, as occurs in prolonged exercise, the alpha cells of the pancreas are stimulated to secrete glucagon. This will cause an almost instantaneous release of glucose from the liver.

Once absorbed across the small intestine, glucose can be used by the cell for energy, or stored in the muscle and liver in the form of glycogen or converted into fat. Fructose, occurs in large amounts in fruit. About two thirds of fructose is converted in the liver to glucose which may accumulate as glycogen or released as glucose. The rest of the metabolised fructose will be released by the liver as lactate (Henry, Crapo and Thorburn 1991).

Polysaccharides are composed of ten to thousands of monosaccharides and can be of plant or animal origin. The plant polysaccharides are starch and fibre. In plants CHO is stored in the form of starch contributing to around 50% of the total human CHO intake. Dietary starch, a polymer of glucose, commonly referred to as complex CHO, exists in two forms: amylose and amylopectin. Amylose hydrolyses relatively slowly in the gut and is considered a resistant starch. Amylopectin is a highly branched form and its structure allows a greater surface exposure to digestive enzymes compared with amylose, allowing a faster digestion and absorption. Ingestion of starch high in amylopectin will result in a larger glycaemic and insulinaemic response than starch rich in amylose (Behall, Scholfield, van der Sluijs and Hallfrisch 1998; Granfeldt, Drews and Bjorck 1995). In comparison, resistant starch, an indigestible form of starch will reduce postprandial glycaemic and insulinaemic response by slowing down absorption and digestion (Raben, Andersen, Christensen, Madsen, Holst and Astrup 1994). Fibre is a structural polysaccharide, that exists exclusively in plants giving the structure of leaves, stems, roots, seeds and fruit covering. It is located mostly in the cell wall, (giving its rigidity) as cellulose, gums, hemicellulose and pectin. It resists hydrolysis by human digestive enzymes although a part of it can undergo bacterial fermentation in the large intestine. Dietary fibre, water soluble like pectin or water insoluble like cellulose, is necessary in keeping a healthy

digestive system and also helps reduce the incident of obesity, diabetes and heart disease (Salmeron, Manson, Stampfer, Colditz, Wing and Willett 1997b).

There has been an ongoing debate on the effect of dietary fibre on glycaemic response (Pi-Sunyer 2002). There is a very weak relationship between fibre content and GI. However Wolever and colleagues (1990) found that when fibre is broken down to its components, one of them, insoluble fibre has a much stronger relationship with GI ($r=0.584$, $P<0.001$) (Wolever 1990). The outer coat of grains is a source of insoluble fibre, often referred to as roughage. Insoluble fibres will lower GI by acting as a physical barrier, delaying access of digestive enzymes and water to the starch within the cereal grain.

In contrast a review by Nuttall (1993) found that when large quantities of fibre are added to a CHO meal it was only the soluble fibre that had an effect on the glycaemic response (Nuttall 1993).

2.3 The Glycaemic Index

Carbohydrates are digested and absorbed at different rates. Almost all carbohydrates, regardless of the form in which they are consumed, are broken down to glucose, which enters the systemic circulation. The rise in blood glucose levels is known as the glycaemic response. A measure of the glycaemic response, the glycaemic index (GI), was first defined by Jenkins (1981) and documented as a tool for managing type 1 diabetes (Jenkins, *et al.*, 1981).

The GI is defined as the incremental area under the blood glucose curve (IAUC) after consumption of a 50 g available-carbohydrate portion of a food expressed as a percentage of that after 50 g of glucose. Carbohydrate foods received a numeric value (1-100, where glucose =100) and were then

arbitrarily classified as having a high (>70), moderate (56-69), or low GI (<55) (Jenkins, Wolever, Jenkins, Josse and Wong 1984). Ongoing research into the effect of GI on postprandial glycaemia resulted in the publication of the first edition of the International Tables of Glycemic Index that included 565 food (Foster-Powell and Miller 1995) and the revised version in contained 750 different types of food (Foster-Powell, *et al.*, 2002).

The use of GI for the classification of CHO-rich foods has been endorsed by the Food and Agriculture Organization (FAO) of the United Nations and World Health Organisation (WHO) (FAO/WHO 1998). In order to promote good health, they recommended the consumption of a high CHO diet (≥55% energy from CHO) mostly consisting of low GI CHO foods. Information about food composition together with GI values of foods was recommended as a more useful guide to food choices (Foster-Powell, *et al.*, 2002).

2.3.1 Factors influencing the Glycaemic Index

Type of reference food

The 'reference food' is the CHO against which other foods are compared. Glucose was the original reference (Jenkins, *et al.*, 1981) but this was changed to white bread because it was considered a CHO source that is used in normal habitual diet (Jenkins, Wolever, Jenkins, Thorne, Lee, Kalmusky, Reichert and Wong 1983). Both reference foods have been assigned the value of 100. Glycaemic index values based on white bread as a reference food are 1.4 times greater than those based on glucose, because glucose has a glycaemic response that is 40% higher than that of white bread. For the purpose of standardisation GI values can be adjusted and expressed on a standard glucose scale by dividing the GI values obtained using white bread as reference food by 1.4 (Wolever 2003b).

The blood glucose response to a CHO food, and thus the GI, can be affected by a number of factors. These include the types of monosaccharides (fructose and galactose give lower GI compared with glucose) (Englyst, Vinoy, Englyst and Lang 2003); the amylose to amylopectin ratio (the branched amylopectin is more rapidly digested than the straight chain amylose and results in a higher GI) (Behall, Scholfield and Canary 1988; Granfeldt, *et al.*, 1995).

Carbohydrates: availability and amount

The term 'available CHO' represents part of the CHO that can be digested by human enzymes and absorbed from the small intestine. It does not include dietary fibre, which can be an energy source only after fermentation in the large intestine.

Blood glucose responses are influenced by the amount of ingested CHO. Jenkins and colleagues (1981) have shown that the increase in glycaemic response is almost linear for a CHO intake of 0-50 grams following which a levelling off occurs as CHO intake increases from 50-100grams (Jenkins, *et al.*, 1981). As a result test meals used in most studies contain 50g available CHO, excluding CHO portions that cannot be digested and absorbed by the small intestine (dietary fibre, resistant starch). However in real life, people consume more than 50 g CHO, usually in a mixed meal and without knowing its fibre or resistant starch content. Consideration also has to be given to the quantity of the test meal. For example 50 g of available CHO might be adequate for a small female or a child but is inadequate for a growing teenager, a larger male or people with large energy expenditures.

Food origin and maturation

Pi-Sunyer and colleagues has shown that rice produced very different GI values due to its amylose to amylopectin ratio (Pi-Sunyer 2002). Foster-Powell

(2002) reported a large variations in the GI values with potato variety. For example a non-specific type of boiled potato from Kenya with a GI of 24 was ~78% lower than the GI of 111 for potatoes from the US. Differences were seen even in the same variety of potatoes as GI ranged between 78 and 111 for U.S. Russet potatoes (Foster-Powell, *et al.*, 2002). Similarly Henry and colleagues found that GI values of UK potatoes varied between 56 and 94. The more waxy textured potatoes produced medium GI whilst floury potatoes had high GI values.

With such large variation in GI, the reliability of GI was questioned following a study conducted by seven separate laboratories, which tested four centrally distributed foods: instant potatoes, rice, spaghetti and barley (Wolever 2003b). The difference between the highest and lowest GI of instant potato was 33 units (Wolever 2003b). According to the arbitrary categorization of GI into Low-GI (<55), Medium GI (56-69) and High-GI (>70), the mash potato mentioned could be in any of the three categories.

An important determinant of GI is the state of maturation or ripeness of food. It is well known that fruit starch gradually turns to sugar during the ripening process, thus a green banana will have a lower GI than a ripe banana (Englyst and Cummings 1986).

Fat and protein

The addition of protein and fat to CHO has been demonstrated to affect postprandial responses. Several studies have shown that protein added to CHO will result in a considerable increase in the insulin response to this food without an effect on the glucose response (van Loon, Saris, Verhagen and Wagenmakers 2000). Protein added to CHO food has been shown to significantly increase insulin responses and therefore enable a greater glucose

uptake and possibly a greater muscle glycogen synthesis (Betts, Stevenson, Williams, Sheppard, Grey and Griffin 2005; Betts, Williams, Boobis and Tsintzas 2008; Ivy, Goforth, Damon, McCauley, Parsons and Price 2002; van Hall, Shirreffs and Calbet 2000). It is generally considered that fat reduces (Betts, *et al.,* 2008)glycaemic responses by delaying gastric emptying rate (Welch, Bruce, Hill and Read 1987). However the protein and fat content of normal meals are not really large enough to have any detectable effect on postprandial glucose and insulin responses in healthy individuals (Wolever 2000). For example Wolever and Bolognesi fed five unmatched meals (0, 25, 50, 75 or 100g CHO) to healthy individuals. The five meals varied in energy (1650-2550 kJ), fat (8-24 g), protein (12-25 g) carbohydrate (38-104 g) and glycemic index (43-99). The Statistical analysis of the data revealed that both source and amount of CHO had significant effect on the incremental area under the curve for capillary and plasma glucose and plasma insulin. The CHO source accounted for 85-94% of the variability of the mean insulin and glucose responses (Wolever and Bolognesi 1996).

Food variability

The glycaemic index was originally conceived as a natural property of food and not as a metabolic response of an individual to that food. Based on this the GI of a food should be reproducible and consistent. According to the most recent publication of the International Table of Glycemic Index values, there is a wide range of GIs for the same CHO food (Foster-Powell, *et al.,* 2002). Even glucose, chosen as a reference food because of its ease of measurement and good coefficient of variation compared with other carbohydrates, showed a fairly large within participant variability in its glycaemic response. For 11 normal participants the mean (± SEM) within participant coefficient of variation (CV) for repeated tests of 50 g glucose was 25 ±4 % (Wolever 1985) and in 47 participants 23.4 ± 2.1% (Wolever,

Vorster, Bjorck, Brand-Miller, Brighenti, Mann, Ramdath, Granfeldt, Holt, Perry, Venter and Xiaomei 2003). It is known that the GI of CHO rich foods can vary greatly depending on the method of preparation, processing, variety, origin, maturation, degree of ripeness and time of day the GI is measured (Pi-Sunyer 2002). There is also a large between participants and within participant variation. Hence dietary planning cannot be useful unless the GI has a predictable effect on blood glucose it is valid and most of all reliable.

Processing and preparation

Processing like grinding, rolling, mashing, chewing, canning of CHO rich in starch will disrupt the amylase and amylopectin molecules making them more available for hydrolysis which in turn will increase their GI (Pi-Sunyer 2002). Juntunen and colleagues found decreased postprandial insulin responses with ingestion of less processed whole grain products and concluded that the postprandial responses of insulin are determined by the structure of the grain rather then the fibre composition or type of cereal in food. (Juntunen, Niskanen, Liukkonen, Poutanen, Holst and Mykkanen 2002).

Cooking has been shown to alter the GI of CHO rich foods especially if they are high in starch. For example the GI of raw potato has a relatively low GI because in its uncooked form its starch is resistant to hydrolysis by digestive enzymes, however the potato is also very unpalatable to eat in this form. Fernandes (2005) and colleagues found that during cooking starch molecules in the potato become gelatinised, increasing starch digestibility, GI and the palatability. Some studies found that while the potato is cooled this gelatinisation is reversed, resistant starch is formed resulting in a lower GI (Fernandes, Velangi and Wolever 2005) According to Fernandes, individuals wishing to lower the dietary GI could do so by, for example by precooking

potatoes and eating them later cold or reheated, however this results in change in taste.

Canning process also have a sizable effect on food items. For example fruits when thermally processed, will also be subjected to the effects of ripening as well as those of processing. Increase in temperature will stimulate the activity of ripening-related as well as wall-degrading enzymes. Most importantly softening of the fruit occurs as a result of an easier cell separation due to degradation of pectic polysaccharides which plays an important role in cell adhesion. The softening is also attributed to acid hydrolysis of glycosidic bonds in the cell-wall polysaccharides (Waldron, Parker and Smith 2003). This process enables access to the cell contents increasing the amount of readily available glucose.

Diets that contain large amounts of highly processed hence rapidly digestible CHO, which elevate blood glucose and insulin, maybe detrimental to health. Englyst and colleagues investigated the glucose availability upon digestion of different foods. Measuring different starch fraction they concluded that there was a highly significant correlation between rapidly available glucose (RAG) and GI (Englyst, Veenstra and Hudson 1996; Englyst, Englyst, Hudson, Cole and Cummings 1999; Englyst, et al., 2003). In vitro measurement of RAG was based on the measurement by HPLC of the glucose released from a test food during timed incubation with digestive enzymes under standardized conditions (Englyst, *et al.*, 1999). Englyst suggested the use of RAG value instead of GI might be more useful indicator of the glucose and insulin raising capacity of the CHO foods.

On the whole starch is considered to be digested slowly resulting in a modest glycaemic response. However the extent to which starch is digested and

absorbed depends on the source and degree of processing (Englyst, *et al.*, 1986). Simple sugars like glucose and sucrose are rapidly absorbed hence breakfast cereals possibly containing hidden sugars could raise blood glucose and insulin concentrations. (Englyst, *et al.*, 1996). In vitro Englyst and colleagues (1996) measured three fractions of starch and classified them as: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). The same technique can give a value for rapidly available glucose (RAG) and slowly available glucose (SAG). Following the analysis of 39 foods a highly significant positive correlation was found between GI and RAG ($r=0.981$, $P<0.0001$). According to the authors this direct measurement of RAG in vitro can provide value for a direct calculation of glucose likely to be absorbed by the human small intestine, influencing blood glucose and insulin levels (Englyst, *et al.*, 1996). The significance of RAG in the measurement in GI was demonstrated by analysing 23 commercially available products (five breakfast cereals, six bakery products and crackers, and twelve biscuits). In vitro measures describing the rate of glucose release from these food products were found to be the main determinants of glycaemic index (GI) and insulinaemic index for cereal products (Englyst, et al., 2003).

Time of day

Absolute glycaemic response (i.e., the AUC) as well as the relative glycaemic response (i.e., the GI) is affected by the time of the day in which the glycaemic response is measured (Gannon, Nuttal, Westphal, Fang and Ercan-Fang 1998; Wolever, *et al.*, 1996). Wolever and Bolognesi (1996) comparing the glycaemic responses to two different breakfasts under two conditions (after 12 h fast and 4 hrs after breakfast) found that the mean AUC for the high fibre cereal was 50% lower than the low fibre cereal after 12 hour fast, however this difference was reduced to 21% at midday (Wolever, *et al.*, 1996). This study suggests that GI values are only valid if they are determined in the morning.

It has been recognized for more than two decades that, in normal subjects, the response to an oral glucose tolerance test varies according to time of day (Jarrett and Keen 1969). In the afternoon and evening, blood glucose levels 1–2h after ingestion of a 75-g oral glucose load are generally 1.7–2.8 $mmol.l^{-1}$ higher than when the test is performed in the morning. Tis increased potential of a false-positive diagnosis of diabetes in the afternoon, as compared with the morning (Jarrett, Viberti and Sayegh 1978). The insulin response to oral glucose is also affected by time of day, the increase being generally higher and of shorter duration in the morning and lower, delayed, and more prolonged in the evening.

Glucose kinetics

The GI of a food is thought to directly reflect the rate of digestion and glucose entry into the systemic circulation. However the blood glucose concentration represents both entry and removal of glucose from the blood. Schenk and colleagues (2003) measured plasma glucose kinetics (by constant-rate infusion) and insulin concentration in six healthy males during 180 min following the ingestion of 2 test breakfasts. Two popular and commercially available breakfasts cereals were used. Cornflakes (CF) and a bran cereal (BC)(All-Bran) breakfast containing 50g of available CHO were consumed following an overnight fast. The results showed that the glycaemic index of CF was more than twice that of BC, despite of the fact that there was no significant difference in the rate of glucose appearance. An earlier rise in postprandial insulin caused a 31% increase in the rate of glucose disappearance in the BC trial compared with CF trial. The earlier increase in insulin aided the increased glucose uptake thus reducing circulating glucose levels. (Schenk, *et al.*, 2003).

In summary, differences in GI of carbohydrate foods can be influenced not just by the rate of appearance of glucose but also by the rate of its disappearance.

Blood sampling

Venous plasma glucose concentrations are used in oral glucose tolerance test (OGTT) and often in the clinical management of diabetic patients. Most metabolic studies use venous blood sampling as several metabolites can be measured from one sample. With the constant development of micro-assays it is now possible to measure a range of metabolites from small samples of finger tip blood. However the concentration of blood glucose can be site and method specific (Ellison, Stegmann, Colner, Michael, Sharma, Ervin and Horwitz 2002). Kuwa and colleagues (2001) found that blood glucose concentrations following an OGTT were higher in fingertip whole blood samples compared with whole blood venous samples. The difference was the highest at 60 min (20%) into the postprandial period, diminishing to 11% at the end of the 180 min. (Kuwa, Nakayama, Hoshino and Tominaga 2001). Furthermore data from an inter-laboratory study by Wolever and colleagues found that the within-participant coefficient of variation (CV) was significantly lower using capillary blood samples (23.4 ± 2.1%) compared with venous blood samples (56.8 ± 4.4%) (Wolever, Jenkins, Jenkins and Josse 1991).

Therefore it appears that the method of measurement as well as the blood collection site can effect blood glucose results, especially immediately following ingestion of a meal when blood glucose concentration is at the highest.

2.4 Glycaemic Index and mixed meals

It has been suggested that the GI, based on tests on single foods, may not apply to mixed meals containing fat and protein (Coulston, Hollenbeck, Liu, Williams, Starich, Mazzaferri and Reaven 1984a; Coulston, Hollenbeck and Reaven 1984b). However mixed meals have been used successfully in several studies, the calculation of the meal's GI is based on the sum of the GI contributions of each CHO component of the meal (Wolever and Jenkins 1986).

Wolever and colleagues (1986) found that GI of a mixed meal correlated positively with the actual plasma glucose response ($r=0.987$; $P<0.020$) (Wolever, *et al.*, 1986). However, there are several studies that found that GI cannot be predicted from mixed meals (Coulston, *et al.*, 1984b; Flint, Moller, Raben, Pedersen, Tetens, Holst and Astrup 2004; Hollenbeck and Coulston 1991). Meals in some studies contained only 50 gram of available CHO and were not matched for energy (Flint, Moller, Raben, Pedersen, Tetens, Holst and Astrup 2004) and macronutrient composition. Studies from our laboratory successfully used HGI and LGI mixed meals with an available CHO content of $2g.kg^{-1}BM$ (Stevenson, Williams and Nute 2005; Stevenson, *et al.*, 2006; Wee 2005; Wu, *et al.*, 2003).

The GI as a tool, was meant to predict the glycaemic and insulinaemic response to a meal. While GI can predict the glycaemic response to a meal it cannot predict the insulinaemic response, especially when macronutrients other than CHO are present (Pi-Sunyer 2002). For example the addition of protein to a meal is known to increase the insulin response and reduce the glycaemic response (Nuttall, Mooradian, Gannon, Billington and Krezowski 1984; Pi-Sunyer 2002). Added protein increases the insulin response, however the glucose response does not change (van Loon, *et al.*, 2000). Milk products

have a strong insulinotropic effect (Gannon, Nuttall, Krezowski, Billington and Parker 1986; Nilsson, Stenberg, Frid, Holst and Bjorck 2004; Ostman, Liljeberg Elmstahl and Bjorck 2001) and have the ability to increase insulin response also when supplied in a mixed meal (Hoyt, Hickey and Cordain 2005; Liljeberg Elmstahl and Bjorck 2001; Nilsson, Elmstahl and Bjorck 2005). For example semi-skimmed milk added to a cereal breakfast can reduce its GI by 30%-50% (Henry, Lightowler, Dodwell and Wynne 2007).

The fibre content of the meal can effect the glycaemic response. Jenkins et al (1981) found that insoluble fibre had little or no impact on the glycaemic response compared with soluble fibre which tends to lower GI (Jenkins, *et al.*, 1981). However, examining the relationship between GI and dietary fibre Wolever (1990) found just the opposite, a significant but week correlation between insoluble fibre and GI (r=0.584) (Wolever 1990).

For many foods the presence of naturally occurring soluble or insoluble fibre has little impact on the GI (Foster-Powell, *et al.*, 2002) based on 50g food portions. Differences in the behaviour of starchy foods in mixed meals are similar to those in single meals, providing the mixed meals have identical macronutrient contents. Most LGI foods contain a varying amounts of dietary fibre and its effects in slowing carbohydrate absorption depends on the amount of processing hence its effect on GI is variable.

In summary, it is possible to measure and compare the glycaemic responses to mixed meals, providing they contain the same amount of energy and macronutrients.

2.5 Glycaemic Index and satiety

A number of studies have addressed the question whether or not the consumption of LGI food reduces hunger and promotes satiety compared with the consumption of HGI foods. Bornet and colleagues, in their recent review paper stated that based on their findings wordings such as "This is a low-GI food. Low-GI foods help one to feel fuller for longer than equivalent high-GI foods' are substantiated health claims (Bornet, Jardy-Gennetier, Jacquet and Stowell 2007).

Some studies have found a significant and others a non-significant reduction in hunger and increased satiety following the ingestion of a LGI compared with HGI foods. Using single foods such as glucose, fructose, amylose v amylopectin (rather than of normal everyday foods) makes the explanation of the results from early studies difficult to interpret in relation to the results from the mixed meal studies. More importantly, foods used in early studies were less palatable/appetising than normal foods (e.g. lentils) and were not match for nutrients and energy content.

Evidence from one day studies have shown that LGI food/meal are more satiating than HGI food/meals (Roberts 2003). High glycaemic index foods (highly refined) common in the Western diet produce high glycaemic responses promoting CHO oxidation at the expense of fat oxidation. The intake of high energy density food intake coupled with a more sedentary lifestyle can lead to excess weight gain in susceptible individuals (Roberts 2003). Prolonged postprandial satiety and increased fat oxidation during exercise are associated with LGI foods (Wu, *et al.*, 2003) and may prove an effective method in reduction of energy intake (Ball, Keller, Moyer-Mileur, Ding, Donaldson and Jackson 2003).

In summary low glycaemic index food intake is to be recommended to reduce the glycaemic and insulinaemic effects on the body and generally increase the feeling of satiety.

2.6 Glycaemic Index, health and disease

Low glycaemic index foods are less processed than HGI foods and tend to contain more dietary fibre. The health benefits of fibre-rich foods is well documented and it should replace low fibre alternative. Epidemiological studies have shown that dietary fibre can reduced the risk of onset of type 2 diabetes (Salmeron, Ascherio, Rimm, Colditz, Spiegelman, Jenkins, Stampfer, Wing and Willett 1997a; Salmeron, *et al.*, 1997b; Wolever 1997). Examining the associations between the intakes of dietary fibre and whole or refined grain products and weight gain over time, Liu and colleagues found weight gain is inversely associated with the intake of high-fibre and whole-grain foods but positively related to the intake of refined-grain foods, important for the management of weight control (Liu, Willett, Manson, Hu, Rosner and Colditz 2003).

Following a meta-analysis of 14 studies, Brand-Miller (2003) concluded that substituting HGI foods with LGI foods has a small but clinically useful effect on medium-term glycaemic control in diabetic patients. The incremental benefits are comparable to those offered by pharmacological agents (Brand-Miller, *et al.*, 2003).

2.7 The Glycaemic Load

The glycaemic index represents the glycaemic response to CHO foods but does not consider the amount of CHO consumed. To quantify the overall glycaemic effect of a portion of food Salmeron (1997) introduced the concept of Glycaemic Load (GL) (Salmeron, *et al.*, 1997a; Salmeron, *et al.*, 1997b). The

glycaemic load of a typical serving of food is the product of the amount of available CHO and the GI of component foods divided by 100, providing an estimate of the glycaemic impact of a meal.

The Glycaemic Load (GL) was considered as an indicator of the glucose response and insulin demand induced by a serving of food. The introduction of GL significantly alters the rankings of several CHO rich foods. Medium GI sucrose becomes low GL and LGI spaghetti turns into high GL, while the HGI cornflakes comes out as high GL, and LGI kidney beans as low GL.

Ongoing research into the effect of GI on postprandial glycaemia has seen the publication of Glycaemic Load (GL) values of several single foods and ready meals (Foster-Powell, *et al.,* 2002; Henry, *et al.,* 2007; Henry, Lightowler, Strik, Renton and Hails 2005).

Brand-Miller and colleagues (2003) examined the glycaemic response (as determined by area under the curve) to 10 different foods, all with a calculated glycaemic load equivalent to one slice of white bread. They found no difference between the glycaemic responses of the 9 foods compared to that of white bread. Only lentils produced lower than predicted responses (Brand-Miller, Thomas, Swan, Ahmad, Petocz and Colagiuri 2003). They have also tested the glycaemic responses to different glycaemic loads and found that a stepwise increase in glycaemic load produced significant and predictable increases in both glycaemia and insulinaemia. In order to prove the validity of the GL concept all meals should have produced the same glycaemic response. These finding support the concept that dietary GL can be used as a measure of overall glycaemic response and insulin demand (Brand-Miller, *et al.,* 2003).

In summary the GL is a useful tool to measure the overall impact of the amount of carbohydrate ingested and it can indicate the overall demand for insulin, however its value depends on the GI value of the food.

2.8 Fat and carbohydrate: fuels for physical activity.

2.8.1 Substrate oxidation at rest.

Following an overnight fast, plasma insulin concentrations are at their lowest and free fatty acid (FFA) concentrations, the highest. Originating mostly from the adipose tissue, circulating FFA provide the primary fuel for muscle, liver, heart and kidneys (Jensen 2003).

Homeostasis maintains circulating blood glucose concentration at about 5 $mmol.l^{-1}$ by the breakdown of liver glycogen (Frayn 2003). In the fasted state it is the brain that takes up most of the circulating glucose with minimal use by skeletal muscle because when there is a low concentration of glucose the brain and the central nervous system take priority.

Significant metabolic changes occur with the ingestion of a meal. The magnitude of these changes will depend on the composition of the ingested meal and an individual's level of activity. Nutrients in the meal will be absorbed at different rates. Glucose and amino acids enter the general circulation within the first 15 – 30 min of the postprandial period. Some CHO meals cause elevated plasma glucose for 3 – 4 hours (Frayn 2003), resulting in the rapid release of insulin from the pancreas. Insulin inhibits mobilization of FFA, limits their circulation and oxidation (Coyle, *et al.,* 1997; Sidossis and Wolfe 1996). The low circulating FFA and increased insulin levels facilitate glucose uptake by the muscle resulting in increased CHO oxidation and lactate production. Sidossis and colleagues (1996) reported that the nature of

substrate oxidation in humans is determined by the intracellular availability of glucose, and not FFA as was previously thought (Sidossis, *et al.*, 1996).

2.8.2 Substrate oxidation during exercise

The transition from a resting state to exercise results in an increased energy demand and an elevation in CHO and fat metabolism (for review see Spriet 2002). Carbohydrate is considered a more adaptable fuel than fat as it can contribute to ATP production both aerobically and anaerobically (Spriet and Watt 2003). However, CHO stores in the body are limited and can be depleted within 90-120min of heavy exercise (Hermansen, Hultman and Saltin 1967). Any sparing of the limited CHO stores by producing energy via fat oxidation is therefore desirable.

The relationship between fat oxidation and exercise intensity has been extensively studied. Romijn and colleagues (1993), using isotope tracer techniques, investigated substrate oxidation during 30 min of cycling at 25%, 65% and 85% $\dot{V}O_2$max. They found that plasma glucose uptake, muscle glycogen oxidation and breakdown of fat (lipolysis) increased in relation to exercise intensity (Romijn, Coyle, Sidossis, Gastaldelli, Horowitz, Endert and Wolfe 1993). At exercise intensities of 25% and 65% of $\dot{V}O_2$max plasma FFA concentrations remained high, ensuring the delivery of FFA to the working muscle. However, at an exercise intensity of 85% $\dot{V}O_2$max plasma FFA concentrations decreased by ~50% even though adipose tissue lipolysis stayed the same during all exercise intensities. The authors suggested that the reduction in FFA concentrations in the blood was due to decreased adipose tissue blood flow which in turn prevented the FFAs from reaching the systemic circulation. Romijn and colleagues studied the delivery of FFA during the 85% $\dot{V}O_2$max exercise by artificially maintaining the FFA level measured in the 65%$\dot{V}O_2$max exercise. Although FFA uptake and oxidation

were higher in the FFA maintained state, fat oxidation was lower than levels seen in the 65% $\dot{V}O_2$max exercise seen in a previous study (Romijn, Coyle, Sidossis, Zhang and Wolfe 1995). This suggests that the rate of FFA uptake and fat oxidation at an exercise intensity of 85% $\dot{V}O_2$max might be determined by events happening in the working muscle itself.

Van Loon and colleagues (2001), using staple isotope techniques in combination with muscle biopsies, investigated substrate utilization and the regulation of fuel selection during exercise (van Loon, Greenhaff, Constantin-Teodosiu, Saris and Wagenmakers 2001). Fuel selection was studied in eight cyclists, at rest and in three consecutive 30 min exercise periods at the equivalent of 40%, 55% and 75 % of maximal workload (W_{max}). Plasma glucose and muscle glycogen oxidation rates increased proportionally with the intensity of exercise. The relative contribution of fat oxidation to total energy expenditure did not change from rest and remained the same up to an exercise intensity of 55% W_{max}. However as exercise intensity increased to 75% W_{max}, total body fat oxidation rate decreased by 34% compared to that at 55% W_{max}. This decline was due to a decrease in plasma FFA concentration as well as a decrease in the rate of intramuscular lipid oxidation. The authors concluded that the reduction in fat oxidation during high intensity exercise might be the result of a down-regulation of specific proteins involved in FFA transfer into the mitochondria .

Several studies have investigated the effect of exercise intensity on maximal fat oxidation. In a study by Achten and colleagues (2002), a group of moderately trained men, performed graded exercise in a fasted state and reached maximal fat oxidation at an exercise intensity of about 64% $\dot{V}O_2$max (Achten, Gleeson and Jeukendrup 2002). Exercising at levels beyond 64% $\dot{V}O_2$max resulted in a sharp decrease in fat oxidation.

The same research group also studied the effect of CHO ingestion before exercise on maximal fat oxidation (Achten and Jeukendrup 2003a). They found that ingestion of 75g of glucose 45 min before exercise not only decreased maximal fat oxidation by ~28% but also decreased the exercise intensity at which fat max occurred by 8% compared with the placebo trial (Achten, *et al.*, 2003a).

Although a number of important factors regulating fat oxidation have been identified, it is apparent that there is a considerable between-participant variation in substrate utilisation. Using a group of 55 endurance-trained men, Achten and colleagues investigated the reliability and reproducibility of the maximal fat oxidation rates (Achten and Jeukendrup 2003b). They found that maximum fat oxidation occurred at ~62% $\dot{V}O_2$ max and minimum fat oxidation at ~86% $\dot{V}O_2$ max. The difference in relative exercise intensity between maximal and minimal fat oxidation was close to 24%. However the coefficient of variation for fat oxidation was surprisingly large ~10%. It means exercise at the same relative intensity could elicit maximal fat oxidation in one individual and minimal fat oxidation in another. This study also highlighted the significant correlation between maximal rate of fat oxidation and $\dot{V}O_2$ max ($r=0.636$, $P<0.05$). The relationship between maximal fat oxidation and $\dot{V}O_2$ max was further confirmed when participants were divided into a low and high $\dot{V}O_2$ max group. The group of participants with a high $\dot{V}O_2$ max (72 $\pm$6 $ml.kg^{-1}.min^{-1}$) had significantly higher fat oxidation rates than the group with lower $\dot{V}O_2$ max (59 $\pm$ 5 $ml.kg^{-1}.min^{-1}$) (Achten, *et al.*, 2003b). These finding agree with results from other studies (Friedlander, Casazza, Horning, Huie and Brooks 1997). Maximal fat oxidation occurred at similar relative workloads i.e. 63 $\pm$ 10% for the high $\dot{V}O_2$ max group and 62 $\pm$ 10% in the low $\dot{V}O_2$ max group. However whilst absolute fat oxidation rate was significantly higher in the high compared with the low $\dot{V}O_2$ max groups (0.56

± 0.1 g.min^{-1} versus 0.48 ± 0.2 g.min^{-1}) there was no difference in the relative contribution of fat to total energy expenditure between the two groups.

2.9 Sex differences in substrate oxidation at rest and exercise

Several studies have investigated whether sex differences exist in fuel selection and utilization during sub-maximal exercise (Roepstorff, Steffensen, Madsen, Stallknecht, Kanstrup, Richter and Kiens 2002). Postprandial substrate oxidation has been reported to be different in males and females. Toth and colleagues (1998) found that at rest older men (70 ± 4 years) oxidised more fat than older women (66 ± 4 years) in absolute terms. Similarly exercising at 45% $\dot{V}O_2$ peak for 30 min men oxidised more fat compared with women (0.88 ± 19 versus 0.51± 0.15 mmol.min^{-1}; $P<0.01$) in absolute terms due to their higher absolute work load. However there was no difference in the rate of FFA appearance and relative rate of fat oxidation between men and women.

The majority of studies have reported that during moderate intensity long duration exercise women use a greater amount of fat and less CHO than equally trained males (Tarnopolsky, MacDougall, Atkinson, Tarnopolsky and Sutton 1990; Tarnopolsky, Atkinson, Phillips and MacDougall 1995).

However Mittendorfer and colleagues found that men and women, exercising at similar relative workload (52 ± 3% and 53 ± 2% $\dot{V}O_2$ peak) for 90 min, used similar relative amounts of CHO and fat (Mittendorfer, Horowitz and Klein 2002). Five men and five women matched on adiposity (24 ± 2 and 25 ± 1% body fat, respectively) and peak oxygen uptake (49 ± 2 and 47 ± 1 ml. kg^{-1}. min^{-1}, respectively) took part in the study. The study revealed an increase in the rate of appearance of glycerol and free fatty acid (FFA) in plasma during exercise. This was approximately 65% greater in women than in men (glycerol R(a): 317 ± 40 and 195 ± 33 micromol.kg^{-1}, respectively; FFA R(a):

652 ± 46 and 453 ± 70 micromol.kg^{-1}, respectively; both $P < 0.05$). Although the rate of whole body total fatty acid oxidation was similar in men and women the source of fatty acids used was different between the sexes: women oxidised more FFA, the probable source of which was adipose tissue triglycerides.

2.10 Effect of menstrual cycle on substrate oxidation during rest and exercise

Some research suggest that there is a tendency towards increased fat metabolism in eumenorrheic compared with amenorrheic women. In the follicular (pre-ovulatory) phase plasma oesterogen and progesterone concentrations are low, while in the luteal (post-ovulation) phase these hormones increase considerably (Redman, Scroop and Norman 2003). Although the main role of these hormones are in reproduction they also have a significant influence on metabolism (Campbell, Angus and Febbraio 2001). Cyclical variations in oestrogen and progesterone levels can affect CHO and lipid metabolism (Horton, Miller, Glueck and Tench 2002). However, some experiments have found no differences in rate of FFA turnover in the early follicular, mid follicular or mid luteal phases of the menstrual cycle, either at rest or during 90 min of moderate intensity (50% $\dot{V}O_2$max) exercise (Horton, Miller and Bourret 2006).

Several studies investigating the effect of menstrual cycle phase on fat and CHO oxidation at rest, have found no difference between the luteal and follicular phase. Melanson and colleagues (1996) reported no cycle phase effect on postprandial fat, CHO and protein oxidation following four different test meals unmatched for energy content (Melanson, Saltzman, Russell and Roberts 1996). Similarly no differences have been reported in whole body RER (Piers, Diggavi, Rijskamp, van Raaij, Shetty and Hautvast

1995) or glucose turnover (Zderic, Coggan and Ruby 2001) in the different phases of the menstrual cycle.

It has been reported that insulin sensitivity and glycaemic control is affected by the sex steroid hormones. Women with insulin-dependent diabetes mellitus have a poorer glycaemic control in the luteal phase of the cycle compared with the follicular phase of the menstrual cycle (Widom, Diamond and Simonson 1992). High levels of oesterogen and progesterone in the luteal phase caused a decrease in insulin sensitivity but not glucose tolerance (as measured by an intra-venous glucose tolerance test).

While at rest there is little evidence to support that the menstrual cycle influences substrate oxidation this does not appear to be the case when exercising. Oesterogen and progesterone levels in women are thought to have an important role in regulating substrate oxidation during exercise (D'Eon, Sharoff, Chipkin, Grow, Ruby and Braun 2002). Oesterogen lowers CHO oxidation by reducing the rate of glucose uptake (D'Eon, *et al.,* 2002), also promotes increased fat oxidation during exercise thus sparing muscle glycogen (Kendrick and Ellis 1991). In contrast progesterone has been reported to reduce the fatty acid availability (Hatta, Atomi, Shinohara, Yamamoto and Yamada 1988).

Zderic and colleagues found that there were differences in glucose kinetics during the different phases of the menstrual cycle. There were no differences in the rate of plasma glucose appearance (Ra) or disappearance (Rd) at rest and during exercise at 70% of lactate threshold. Similarly there were no differences in CHO or fat oxidation (Zderic, *et al.,* 2001). However the large intra-individual variations in studies does not allow a definitive statement on the effect of menstrual cycle on metabolism (D'Eon, *et al.,* 2002).

Therefore, while the hormonal changes associated with the menstrual cycle in women might be expected to influence substrate oxidation there seems little evidence to support this.

2.11 Effect of oral contraceptives on substrate utilisation

Given the fact that metabolic responses vary during the different phases of the menstrual cycle considerations needs to be taken regarding the use of synthetic steroid hormones in the forms of the oral contraceptive pill. The presence of these synthetic steroids in oral contraceptives might effect CHO and lipid oxidation (Bonen, Haynes and Graham 1991). Bonen and colleagues reported no change in substrate utilisation despite the fact that FFA concentrations were consistently higher and glucose concentrations were lower during low intensity exercise in the oral contraceptive group compared with the controls (Bonen, *et al.*, 1991). Similarly lower blood glucose concentrations were observed in oral contraceptive users exercising for 90 min at 50% of $\dot{V}O_2$ max (Bemben, Boileau, Bahr, Nelson and Misner 1992). However ingestion of 0.5 $g.kg^{-1}$ BM of CHO 30 min before exercise produced no difference in plasma glucose or insulin between the oral contraception and control groups. The authors concluded that CHO ingestion before exercise induced similar glucose tolerance with or without oral contraceptives (Boisseau, Rannou, Delamarche, Bentue-Ferrer and Gratas-Delamarche 2001). Using stable isotopic tracers a longitudinal study by Suh and colleagues (2003) reported that in women fed several hours before moderate-intensity exercise OC decreases glucose flux, but not overall CHO and lipid oxidation rates during exercise (Suh, Casazza, Horning, Miller and Brooks 2003). Casazza and colleagues (2004) reported that four months of contraceptive pill usage did not effect overall whole body RER during moderate –intensity exercise at 45% $\dot{V}O_2$ peak or at 60% $\dot{V}O_2$ peak (Casazza, Jacobs, Suh, Miller, Horning and Brooks 2004).

In summary, there is conflicting evidence on the influence of oral contraceptives on metabolism. Further research is needed, preferably on a longitudinal bases to investigate the influence of oral contraceptives on glycaemia and metabolism during rest and sub-maximal exercise. However the constantly changing composition of tablets make longitudinal studies fraught with difficulties.

2.12 Pre-exercise carbohydrate intake – effect on metabolism and exercise

Several studies investigated the effect of CHO on muscle glycogen utilization during endurance exercise. In some studies pre-exercise CHO ingestion has been demonstrated to increase (Costill, *et al.,* 1977; Hargreaves, Costill, Katz and Fink 1985), decrease (Devlin, Calles-Escandon and Horton 1986; Febbraio and Stewart 1996; Kirwan, O'Gorman and Evans 1998; Levine, Evans, Cadarette, Fisher and Bullen 1983), or have no effect on muscle glycogen use.

Inconsistencies seen between studies could be due to the variation in the pre-exercise meal and how this alters the plasma glucose and insulin response. A CHO meal contains a combination of individual nutrients which are absorbed and oxidised at different rates (Horowitz and Coyle 1993), effecting the glycaemic and insulinaemic response. As the glycaemic index provides a mechanism of ranking for CHO rich foods it has been widely used in the area of pre-exercise nutrition (Burke, Claassen, Hawley and Noakes 1998; Febbraio, *et al.,* 1996; Thomas, Brotherhood and Brand 1991; Thomas, Brotherhood and Miller 1994; Wee, Williams, Gray and Horabin 1999). Although the pre-exercise meal is chosen and composed according to the GI, results are inconsistent.

Some studies found a relative shift in substrate oxidation from CHO to fat when a LGI meal was ingested before exercise compared with that of a HGI meal (Wee, *et al.*, 1999). Wee and colleagues, using muscle biopsies, found that LGI meal contributed less CHO to muscle glycogen synthesis in the 3 h postprandial period compared with a HGI meal. A sparing of muscle glycogen utilization was observed in the LGI trial, most likely due to a better maintained fat oxidation (Wee, *et al.*, 2005).

Carbohydrate feeding in the hour before exercise produces a large increase in blood glucose and insulin. With the onset of exercise there is a rapid fall in blood glucose due to the high insulin concentration and increased glucose uptake by muscle (Hargreaves, Hawley and Jeukendrup 2004). High insulin levels inhibit lipolysis hence there will only be a small increase in plasma FFA concentration with exercise (Costill, *et al.*, 1977; Horowitz, *et al.*, 1997). Fat oxidation is reduced because of lower plasma FFA availability and inhibition of lipid oxidation in the muscle. Gastro-intestinal discomfort can occur during exercise if a meal is eaten too close to the start of exercise. Eating a meal 3-4 h before exercise provides time for digestion and absorption, preventing gastrointestinal discomfort and allowing plasma glucose and insulin to return to baseline values by the start of exercise. However the onset of exercise can produce a transient fall in plasma glucose, increased CHO oxidation and blunting of FFA mobilization (Coyle, *et al.*, 1985). Increased CHO oxidation occur with increased CHO availability (Richter et al).

Most studies used single foods, however in order to assess the effect of food intake in real life some research studies used mixed meals composed of commonly consumed foods(Stevenson, *et al.*, 2005; Stevenson, Williams, Nute, Swaile and Tsui 2005b; Stevenson, *et al.*, 2006; Wu, *et al.*, 2003).

In summary mixed carbohydrate meals have been successfully used to assess the glycaemic impact of commonly consumed foods. Some studies have shown low GI food to be beneficial in lowering the glycaemic and insulinaemic effect of food and increase fat oxidation during postprandial exercise. Further studies needed to examine their effects on the general population during rest and exercise – hence the purpose of this thesis.

CHAPTER 3

General Methods

3.1 Introduction

This chapter contains a description of the methods and equipment used in the three studies contained in this thesis. All investigations were conducted in the Exercise Physiology Laboratory of the School of Sports and Exercise Sciences at Loughborough University. All studies were approved by the Loughborough University Ethical Advisory Committee (Appendix A1) prior to participant recruitment. The "Code of Practice for Workers having Contact with Body Fluids" was strictly adhered to.

3.2 Study participants

Healthy, recreationally active male and female participants, aged 18 – 50y, were recruited from the student and general population by contacting running clubs, as well as through personal contact. Those interested in the studies were verbally briefed and given a participant information sheet (Appendix A2a) to read at their leisure. Volunteers who agreed to take part in a study were asked to give a written consent indicating that they were fully aware that they could withdraw from a study at any time and without giving a reason (Appendix A2b).

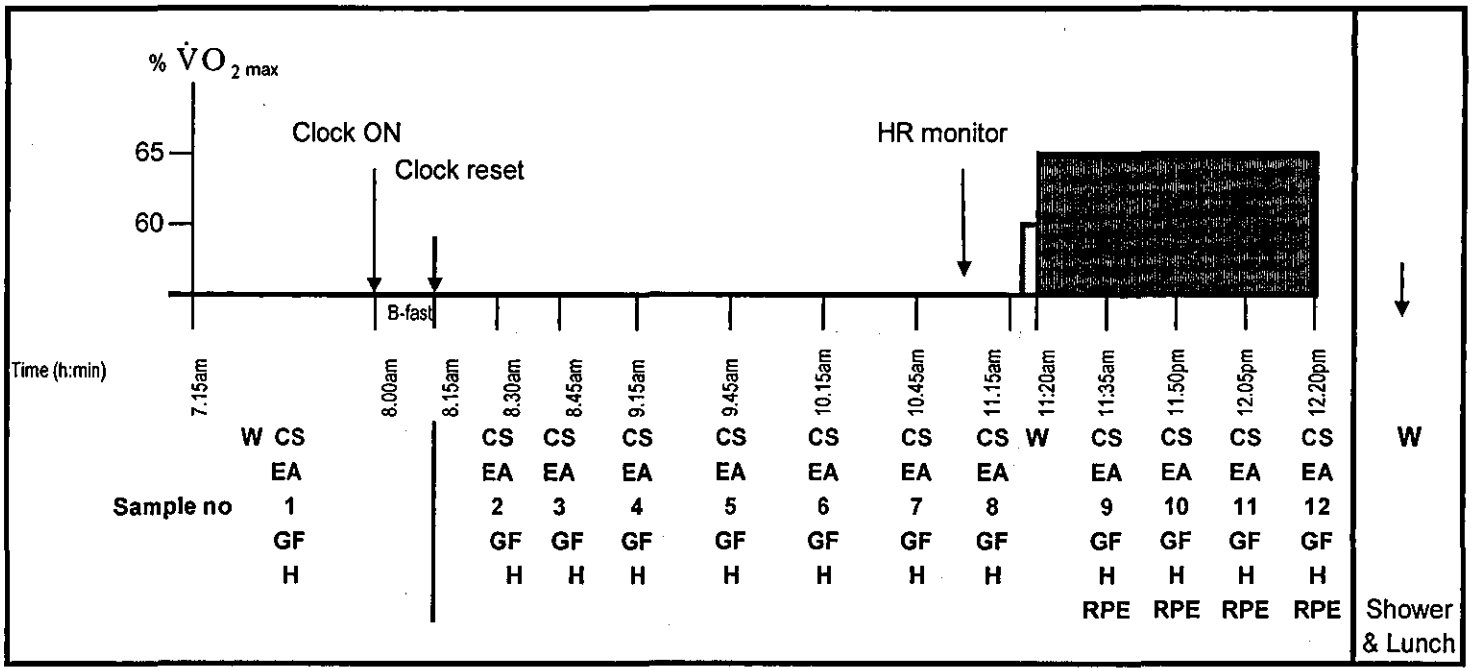
On the first visit to the Exercise Physiology Laboratory all participants completed a compulsory health questionnaire (Appendix A3). Individuals were excluded from involvement in a study if they had diabetes, cardiovascular problems or a chest infection. Participants completed further questionnaires on each visit to the laboratory to ensure that they were in good health on the day of each trial (Appendix A4).

3.3 Experimental design

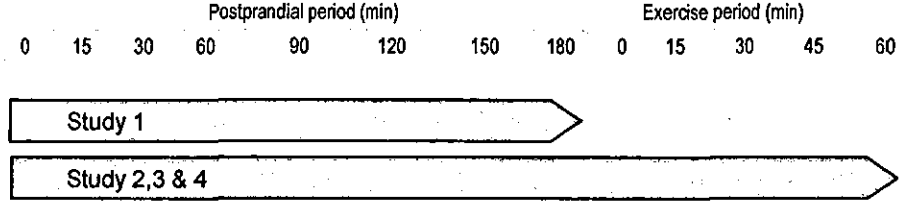
Body Mass (BM) and height were measured on the initial visit to the laboratory. Body mass was also recorded on the day of each trial. All body mass measurements were made using a balance scale (Avery 3306, Avery Ltd., UK) that had a capacity of 120 kg and was accurate to ± 0.05 kg. Height was measured in bare feet, using a wall mounted stadiometer (Holtain Ltd) that had a maximum range of 200 cm and was accurate to ± 0.01 cm. Both height and body mass measurements were used to calculate body mass index (BMI = kg/m^2).

Participants took part in four trials in Study 1 (Chapter 4) and in two trials in studies 2, 3 and 4 (Chapters 5, 6 and 7). Trials were separated by 3 to 7 days. In studies 2, 3 and 4 expired air samples were collected before and during the three hour postprandial period as well as during the subsequent exercise period. At the end of the 3 h postprandial period participants performed a 60 min treadmill run at 65% of $\dot{V}O_2$max. A summary of the experimental protocol can be found in Table 3.1.

Table 3.1 Experimental Protocol



W weight
CS capillary blood sample
EA expired air sample
GF Gut Fullness
H Hunger
RPE Rate of Perceived Exertion
5min warm up at 60% VO_2max
60min run at 65%VO_2 max



3.4 Preliminary tests

Prior to the main trials all participants made two visits to the laboratory for preliminary tests. During the first of these the participant's sub-maximal and maximal oxygen uptake was assessed. The second visit was designed to familiarise participants with the experimental procedures used in the main trials and to check that the calculated running speed did elicit an exercise intensity equivalent to 65% $\dot{V}O_2$ max. Exercise during the preliminary tests and main trials was performed on a motorised treadmill (Run Race 47035, Technogym, Gambettoio, Italy, for Study 2 and Runner 2000, Cavezzo, Modena, Italy for Study 3). Treadmills were calibrated at the start of each study with spot checks conducted at regular intervals.

3.4.1 Sub-maximal oxygen uptake

The relationship between sub-maximal oxygen uptake and running speed was determined using a 16 min incremental test. The test consisted of four stages in which the treadmill speed was increased by 1-1.5 $km.h^{-1}$ every 4 min, depending on the sex and training status of the participants. During the final minute of each 4 min stage an expired air sample and a rating of perceived exertion (Borg 1973) were collected and heart rate response was assessed. Heart rates were recorded using short range telemetry (Polar S810, Finland).

3.4.2 Maximal oxygen uptake

Approximately 30 min after the sub-maximal oxygen uptake test participants completed a maximum oxygen uptake ($\dot{V}O_2$ max) test, which was a modified version of that used by Taylor and colleagues (Taylor, Buskirk and Henschel 1955). The test consisted of a continuous uphill incremental treadmill run to voluntary fatigue (Williams, Nute, Broadbank and Vinall 1990). Treadmill speed was kept constant. The test began with the treadmill gradient at 3.5% and increased by 2.5% every 3 min. Expired air samples were collected

between 1:45 – 2:45 of each 3 min block. The final air sample was taken immediately the participants signalled that they could not sustain the speed for more than one minute. Participants received verbal encouragement throughout the test. Participants were considered to have reached $\dot{V}O_2$ max when the respiratory exchange ratio (RER) reached the value of 1.15 and if the increase in $\dot{V}O_2$ in response to an increased gradient was $\leq$ 2.5 ml.kg^{-1}.min^{-1} .

3.4.3 Familiarization run

Oxygen uptake values were plotted against running speeds for the 16 min test. The linear regression line obtained was used to calculate the running speed equivalent to 65% $\dot{V}O_2$ max for each participant. Participants were asked to run at this calculated speed for 45 min thus providing confirmation that the calculated speed corresponded to the appropriate relative exercise intensity ($\pm$ 2%).

3.5 Main trials

The first study included in this thesis used a Latin Square design, fully randomised and counterbalanced as much as possible. In studies 2, 3 and 4 trials were conducted in randomised cross-over design. In all main trials food intake was followed by a 3 h postprandial period at rest. In studies 2, 3 and 4 (Chapter 5, 6 and 7) the 3 h postprandial period was followed by exercise which consisted of a 60 min run at a speed equivalent to 65% $\dot{V}O_2$ max.
On test days, participants arrived in the laboratory between 7.00-8.00 am after an overnight fast of 10-12 h . Participants maintained their normal exercise pattern, but refrained from strenuous exercise the day before each trial.

On arrival at the laboratory participants completed a pre-test health questionnaire. Following a 20 min standardised rest period a 5 min expired air

sample was collected, followed by the collection of a fingertip blood sample from a pre-warmed hand. After providing this initial blood sample participants consumed the prescribed breakfast within 15 min and the clock was re-started. Further expired air and fingertip blood samples were collected at 15, 30, 60, 90, 120, 150 and 180 min of the postprandial period. During the expired air collection perceived gut fullness (Appendix A5) and hunger (Appendix A6) were recorded using a 6-20 scale. In addition in study 3 ratings of gut fullness and hunger were recorded using a visual analogue scale (VAS) (Appendix A7). Ambient temperature of the laboratory during the trials remained between 22^{0}C and 23^{0}C and humidity between 48 – 53%. During the postprandial period participants remained seated except for toilet breaks.

At the end of the 180 min postprandial period (studies 2, 3 and 4), participants were weighed and fitted with a short-range telemetric heart rate monitor (Polar S810, Finland). After a 5 min warm up on the treadmill (60% $\dot{V}O_2$ max), the speed was adjusted to the equivalent of 65% $\dot{V}O_2$ max for the 60 min run. Expired air samples, finger tip blood samples, heart rate, rating of perceived exertion (RPE) (Appendix A8), hunger and gut fullness ratings were obtained at 15, 30, 45 and 60 min during exercise. At the end of the trial, participants showered, changed and were served lunch before leaving the laboratory.

3.6 Test meals

The test breakfasts consisted of two high glycaemic index (HGI) and two low glycaemic index (LGI) breakfasts with an available CHO content of 1g.kg^{-1} BM. The HGI breakfasts were : toast and jam (HGI-T) and cornflakes, milk and a slice of toast (HGI-C). The LGI breakfasts were: muesli, milk and chunks of fresh apple (LGI-M) and fruit and yoghurt (LGI-F) (Table 3.2). All breakfasts were served with 180 ml of hot tea that included 30ml of skimmed

milk. The meals were isoenergetic, matched, for available CHO, fat and protein content (Table 3.2). The nutritional content of each breakfast was calculated from the information provided by the manufacturer. Test meals were prepared on site, just before the trial and were randomly assigned to the participants.

The GI of each food was obtained from the published International GI tables (Foster-Powell, *et al.*, 2002) and were used to choose LGI and HGI foods for the mixed meals. The GI of the mixed meals were calculated by using the sum of the GI contribution of each carbohydrate in the meal (Wolever, *et al.*, 1986). The GI of the breakfast was calculated as follows:

1. Calculate percentage of total CHO in the meal contributed by each food.
2. Multiply the GI of the food with the % contribution of the food.
3. Add the results together to arrive at the predicted GI of the meal.

EXAMPLE:

Cornflakes	CHO 42.9 g	42.9 / 70 * 100 = 61% of total CHO
		61 x 81 (GI) / 100 = **49**
Bread	CHO 17.1 g	17.1 / 70 * 100 = 24% of total CHO
		24 x 70 (GI) / 100 = **17**
Ssk-milk	CHO 10.0 g	10.0 / 70 * 100 = 14% of total CHO
		14 x 32 (GI) / 100 = **5**
Total CHO	70.0 g	**GI of the meal = 71** (49+17+5)

The glycaemic load is calculated by taking the percentage of the food's carbohydrate content per portion and multiplying it by its Glycaemic Index value (Foster-Powell, *et al.*, 2002).

$$GL = \frac{\text{CHO content per portion} \times GI}{100}$$

EXAMPLE:

Cornflakes	CHO	42.9g	42.9 x 81 (GI) / 100 = **35**
Bread	CHO	17.1g	17.1 x 70 (GI) / 100 = **12**
Ssk-milk	CHO	10.0g	10.0 x 32 (GI) / 100 = **3**
Total CHO		70.0g	**GL of the meal = 50** (35+12+3)

Table 3.2 Characteristic of breakfast (for a 70kg individual)

Breakfasts	**HGI-T** (Toast)	**HGI-C** (Cornflakes)	**LGI-M** (Muesli)	**LGI-F** (Fruit)
Food Items	(g)	(g)	(g)	(g)
white bread[a]	123	39	-	-
strawberry jam[c]	25	-	-	-
Corn flakes[b]	-	51	-	-
skimmed milk[c]	-	200	210	-
low fat margarine[e]	-	3	-	-
Muesli[d]	-	-	69	-
Apples[c]	-	-	133	150
canned peaches[c]	-	-	-	227
Yoghurt[c]	-	-	-	210
black tea[e]	180	180	180	180
skimmed milk[c]	30	30	30	30
Macronutrients				
E (kcal)	350	365	380	362
E (MJ)	1.5	1.5	1.6	1.5
CHO (g)	70.0	70.0	70.0	70.0
Fat (g)	3.0	3.4	4.4	3.7
Protein (g)	11.0	13.6	15.0	11.0
Glycaemic Index	**72[f]**	**71[f]**	**48[f]**	**36[f]**
Glycaemic Load	**51[g]**	**50[g]**	**34[g]**	**25[g]**
Weight of breakfast (excluding cup of tea)	**148**	**293**	**412**	**587**

[a] White bread, (Kingsmill, Allied Bakeries Ltd, UK);
[b] Cornflakes (Kelloggs' UK Ltd.)
[c] Strawberry jam, Braeburn apple, tinned peaches, low fat yoghurt, skimmed milk (TESCO Ltd, UK),
[d] Alpen muesli (No added sugar) (Weetabix Ltd, UK),
[e] Flora margarine, PG tips - black tea (Unilever UK Ltd)
[f] Calculated by the method described by Wolever (1986) with GI values taken from Foster-Powell et all. (2002)
[g] Calculated by the method described by Foster-Powell et all. (2002)

3.7 Dietary control

In order to ensure the standardisation of their nutritional intake participants weighed (scales used: Salter 3007, UK) and recorded everything they ate and drank (abstaining from alcohol) for three days prior to the experiment (Appendix A9). Participants were asked to stop eating at 10 pm the night before their main trial, fast overnight and drink only water. Participants reproduced the 24 h pre-trial 1 food intake before subsequent trials. In study 1 (Chapter 4) food and fluid intake was only recorded between 5 pm and 10 pm the day before the main trial, and food intake was reproduced during the day before subsequent main trials.

In study 2, participants were given a standardised evening meal consisting of pasta, pasta sauce and a small amount of cheese (55% CHO, 27% fat, 18% protein). The meal contained 1.5 g. kg^{-1} BM of available CHO, and had a calculated GI value of 35 (Table 5.1).

Each dietary record was analysed for energy macro and micro-nutrient intake using a computer software (CompEat 4.0, Nutrition Systems, UK).

3.8 Expired air analysis

Expired air samples were collected using a two-way, lightweight respiratory valve connected via Falconia tubing (Baxter, Woodhouse and Taylor, UK) to a 200 l Douglas bag (Harvard Apparatus, UK)(Williams and Nute 1983). Percentage of O_2 and CO_2 were measured using a paramagnetic and an infer-red analysers (Servomex 1440, UK). The analysers were calibrated via a two point calibration using 100% N_2 and a mixed gas containing 16.0% O_2 and 4% CO_2 (BOC Gases Ltd, UK). The analysers were checked with atmospheric air and were recalibrated after 3 h. Expired air volume was measured using a dry gas meter (Harvard Apparatus, UK). The gas meter was routinely checked

against a precision 3 l calibration syringe (5530 Hans Rudolph Inc., USA). Temperature of the expired air was recorded using a thermistor probe located in the dry gas meter system. Barometric pressure was measured using a wall mounted barometer (Griffin and George BHL-340-X, UK). All values were converted to standard pressure and temperature for a dry gas. Following the analysis of expired air, substrate oxidation rates and energy expenditure were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ values using the following stoichiometric equations (Frayn 1983):

CHO oxidation rate ($g.min^{-1}$) = 4.585 x $\dot{V}CO_2$ - 3.226 x $\dot{V}O_2$

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

3.9 Collection and analysis of blood samples

In the studies described in this thesis capillary rather than venous blood sampling was used, because it is more sensitive to glycaemic responses (Kuwa, *et al.*, 2001; Wolever, *et al.*, 1991). Data from an inter-laboratory study by Wolever and colleagues found that the within-participant coefficient of variation (CV) was significantly lower using capillary blood samples (23.4 ± 2.1%) compared with venous blood samples (56.8 ± 4.4%).

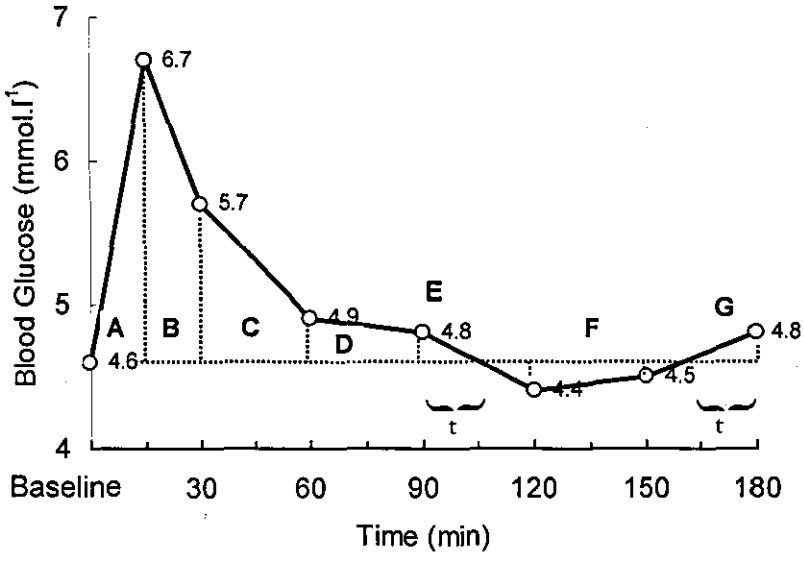
Blood samples were taken from a finger tip of a pre-warmed hand via single use safety lancets (Unistik Extra 21 gauge, 2mm depth, Owen Mumford Ltd, UK) and a Microvette CB300, ETA coated capillary blood collection tube (Sarstedt Ltd, UK). From the ~300 µl of collected whole blood two 20µl samples were removed, immediately deproteinised in 2.5% ice cooled perchloric acid, centrifuged at 10000 rpm (Eppendof 5415C , Hamburg, Germany) and frozen at -20^{0}C until analysis. In study 4 (Chapter 7) the remaining ~250 µl EDTA treated blood samples were centrifuged at 3000 rpm for 15 min in a refrigerated centrifuge (Beckman-Coulter Allegra X –

22R, Germany). Plasma (~125 µl) was transferred to plastic vials and stored at -80°C for insulin analysis.

Blood glucose concentration was determined photometrically using a commercially available kit (GOD-PAP method, GL 2610, Randox, Ireland). Blood lactate concentration was determined via a fluorimetric method (Maughan 1982). Plasma insulin was measured using an Enzyme-Linked Immunosorbent Assay (ELISA) (Insulin kit no.10-1113-01 MERCODIA, Sweden).

3.10 Calculation of the incremental area under the curve

The incremental area under the curve (IAUC) was calculated throughout this thesis in accordance with the method recommended by Wolever (Wolever 2004; Wolever, *et al.*, 1986). The area under the blood glucose curve includes the area above the fasting level only. Blood glucose values below the fasting level are ignored. The method used to calculate the incremental area under the blood glucose response curve is illustrated below; A, B, C, D...represents the increment in blood glucose above the fasting level.



The total IAUC was calculated from the sum of areas.

IAUC = A + B + C + D + E + F + G

Triangle A = [(start conc. + end conc.) x ½ time)]min - baseline area

= [(4.6 + 6.7) x 7.5 – (4.6 x 15)] = **15.8 mmol · 15 min · l^{-1}**

Triangle B = [(6.7 + 5.7) x 7.5 – (4.6 x 15)] = **24.0 mmol · 15 min · l^{-1}**

Triangle C = [(5.7 + 4.9) x 15 – (4.6 x 30)] = **21.0 mmol · 30 min · l^{-1}**

Triangle D = [(4.9 + 4.8) x 15 – (4.6 x 30)] = **7.5 mmol · 30 min · l^{-1}**

Triangle E = [(start conc. – baseline conc.) x t/2]

= [(4.8 – 4.6) x 15/2] = **1.5 mmol · 30 min · l^{-1}**

Where t = (start conc. – baseline conc.) / (start conc. + end conc.) x time (min) =(4.8 – 4.6) / (4.8 - 4.4) x 30 = 15 min

Period F the start and end concentrations of this period are below baseline so the IAUC = **0.0 mmol · 30 min · l^{-1}**

Triangle G = (end conc. – baseline conc.) x t/2

= (4.8 – 4.6) x 20/2 = **2.0 mmol · 30 min · l^{-1}**

Where t = (end conc. – baseline conc.) / (end conc. - start conc.) x time (min)

=(4.8 – 4.6) / (4.8 - 4.5) x 30 = 20 min

Therefore the total IAUC for the 180 min postprandial period =

A	15.8 mmol · 15 min · l^{-1}
B	24.0 mmol · 15 min · l^{-1}
C	21.0 mmol · 30 min · l^{-1}
D	7.5 mmol · 30 min · l^{-1}
E	1.5 mmol · 30 min · l^{-1}
F	0.0 mmol · 30 min · l^{-1}
G	2.0 mmol · 30 min · l^{-1}

IAUC 71.8 mmol · 180 min · l^{-1}

3.11 Subjective data

During each period of expired air collection (during rest and exercise) participants' sensation of gut fullness, hunger and fatigue were monitored. Feelings of gut fullness and hunger were measured using an adapted version of the Borg scale (Borg 1973). The anchor terms here were "not full" to "very, very full" (Appendix A5) and "not hungry" to "very, very hungry" (Appendix A6). In addition, in study 4 gut fullness and hunger ratings were also measured with a visual analogue scale (VAS scale, 0-100mm, Appendix A7). Participants rating of perceived exertion (RPE) were measured using the Borg scale where the anchor terms were "very, very light" to "maximum" (Borg 1973) (Appendix A8).

3.12 Intra-assay variations

In order to evaluate the degree of variation from one data series to another, the coefficient of variation was calculated for resting oxygen uptake and blood metabolites using the formula CV = SD/mean x 100 (Table 3.3). Insulin could not be measured from study 3 as the samples were lost during analysis. There are insulin measurements only from Study 4.

Table 3.3 Intra-Assay coefficient of variations for blood metabolites.

Metabolite	Concentration	Unit of measure	CV %
Blood Glucose	4.5	$mmol \cdot l^{-1}$	0.5
Blood Lactate	2.0	$mmol \cdot l^{-1}$	1.5
Plasma Insulin-rest	6.6	$mU \cdot l^{-1}$	6.9
Plasma Insulin-postprandial	47.4	$mU \cdot l^{-1}$	2.9

Table 3.4 Coefficient of variations for expired air parameters.

Parameters	Value	Unit of measure	CV %
Resting $\dot{V}O_2$	4.0	$ml \cdot kg^{-1} \cdot min^{-1}$	5.0
RER	0.846		3.7

3.13 Statistical analysis

Statistical analysis of data was performed using a commercially available software package (SPSS version 12; SPSS, Chicago, USA). For multiple comparison a two-way general linear method for repeated measures (Meal x Time) was used to analyse differences in the metabolic responses to the trials. If a significant interaction was found the Bonferroni step-wise post-hoc test was used to determine the location of the variance. Differences were considered significant at $P<0.05$. All results are presented as mean ± SEM.

CHAPTER 4

The influence of high and low glycaemic index carbohydrate breakfasts on blood glucose responses in young men

4.1 Introduction

There is an increasing prevalence of type II diabetes in populations living a 'western lifestyle'. The concept of glycaemic index was conceived to provide guidance to diabetics about which carbohydrate containing foods could be consumed; as foods with a low glycaemic index result in lower peak blood glucose concentrations when compared with HGI foods. The glycaemic index of individual foods has been extensively studied, but meals (the typical format in which individuals' consume nutrients) are rarely composed of a single food type. The glycaemic responses associated with mixed meals, with varied macronutrient content, has yet to be thoroughly investigated.

In previous studies that have examined the effect of HGI and LGI pre-exercise meals in trained athletes, based on their daily energy intake and activity levels, the available carbohydrate content of the test meals was set at 2g.kg^{-1} body mass (BM) (Stevenson, *et al.*, 2006; Wee, *et al.*, 1999; Wu, *et al.*, 2003). However, this amount of CHO would probably result in a meal that was too large for recreationally active or sedentary individuals. As a result the amount of CHO in the test meal used in this study was reduced to a more realistic amount 1g.kg^{-1} BM, in line with the recommended intake for these populations.

Therefore the aim of this study was to examine the postprandial responses to HGI and LGI mixed breakfasts, containing 1g.kg^{-1} BM of available CHO, and composed of commonly consumed breakfast foods.

4.2 Methods

Participants

Eight healthy and recreationally active males took part in the study (age: 24.8 ± 1.3 years; height: 1.80 ± 0.01 m; weight: 80.1 ± 4.6 kg; BMI: 24.8 ± 1.3 kg.m^2). Each individual participated in four experimental trials in a randomised design (using a Latin Square approach) separated by 3 to 7 days. Participants recorded everything they ate or drank between 5 pm and 10 pm the evening before the first trial, carefully repeating the same food intake before each subsequent trial. They maintained their normal exercise pattern, but refrained from strenuous exercise and avoided alcohol for 24 h before each trial.

Test meals

In order to use the similar ingredients as in the past studies we split the large HGI and LGI breakfasts from previous studies (2g.kg^{-1}BM available CHO) in four: two based on breakfast cereals (cornflakes; muesli), one based on bread (toast) and based one fruit (mixed fruit). The aim was to test the glycaemic response to all four breakfasts and to establish a HGI and a LGI breakfast with the largest difference in GI for the following study.

Four isoenergetic (2 HGI and 2 LGI) commonly consumed breakfasts were prepared with an available CHO content of 1g.kg^{-1} BM. The two HGI breakfasts were as follows: (1) toast and jam (HGI-T); (2) cornflakes, milk and a slice of toast (HGI-C). The LGI breakfasts consisted of (1) muesli, milk and apple (LGI-M); (2) fruit (apple and tinned peaches) and low fat yoghurt (LGI-

F). A more detailed description of the four breakfasts can be found in Table 3.2. All breakfasts were served with 180 ml of hot tea and 30ml of skimmed milk. The meals were isoenergetic, matched for available CHO, fat and protein content. The GI values of the individual foods were taken from the International Tables for Glycaemic Index and Glycaemic Load (Foster-Powell, *et al.*, 2002). The calculated GI of the meals were **72** (toast breakfast HGI-T), **71** (cornflakes breakfast HGI-C), **48** (muesli breakfast LGI-M) and **36** (fruit breakfast LGI-F) (Table 3.2). The GI of each meal was calculated from the weighted means of the GI values of the meal's component foods (Wolever, *et al.*, 1986)(see Methodology, Chapter 3). The energy and nutrient content of each breakfast was calculated using information provided by the manufacturer. The energy and macronutrient content as well as the GI and GL of the test breakfasts as well as the pre-trial evening meal can be found in Table 4.4.

Main trial

Participants arrived at the laboratory at 8 am following a 10-12 h fast. A finger tip blood sample was collected following a standardised 20 min rest period. Participants were then provided with the test breakfast which was consumed within 15 min and the clock was re-started. Further finger prick blood samples were collected at 15, 30, 60, 90, 120, 150 and 180 min following the end of the meal. All blood samples were collected from a pre-warmed hand. For a detailed description of the blood collection and blood analysis procedures please see Chapter 3 (Methodology). Only 7 participants completed the HGI-C and LGI-M trials. Prior to each blood sample, ratings of perceived gut fullness and hunger were recorded using a 6-20 scale (Appendix A5 and A6). During the postprandial period participants sat quietly reading or watching television.

As the study was set up to run with participants from the general public we decided to use finger tip blood sampling instead of venous blood collected via an indwelling canula. Insulin was only measured in later studies when we worked out the methodology to use with very small finger tip blood samples.

Statistical analysis

For multiple comparison a two-way general linear method for repeated measures (Meal x Time) was used to analyse differences in the metabolic responses following consumption of the two breakfasts. Eight participants started this study, however only 7 finished the HGI cornflakes and LGI muesli trials. Separate comparisons were made between the glycaemic responses to the HGI toast (HGI-T) and LGI fruit (LGI-F) meals (n=8), and between the HGI cornflakes (HGI-C) and LGI muesli (LGI-M) meals (n=7). Finally comparison of the glycaemic responses to all four breakfasts was made using the 7 participants who completed all 4 trials.

4.3 Results

Blood Glucose – 2 way analysis

Following the ingestion of the HGI toast (HGI-T) and LGI fruit (LGI-F) breakfasts blood glucose concentrations increased rapidly and peaked 15 min into the postprandial period. Blood glucose concentrations in the HGI toast (HGI-T) trial increased to a peak of 7.3 ± 0.3 $mmol.l^{-1}$ after which they declined slowly remaining just above fasting level at 180 min of the postprandial period. In the LGI fruit (LGI-F trial) blood glucose concentration increased to 6.6 ± 0.3 $mmol.l^{-1}$ and decreased rapidly reaching fasting values 60 min into the postprandial period. Statistical analysis demonstrated a meal effect ($P<0.05$) (HGI>LGI) and time effect ($P<0.05$) in the HGI toast and LGI fruit trial (Figure 4.1).

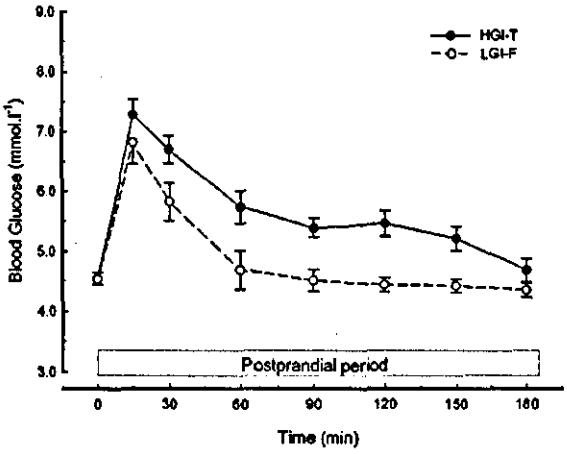


Figure 4.1 Blood glucose concentrations (mmol.l^{-1}) during the 180 min postprandial period following the ingestion of a HGI toast (HGI-T: ●) and a LGI fruit (LGI-F: ○) breakfast (mean ± SEM). Meal effect (P<0.05) (HGI-T > LGI-F) and time effect (P<0.05) .

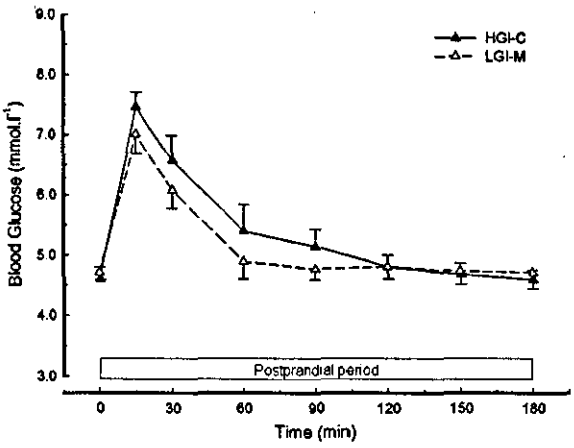


Figure 4.2 Blood glucose concentrations (mmol.l^{-1}) during the 180 min postprandial period following the ingestion of a HGI cornflakes (HGI-C: ▲) and a LGI muesli (LGI-M: Δ) breakfast (mean ± SEM). Time effect (P<0.05),meal x time interaction effect (P<0.05).

The second pair of breakfasts, HGI cornflakes (HGI-C) and LGI (LGI-M) muesli, were based on breakfast cereals. Despite the different GI of the two breakfasts peak blood glucose concentrations in the two trials were similar (HGI-C: 7.5 ± 0.2 mmol.l^{-1} and LGI-M: 7.0 ± 0.3 mmol.l^{-1}). Differences were also seen in the shape of the blood glucose curves. In the LGI trial, blood glucose concentration declined faster reaching fasting levels by 60 min into the postprandial period compared with the 180 min into the postprandial period in the HGI cornflakes trial. Statistical analysis resulted in a meal effect (P<0.05) (HGI>LGI) and a time effect (P<0.05). (Figure 4.2).

The glycaemic response of the HGI-T breakfast was 2.7 times higher than LGI-F (P<0.05), similarly HGI-C was 1.6 times higher then HGI-M (P<0.05) demonstrating measured differences in the GI of the meal (Table 4.1).

Table 4.1 Calculated GI and measured glycaemic effect (IAUC).

IAUC (mmol.l^{-1}.180min)		**HGI/LGI** (IAUC)	Meal GI		**HGI/LGI** (GI)
HGI-Toast	LGI-Fruit		HGI-Toast	LGI-Fruit	
197 *	72	**2.7**	72	36	**2.0**
HGI-Cornflakes	LGI-Muesli		HGI-Cornflakes	LGI-Muesli	
147 *	90	**1.6**	71	48	**1.5**

* HGI significantly different from LGI trial ($P<0.05$)

Blood Glucose – 4 way analysis (n=7)

Despite of the different GI values of test breakfasts, peak blood glucose concentrations were similar in all four trials (HGI-T: 7.3 ± 0.3 mmol.l^{-1} ; HGI-C: 7.5 ± 0.2 mmol.l^{-1} ; LGI-M: 7.0 ± 0.3 mmol.l^{-1} ; and LGI-F: 6.6 ± 0.3 mmol.l^{-1} respectively). However the glycaemic response to the four breakfasts, as expected, were different. The glycaemic response to the HGI toast (HGI-T) breakfast was higher then to that of HGI cornflakes (HGI-C), LGI muesli (LGI-M) and LGI fruit (LGI-F) breakfasts ($P<0.05$) (Figure 4.3, Table 4.1). The glycaemic response to the HGI cornflakes (HGI-C) breakfast was higher than to both LGI breakfasts ($P < 0.05$).

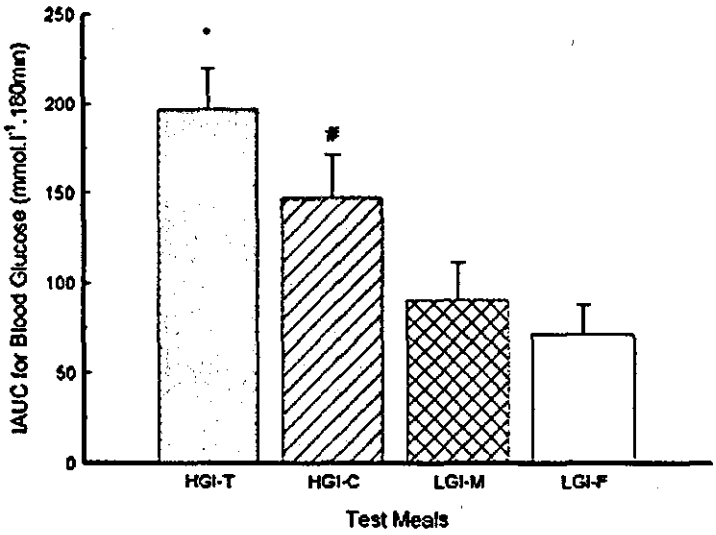


Figure 4.3. Incremental area under the blood glucose curve (mmol.l^{-1}.180min) following the ingestion of two high glycaemic index (HGI-T and HGI-C) and two low glycaemic index breakfasts (LGI-M and LGI-F) (mean ± SEM).
* HGI-T different from all other breakfasts (P<0.05)
# HGI-C different from the two LGI breakfasts (P<0.05)

There was no difference in the glycaemic response between the LGI muesli (LGI-M) and the LGI fruit (LGI-F) breakfasts for the 180 min postprandial period (Figure 4.3).

As the GI of the breakfasts was calculated from the amount of carbohydrate and the GI of the contributing foods, it was expected to be matched by the ratio of the measured glycaemic effect. (Table 4.2). According to Wolever and Jenkins (1986) the GI concept applies well to mixed meals containing fat and protein (Wolever, *et al.*, 1986). In this case all breakfasts were matched for Energy, CHO, fat and protein content and only differed in their GI. In this case the GI ratio between two breakfasts should have matched the ratio of the glycaemic response (IAUC) for the same two breakfasts. Table 4.2 contains examples of different combinations of the breakfasts. The odd one out is he HGI-T having a larger glycaemic response, hence giving a higher ratio than its calculated GI.

Table 4.2 Ratio between measured glycaemic effect (IAUC) and calculated meal GI.

IAUC ($mmol.l^{-1}.180min$)		**Ratio** (IAUC)	Meal GI		**Ratio** (GI)
HGI-T	**HGI-C**		**HGI-T**	**HGI-C**	
197	147	**1.3**	72	71	**1.0**
HGI-T	**LGI-M**		**HGI-T**	**LGI-M**	
197	90	**2.2**	72	48	**1.5**
HGI-T	**LGI-F**		**HGI-T**	**LGI-F**	
197	72	**2.7**	72	36	**2.0**
HGI-C	**LGI-M**		**HGI-C**	**LGI-M**	
147	90	**1.6**	71	48	**1.5**
HGI-C	**LGI-F**		**HGI-C**	**LGI-F**	
147	72	**2.0**	71	36	**2.0**
LGI-M	**LGI-F**		**LGI-M**	**LGI-F**	
90	72	**1.3**	48	36	**1.3**

However when the calculated GI of the mixed breakfasts was plotted against the mean measured glycaemic response (IAUC for 180 min) a strong positive relationship was found. (r = 0.914; $P<0.05$) (Figure 4.4, n=7).

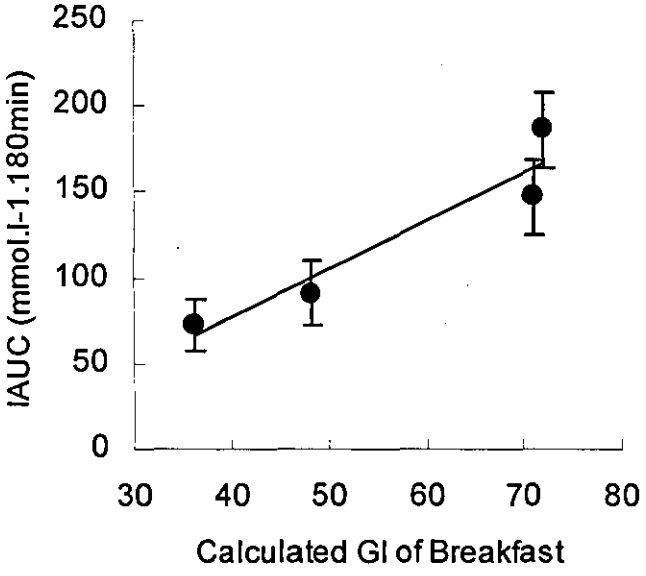


Figure 4.4. Correlation between calculated GI of breakfasts and incremental blood glucose response area (mean ± SEM). (r = 0.914, $P<0.05$, y=2.99x– 41.1)

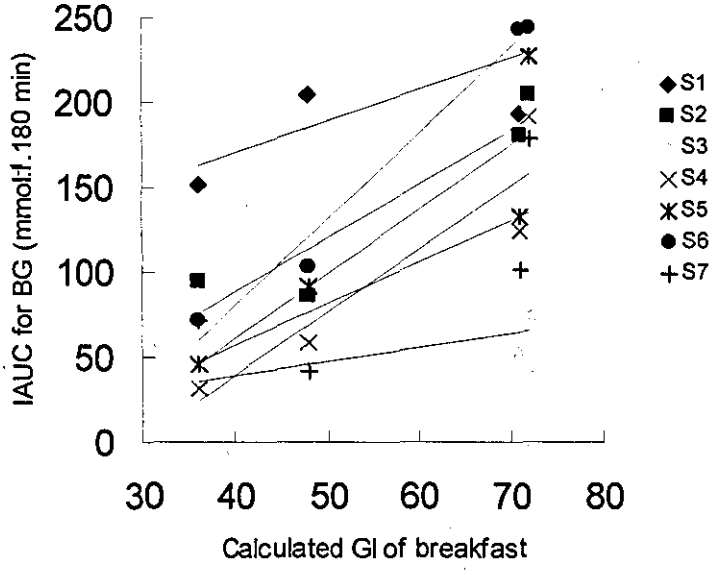


Figure 4.4a. Correlation between calculated GI of breakfasts and incremental are for blood glucose (individual values n=7).

Blood Lactate – 2 way analysis

Blood lactate concentrations increased over time during the postprandial period in the HGI-toast (HGI-T) v LGI fruit (LGI-F) trial ($P<0.05$) (Figure 4.5). From a peak at 15 min, blood lactate concentration remained significantly higher in the LGI-fruit (LGI-F) trial compared with the HGI toast (HGI-T) trial for the first 90 min of the postprandial period (Figure 4.5). Blood lactate concentrations were significantly higher at 15, 30, 60 and 90 min of the postprandial period in the LGI fruit (LGI-F) trial compared to the HGI toast (HGI-T) trial ($P<0.05$)(n=8) (Figure 4.5). Blood lactate concentrations reached fasting values at 120 min and remained at this level for the remainder of the postprandial period in both trials.

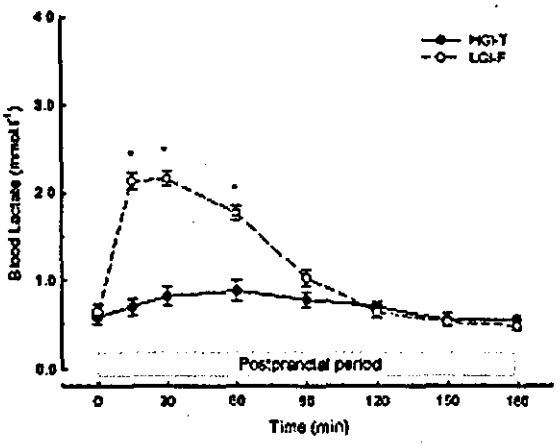


Figure 4.5 Blood lactate concentrations (mmol.l^{-1}) during a 180 min postprandial period following the ingestion of a HGI toast (HGI-T: ●) and a LGI fruit (LGI-F : ○) breakfast (mean ± SEM). Meal by time interaction effect ($P<0.05$).
* HGI significantly different from the LGI trial ($P<0.05$).

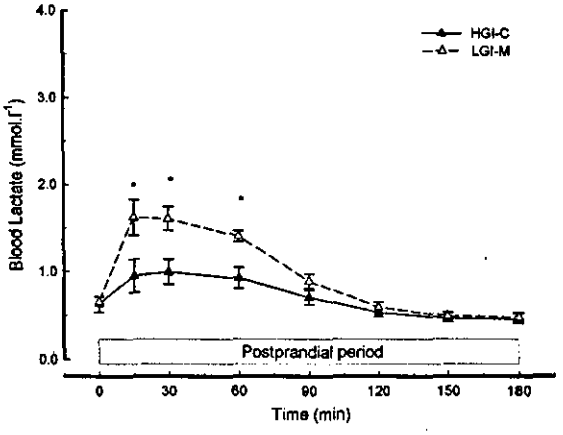


Figure 4.6 Blood lactate concentrations (mmol.l^{-1}) during a 180 min postprandial period following the ingestion of a HGI cornflakes (HGI-C: ▲) and a LGI muesli (LGI-M: Δ) breakfast (mean ± SEM). Meal by time interaction effect ($P<0.05$).
* HGI significantly different from the LGI trial ($P<0.05$).

Blood lactate concentrations increased over time during the postprandial period in HGI cornflakes (HGI-C) v LGI muesli (LGI-M) trial ($P<0.05$) (Figure 4.6). From a peak at 15 min, blood lactate concentration remained

significantly higher at 30, 60 and 90 min of the postprandial period following the LGI muesli (LGI-M) compared to the HGI cornflakes (HGI-C) trial ($P<0.05$) (n=7) (Figure 4.6).

Gut fullness and hunger ratings

Measurement of gut fullness and hunger were not available for the HGI cornflakes (HGI-C) and LGI muesli (LGI-M) trials, so the findings below refer only to the HGI toast (HGI-T) and LGI fruit (LGI-F) trials.

Following the ingestion of the HGI toast (HGI-T) and LGI fruit (LGI-F) breakfast rating of gut fullness increased, peaked at 15 min, then gradually decreased over time in both trials (Table 4.3). There was a meal by time interaction effect ($P<0.05$). A post hoc test confirmed that the largest variation in gut fullness ratings occurred at 15, 30 and 150 min in the postprandial period (LGI-F >HGI-T) ($P<0.05$) (Table 4.3).The perceived rate of hunger showed a meal by time interaction ($P<0.05$). Hunger ratings were lower at 30, 90, 120, 150, 180 min following the LGI fruit (LGI-F) compared with the HGI toast (HGI-T) trial ($P<0.05$, Table 4.3).

Providing breakfasts with different GI but equal amount of available carbohydrate required adjustment in meal sizes (Table 4.4). The mean amount food intake was the lowest for the HGI toast (HGI-T) and highest for the LGI fruit (LGI-F) breakfast (HGI-T: 169 ± 10 g; HGI-C: 336 ± 19 g, LGI-M: 471± 27 g and LGI-F: 672 ± 39 g). Breakfasts sizes were inversely proportional to their calculated GI value ($r=0.945$, $P<0.05$).

Table 4.3 Gut fullness and hunger ratings during the HGI toast (HGI-T) and LGI fruit (LGI-F) trials (mean ± SEM).

		Postprandial Period (min)						
Variable	**Trial**	**15**	**30**	**60**	**90**	**120**	**150**	**180**
Gut Fullness	HGI-T	12 ± 1	12 ± 1	11 ± 1	11 ± 1	10± 1	9 ± 0	9 ± 0
	LGI-F	17 ± 0*	15 ± 1*	13 ± 1	12 ± 1	12 ± 1	11 ± 1*	10 ± 1
Hunger	HGI-T	8 ± 0	8 ± 0	8 ± 0	10 ± 0	10 ± 0	11 ± 0	12 ± 1
	LGI-F	8 ± 1	7 ± 0*	8 ± 0	8 ± 0*	8 ± 0*	9 ± 0*	10 ± 0*

*LGI-F significantly different from HGI-T trial ($P<0.05$)

Table 4.4 Energy and macronutrient intake for test meals and pre trial evening meal (mean ± SEM) (n = 8)

Breakfast	Weight (g)	Energy (kcal)	CHO (g)	Fat (g)	Protein (g)	GI	GL
HGI-T (toast)	169 ± 10	401 ± 23	80.1 ± 4.6	3.4 ± 0.2	12.4 ± 0.7	72 ± 0	58 ± 3
HGI-C (Cornflakes)	336 ± 19	417 ± 24	80.1 ± 4.6	3.9 ± 0.2	15.6 ± 0.9	71 ± 0	57 ± 3
LGI-M (Muesli)	471± 27	435 ± 25	80.1 ± 4.6	5.1 ± 0.3	17.1 ± 1.0	48 ± 0	38 ± 2
LGI-F (Fruit)	672 ± 39	415 ± 24	80.1 ± 4.6	4.3 ± 0.2	12.2 ± 0.7	36 ± 0	29 ± 2
Evening Meal		1281 ± 121	143.6 ± 17.5	51.1 ± 10.3	71.1 ±10.5	60 ± 3	83 ± 12

4.4 Discussion

This study investigated the glycaemic responses to HGI and LGI mixed breakfasts composed of commonly consumed breakfast foods and containing 1g.kg^{-1} BM of available CHO. The major finding was that, despite differences in the GI values of breakfasts, and contrary to expectations, postprandial blood glucose peaks were similar at 15 min into the postprandial period. Ingestion of the first pair of breakfasts resulted in a blood glucose peak of 7.3 ± 0.3 mmol.l^{-1} for the HGI toast and 6.6 ± 0.3 mmol.l^{-1} for LGI fruit. One of the possible explanation would be that the LGI-F breakfasts contained 57% fruit, 25% from fresh apples and 32% from tinned peaches. Most of the glucose probably came from the tinned peaches and this could explain why blood glucose concentration in the LGI fruit (LGI-F) trial rose to a similar level as in the HGI-toast (HGI-T) trial.

There was a marked difference in the shape of the blood glucose curves. Blood glucose concentrations declined rapidly reaching near fasting levels at 60 min into the postprandial period of the LGI fruit trial in contrast with reaching fasting levels only at 180 min in the HGI toast trial.

The second pair of breakfasts resulted in similar peak blood glucose concentrations (HGI-C: 7.5 ± 0.2 mmol.l^{-1} and LGI-M: 7.0 ± 0.3 mmol.l^{-1}). The decline in the blood glucose concentration was similar to that observed in the first pair of breakfasts, reaching fasting values at 60 min (LGI-M) and 180 min (HGI-C) into the postprandial period.

The concentration of plasma glucose in the systemic circulation is tightly controlled and is affected by the rate of glucose appearance as well as the rate of glucose disappearance. Schenk and colleagues (2003) studied glucose kinetics following the consumption of a HGI cornflakes and LGI All-Bran

breakfast (Schenk, *et al.*, 2003). The authors reported that a rapid glucose clearance brought on by an earlier and higher insulin peak (elicited by a larger protein content of the All-Bran) could account for differences in GI foods. In the present study there was a similar rapid decrease in blood glucose concentrations in both LGI trials (LGI-M and LGI-F), which could suggest a more rapid removal of glucose from the systemic circulation. However meals in the present study were matched for energy and macronutrients, so the differences observed cannot be due to differences in protein content unlike the explanation offered by Shenk and colleagues (Schenk, *et al.*, 2003). Their study suggests that the lower IAUC in the LGI meal is due to an increased glucose disappearance from the systemic circulation. In the present study one possible explanation of the low glycaemic response to the LGI fruit breakfast is that processing changed the nature of the fruit. The heat treatment involved in the processing of peaches would have produced a break down of the fruit's cell walls, making its contents more readily available (Kunzek, Kabbert and Gloyna 1999). Readily available glucose, and the early rise in circulating insulin levels (not measured in this study) could have contributed to and increased disappearance of blood glucose hence a small glycaemic response.

A large constituent of the LGI fruit (LGI-F) breakfast was fructose, which does not directly raise blood glucose concentration and is metabolised by the liver to produce glucose (Henry, *et al.*, 1991). One of the effects of this metabolism of fructose can be seen by the large increase in blood lactate concentration during the first 90 min of the postprandial period. Several studies have reported that the ingestion of fructose or a high fructose content CHO meal results in an increased blood lactate concentration (Koivisto, Karonen and Nikkila 1981; Moore, Cherrington, Mann and Davis 2000). Only about 65% of fructose is converted to glucose, the rest is released from the liver as lactate. (Henry, *et al.*, 1991). The high blood lactate concentrations in

the two LGI trials were probably due to fructose being metabolised by the liver and released as lactate. Interestingly high postprandial blood lactate concentrations have been previously reported following ingestion of a mixed meal with a larger CHO content (2g.kg^{-1}BM) containing 160 g of apple and 280 g of tinned peaches (Wu, *et al.*, 2003) and 400 ml apple juice (plus All-Bran). The fruit content of the present LGI fruit breakfast was close to that used in study of Wu (2003) i.e. 150 g apple, 227 g canned peaches (plus low fat yoghurt).

The second pair of breakfasts were cereal based. Starch is considered to be digested slowly resulting in a modest glycaemic response. However the extent to which starch is digested and absorbed depends on the source and degree of processing (Cummings and Englyst 1995; Gannon and Nuttall 1987; Raben, Tagliabue, Christensen, Madsen, Holst and Astrup 1994; Wolever 1991). Simple sugars like glucose and sucrose are rapidly absorbed. Starch rich breakfast cereals, possibly containing hidden sugars, could raise blood glucose and insulin concentrations. In the present study the Alpen Original – no added sugar, LGI muesli could be one of the cereals with hidden sugars that might have elicited the high postprandial peak glucose concentration. Englyst and colleagues (1996) measuring rapidly available glucose (RAG) in carbohydrate foods, found that this correlates highly with the GI of CHO foods ($r=0.981$, $P<0.0001$) (Englyst, *et al.*, 1996). Both white bread (wheat product) and cornflakes (maize product) are highly processed foods, in which starch is fully gelatinised hence both food items are easily digested and absorbed, and were expected to elicit similarly high glycaemic responses. Interestingly in the present study the HGI cornflakes (HGI-C) was calculated to have the same GI as the HGI toast (HGI-T) breakfast (71 compared with 72), but the former resulted in a 25% lower glycaemic response (Figure 4.3). This decrease in glycaemic effect is thought to be due to the milk content in

the HGI cornflake (HGI-C) breakfast. Milk, despite being a LGI food is known to raise plasma insulin levels and hence to enhance glucose uptake (Liljeberg Elmstahl, *et al.*, 2001).

Peak blood glucose as well as changes in glucose concentration during the 3 h postprandial period in the LGI muesli (LGI-M) and LGI fruit (LGI-F) trial did not follow the definition of LGI food i.e. lower peak blood glucose and slower glucose release compared to HGI foods. The consumption of the two LGI breakfasts resulted in high peak blood glucose concentrations followed by a rapid fall, resembling the metabolic responses to HGI rather than LGI CHO foods. These results suggest that foods, although classified as LGI based on the 'incremental area under the curve', may show an initial glycaemic response similar to those expected after consuming HGI CHO foods.

The similarity in the peak blood glucose concentrations following consumption of the 4 breakfasts examined in the present study (Figure 4.1 and 4.2) contrasted with findings from previous studies from our laboratory. Using 2 g of available CHO per kg BM and similar meal composition, Stevenson and colleagues reported significant differences in the peak plasma glucose concentration between HGI and LGI breakfasts (Stevenson, *et al.*, 2005). The difference between the latter and the present study might be explained by the inclusion of a concentrated glucose syrup (Lucozade original) in the HGI trial thus resulting in a higher glucose availability (Stevenson, et al., 2005).

The results from the present study agrees with previous finding from our laboratory, that although the addition of LGI foods lowers the overall GI of the breakfast, the early metabolic responses during the postprandial period are

similar to that following the ingestion of a CHO breakfasts composed entirely of HGI food (Backhouse, Williams, Stevenson and Nute 2007).

The validity of the GI values for mixed meals has been questioned in several studies (Coulston, *et al.*, 1984a; Coulston, *et al.*, 1984b; Hollenbeck, Coulston and Reaven 1988). Previous studies from our laboratory have shown that the ratio between the glycaemic response to HGI and LGI meals closely follows the ratio of their calculated GI values. Wu and colleagues (2003), using a meal with an available CHO content of 2 g per kg BM reported that a 2.2 : 1 ratio in glycaemic response followed the 2.1 : 1 ratio of the corresponding meal GIs (Wu, et al., 2003). Similarly Stevenson (2006) and colleagues reported a ratio of 1.8 : 1 in the glycaemic response was matched by a ratio of 1.8 : 1 of the GI of the corresponding meals (Stevenson, et al., 2006).

In contrast to the two studies mentioned above in the present study out of 6 possible pairing of breakfasts only 3 demonstrated a close relationship between measured and calculated GI response; none of them included the HGI toast (HGI-T) breakfast (Table 4.2). The current data suggests that the discrepancies between the ratio of the calculated breakfast GI and measured glycaemic ratio are due to the high IAUC in the HGI toast trial. These differences in the ratio of measured glycaemic effect are less if the IAUC is calculated only for 120 min of the postprandial period. The present recommended blood sampling interval and measurements of glycaemic response in GI testing is 120 min even though the ingested CHO will have an effect on glycaemia and insulinaemia (especially in diabetic individuals) for a further 2-4 hrs (Gannon, *et al.*, 1987). Following the consumption of a HGI toast breakfast plasma glucose concentration remained elevated for longer than the recommended 120 min cut-off point, hence the IAUC was larger at 180 min compared with 120 min the HGI toast trial. The discrepancy between

ratios of calculated meal GI and those of the measured glycaemic response were effected by the choice of time interval chosen for the calculation of the IAUC. Although these discrepancies were observed over a 120 min period, their magnitude was less then at 180 min.

In conclusion, the measured glycaemic responses in the present study only partly followed Wolever's method of estimating the GI of mixed meals (Wolever, et al., 1986) and in case of the HGI toast breakfast, was effected by the length of the sampling time.

The GI was originally devised and used to aid diabetics with food choices. Several studies have reported that a diet high in rapidly absorbed carbohydrates and low in cereal fibre is associated with an increased risk of type 2 diabetes (Schulze, *et al.*, 2004). Hence choosing low-GI foods in place of conventional or high-GI foods should alleviate this risk. A meta-analysis of randomised controlled trials reported that a LGI diet has a small but clinically useful effect on medium-term glycemic control in patients with diabetes. This benefit is incremental and is similar to that offered by drugs targeting postprandial hyperglycaemia (Brand-Miller, *et al.*, 2003). Yet in the present study peak blood glucose concentration in the HGI and LGI breakfast were similarly elevated with no significant difference between breakfasts. This would be a rather undesirable effect for diabetic individuals following a LGI diet expecting to have low glucose peaks and slow glucose release into the systemic circulation.

Providing breakfasts with different GI but equal amounts of available carbohydrate required adjustment in meal sizes (Table 4.4). The mean food intake (measured in grams) was the lowest for the HGI toast (HGI-T) and highest for the LGI fruit (LGI-F) breakfast (HGI-T: 169 ± 10 g; HGI-C: 336

± 19 g, LGI-M: 471± 27 g and LGI-F: 672 ± 39 g). Breakfasts sizes were inversely proportional to their calculated GI value (r=0.945, $P<0.05$). Some of the larger breakfasts would have taken longer to consume, however participants were asked to pace themselves and eat the meals in a 15 min period. Mechanical gastric distension of the stomach may have played a role in the sensations of gut fullness and hunger. Oesch and colleagues (2006) reported that distending part of the stomach (the fundus) affected the mean Visual Analogue Scale (VAS) in the pre-meal period i.e. participants experienced a reduced degree of hunger and a concomitant feeling of fullness, but the effect was only apparent during distension with larger volumes of 600 ml and 800 ml (Oesch, Ruegg, Fischer, Degen and Beglinger 2006). Gut fullness ratings and increased satiety in the LGI fruit breakfast in the present study may have been effected by the size of the breakfast as the LGI fruit breakfast was 4 times larger than the HGI toast breakfast (LGI-F: 672 ± 39 g and HGI-T: 169 ± 10 g). It is seems unlikely that this would have been the only influence on the gut fullness and hunger ratings.

In conclusion, this study shows that the glycaemic responses (IAUC) following the two HGI breakfasts were significantly larger than the two LGI breakfasts and were similar to those reported previously. Contrary to expectations ingestion of the two LGI breakfasts did not result in lower peak blood glucose concentrations. Furthermore the blood glucose concentration for the LGI breakfasts did not follow the responses expected from a LGI food. The results of the present study suggest that although some foods are classified as LGI based on the 'area under the glucose curve', metabolically they might behave closer to what is expected from a HGI CHO food.

CHAPTER 5

The influence of high and low glycaemic index carbohydrate breakfasts on blood glucose response and substrate metabolism during rest and exercise in recreationally active men

5.1 Introduction

In the previous chapter evidence was presented that the LGI fruit (LGI-F) and LGI muesli (LGI-M) breakfasts elicited a smaller glycaemic response compared with the two HGI breakfasts, based on the 'area under the glucose curve'. However metabolically and in the early part of the postprandial period the two LGI breakfasts were closer to what is expected from a HGI CHO food. Peak blood glucose concentrations were not significantly different between high and low glycaemic index test breakfasts. The rapid rise and fall in blood glucose concentration following the two LGI breakfasts was quite different from the slow rise and fall in blood glucose concentration reported for LGI foods such as lentils (Jenkins, Wolever, Taylor, Griffiths, Krzeminska, Lawrie, Bennett, Goff, Sarson and Bloom 1982). Therefore the blood glucose response of the LGI breakfasts were not those expected of a typical LGI foods.

Previous studies have reported an increase in fat oxidation following ingestion of a LGI meal compared with ingestion of a HGI meal. The reduced glycaemic and insulinaemic response following ingestion of a LGI meal allows an increase in circulating plasma free fatty acids (FFA) and an increase in the rate of fat oxidation during exercise (Febbraio, Chiu, Angus, Arkinstall and Hawley 2000a; Febbraio, Keenan, Angus, Campbell and Garnham 2000b; Febbraio, *et al.*, 1996; Wu, *et al.*, 2003). Interestingly Schenk and colleagues

(2003) found that in some cases the lower GI of food can be due to an earlier increase in the rate of disappearance of glucose which would reduce the increase in blood glucose concentration (Schenk, *et al.*, 2003). An early and short rise in insulin concentration may cause only a temporary and brief blunting of the mobilisation of FFA and so not significantly impair fat oxidation during subsequent exercise. Previous studies from our laboratory using HGI and LGI breakfasts with an available CHO intake of $2g.kg^{-1}BM$ reported increased fat oxidation during exercise following a LGI breakfast compared with a HGI breakfast, in the athletic population (Wu, *et al.*, 2003).

The aim of the present study was to extend the observations described in Chapter 4 beyond the postprandial period to include measurement of metabolic responses to a 60 min sub-maximal run. The study also examined whether or not the increased fat oxidation following a LGI meal seen in athletes, ingesting 2 g of CHO per kg body mass, was also evident in recreationally active people consuming a smaller amount of carbohydrate i.e. $1g.kg^{-1}BM$. The two meals used were the same as described in the previous chapter. The HGI breakfast was toast and the LGI breakfast was fruit based. The choice of these two breakfasts was made on the evidence that they had the greatest difference in glycaemic response.

5.2 Methods

Participants

Six male participants (aged 32.0 ± 2.4 y, height: 1.78 ± 0.02 m, weight: 79.1 ± 2.7kg, BMI: 25.0 ± 0.7 $kg.m^2$, $\dot{V}O_2$max: 55.3 ± 2.4 $ml.kg^{-1}.min^{-1}$) took part in this study. At the time of the study, all participants were involved in recreational running three to four times per week. None of the participants had a history of diabetes mellitus or any other endocrine disorder. The

Loughborough University Ethical Committee approved the protocol and all participants gave their written informed consent.

Test meals

In Study 1 demonstrated the different glycaemic responses to four carbohydrate breakfasts. There was no statistically significant difference between the glycaemic response to the HGI and LGI cereal breakfasts (cornflakes and muesli). As a result we have chosen breakfasts with the largest difference in glycaemic response (as measured by the IAUC) (Chapter 4 (Figure 4.3).

The two test breakfasts used in this study contained 1 $g.kg^{-1}$BM of available CHO and were chosen because they showed The two breakfasts (HGI toast (HGI-T) and LGI fruit (LGI-F)) were prepared on site, just before each trial. The calculated glycaemic index of the HGI and LGI meals were GI 72 and GI 36 respectively (Table 3.2).

Main trials

On the day of each trial participants were provided with a breakfast in a randomised cross-over design after which they rested for 3 h. Expired air and fingertip blood samples were taken pre-meal and at regular intervals during the 180 min postprandial period. At the end of this period participants completed a 60 min run at 65% $\dot{V}O_2$max on a motorised treadmill. A schematic view of the experimental protocol (Figure 3.1) as well as a detailed description of methods used can be found in the methodology section of this thesis (see Chapter 3).

In order to minimise the variation in dietary intake prior to each trial, a three day weighed food intake was recorded by each participant before the first

main trial. Participants were asked to repeat the same diet before the following trial in order to minimise variation in muscle and liver glycogen concentrations. Previous studies have demonstrated that a single LGI meal can improve glucose tolerance and therefore reduce hyperinsulinaemia following a second meal (Jenkins, *et al.*, 1982; Liljeberg, Akerberg and Bjorck 1999; Wolever, Jenkins, Ocana, Rao and Collier 1988). Furthermore Stevenson and colleagues (2005b) reported that consumption of a LGI evening meal (compared with consumption of a HGI evening meal) had an overnight effect lowering the glycaemic and insulinaemic responses to a HGI standard breakfast consumed the next morning (Stevenson, *et al.*, 2005b). Therefore the evening meal in this study was standardised. The day before the trials participants were given the ingredients for an evening meal consisting of pasta, pasta sauce and a small amount of cheese for the main course and a low fat fruit yogurt for dessert. The meal contained an available CHO content of 1.5 g. kg^{-1} BM, (55% CHO, 27% fat, 18% protein) with a GI value of 35 and GL 42 (Table 5.1). The pasta meal was a popular choice with our subjects and was easy to prepare.

Table 5.1 Energy and macronutrient intake for test meals and pre trial evening meal (mean ± SEM) (n = 6)

Breakfast	Weight (g)	Energy (kcal)	CHO (g)	Fat (g)	Protein (g)	GI	GL
HGI-T (toast)	167 ± 6	396 ± 14	79.1 ± 2.7	3.3 ± 0.1	12.2 ± 0.4	72 ± 0	57 ± 2
LGI-F (Fruit)	663 ± 23	409 ± 14	79.1 ± 2.7	4.2 ± 0.1	12.1 ± 0.4	36 ± 0	28 ± 1
Evening Meal		818 ± 28	118.7 ± 4.1	22.1 ± 0.8	35.2 ±1.2	35 ± 0	42 ± 1

Statistical analysis

A two-way analysis of variation for repeated measures was used to analyse difference in the physiological and metabolic responses in the HGI and LGI trials. More details can be found in Chapter 3 (General Methods).

5.3 Results

Blood Glucose

Following the ingestion of both breakfasts, blood glucose concentrations increased rapidly and peaked at 15 min into the postprandial period. The peak concentration was 6.6 ± 0.6 mmol.l^{-1} for the HGI-T trial and 5.9 ± 0.6 mmol.l^{-1} for the LGI-F trial. (Figure 5.1).

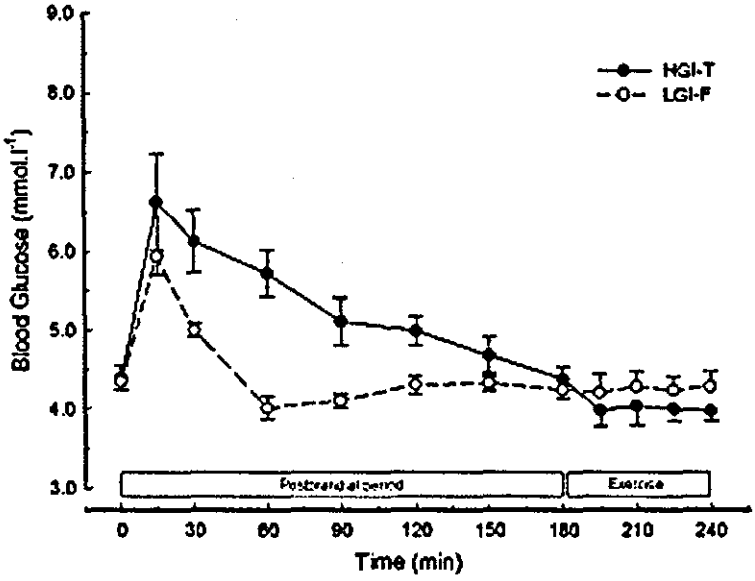


Figure 5.1. Blood glucose concentrations (mmol.l^{-1}) during the 180 min postprandial period following the ingestion of a HGI toast (HGI-T: •) and a LGI fruit (LGI-F: ○) breakfast (mean ± SEM). There was a meal effect (P<0.05), time effect (P<0.05) and a meal x time interaction effect (P<0.05).
* HGI significantly different from LGI trial (P<0.05)

From this peak, blood glucose concentration rapidly decreased in the LGI fruit (LGI-F) trial falling below fasting values within the first hour of the postprandial period. However blood glucose concentrations in the HGI toast (HGI-T) trial remained higher falling to fasting values after 180 min of the

postprandial period. Statistical analysis confirmed the change in blood glucose concentrations over the 180 min postprandial period in both trials ($P<0.05$). Furthermore there was a meal by time interaction effect, HGI-T > LGI-F trial ($P<0.05$) (Figure 5.1). The results of the post-hoc test showed no significant variance at the different time points. Variance at 60 min ($P=0.064$) or at 90 min ($P=0.065$) was close to but did not reach the $P=0.05$ level of statistical significance.

Blood glucose concentrations during exercise were 4.0 ± 0.2 mmol.l^{-1} and 4.2 ± 0.2 mmol.l^{-1} for the HGI-T and LGI-F respectively and there was no difference between the two trials.

The glycaemic response (IAUC), calculated over the 180 min postprandial period, was 4.4 times larger in the in the HGI toast (HGI-T) compared with the LGI fruit (LGI-F) trial ($P<0.05$) (Figure 5.2, Table 5.2). Calculating the IAUC over the recommended 120 min resulted still in a 4 fold difference in glycaemic response to the HGI-T compared to the LGI-F breakfast.

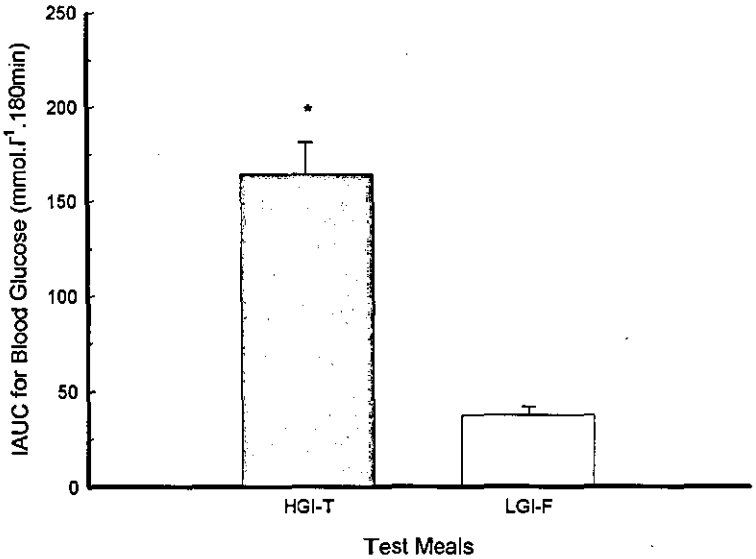


Figure 5.2. The incremental area under the blood glucose curve (mmol.l^{-1}.180min) for the 180 min postprandial period following the ingestion of a high glycaemic (HGI-T) and low glycaemic index meal (LGI-F) (mean ± SEM).
* HGI significantly different from the LGI trial ($P<0.05$).

Table 5.2 Incremental area under the blood glucose curve calculated for 180 min and 120 min of the postprandial period (mmol.l^{-1}.min).

		180 min		120 min	
Breakfasts	Meal GI	IAUC (mmol.l^{-1}.min)	% lower than HGI-T	IAUC (mmol.l^{-1}.min)	% lower than HGI-T
HGI-T (toast)	72	164 ± 17*		144 ± 14*	
LGI-F (fruit)	36	38 ± 4	77	36 ± 4	75

* HGI significantly different from LGI $P<(0.05)$

Blood Lactate

Following ingestion of the LGI fruit (LGI-F) breakfast blood lactate concentration increased more rapidly then following the HGI toast (HGI-T) breakfast and reached peak values at 30 min into the postprandial period. The peak lactate of 2.5 mmol.l^{-1} in the LGI fruit trial was 2.8 times larger compared with 0.9 mmol.l^{-1} in the HGI toast trial. Furthermore, blood lactate concentrations were higher at 15, 30 and 60 min of the postprandial period in LGI trial compared with the HGI trial ($P<0.05$) (Figure 5.3). Following both meals, the concentrations of this metabolite returned to fasting values by the end of the 180 min postprandial period. There was no difference in blood lactate concentration between trials during exercise.

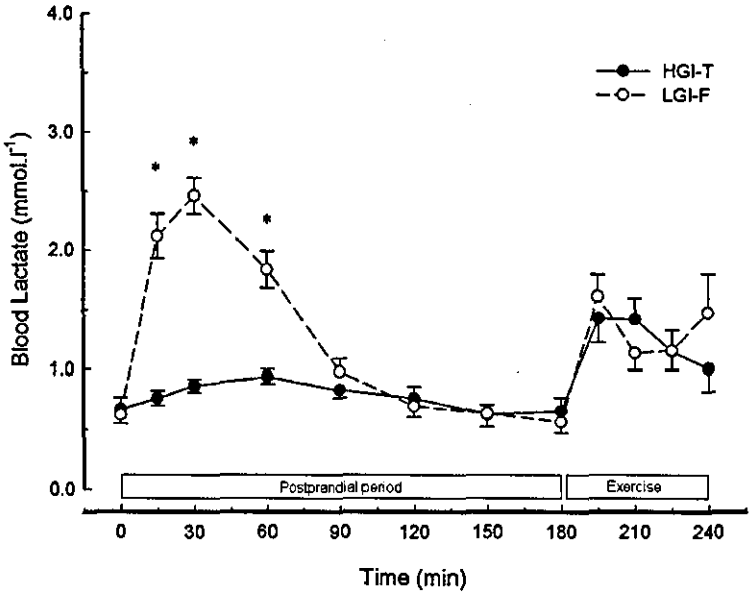


Figure 5.3 Blood lactate concentrations (mmol.l^{-1}) following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-F : ○) (mean ± SEM) during the 180 min postprandial period and the 60 min exercise period. * HGI significantly different from the LGI trial ($P<0.05$).

Oxygen uptake

There was no difference in fasting oxygen uptake, during the postprandial period or during exercise under the two conditions (Table 5.3 and Table 5.4).

Respiratory Exchange Ratio (RER)

Fasting RER values were similar in both trials: 0.80 ± 0.01 (HGI-T) and 0.81 ± 0.01 (LGI-F). There was a significant change in RER values with time during the postprandial period in both trials ($P<0.05$). There was a meal by time interaction effect, suggesting that the HGI trial was significantly different from the LGI trial ($P<0.05$).

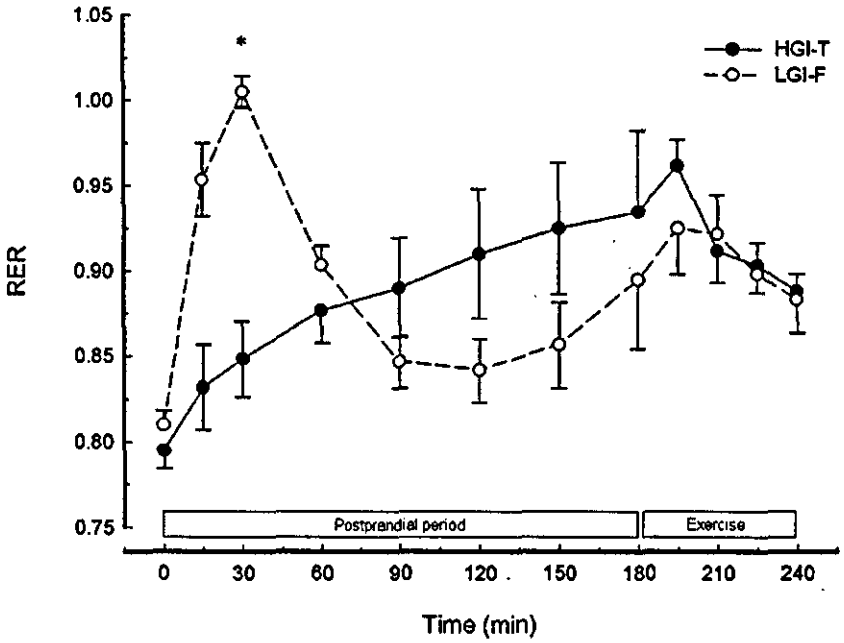


Figure 5.4. Respiratory exchange ratio (RER) following the ingestion of a high glycaemic (HGI-T: •) and low glycaemic index meal (LGI-M :○) during the 180 min postprandial period and the 60 min exercise period (mean ± SEM). Time and meal by time effect during the postprandial period ($P<0.05$).
* HGI significantly different from the LGI trial ($P<0.05$).
Time effect only during exercise ($P<0.05$).

The post-hoc test revealed that the greatest variation occurred at 30 min into the postprandial period ($P<0.05$). In the LGI trial RER values were higher for most of the first 90min of the postprandial period compared with the HGI-T trial. From 60 min onwards RER values in the LGI-F trial fell below and remained lower than the values for the HGI trial (Figure 5.4) (Table 5.3).

During the 60 min exercise RER values decreased in both trials with time ($P<0.05$). There was a trend for higher gut fullness ratings during the LGI trial no difference in RER during exercise in the two trials (Figure 5.4) (Table 5.3).

Table 5.3 Oxygen Uptake ($\dot{V}O_2$), Expired Carbon Dioxide ($\dot{V}CO_2$) and respiratory exchange ratio (RER) during the high (HGI-T) and low glycaemic index (LGI-F) trials.

			Postprandial period (min)						
Variable	Meal	Fast	15	30	60	90	120	150	180
$\dot{V}O_2$ ($l.min^{-1}$) *	HGI-T	0.27 ± 0.01	0.30 ± 0.00	0.32 ± 0.01	0.29 ± 0.02	0.30 ± 0.01	0.31 ± 0.01	0.29 ± 0.01	0.31 ± 0.01
	LGI-F	0.27 ± 0.01	0.33 ± 0.01	0.35± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.29 ± 0.02	0.30 ± 0.01
$\dot{V}CO_2$ ($l.min^{-1}$) *	HGI-T	0.22 ± 0.01	0.25 ± 0.02	0.27 ± 0.03	0.26 ± 0.05	0.27 ± 0.03	0.28 ± 0.03	0.26 ± 0.02	0.29 ± 0.06
	LGI-F	0.22 ± 0.02	0.32 ±0.04#	0.35± 0.03#	0.30 ± 0.03	0.27 ± 0.03	0.25 ± 0.03	0.25± 0.04	0.27 ± 0.06
RER *	HGI-T	0.80 ± 0.01	0.83 ± 0.02	0.85 ± 0.02	0.88 ± 0.02	0.89 ± 0.03	0.91 ± 0.04	0.93 ± 0.04	0.94 ± 0.05
	LGI-F	0.81 ± 0.01	0.95 ±0.02#	1.01± 0.01	0.90 ± 0.01	0.85 ± 0.01	0.84 ± 0.02	0.86 ± 0.02	0.90 ± 0.04

* $\dot{V}O_2$, $\dot{V}CO_2$ and RER values changed with time ($P<0.05$) and all indicated a meal by time interaction effect ($P<0.05$)

HGI different from LGI ($P<0.05$)

Table 5.4 Oxygen Uptake ($\dot{V}O_2$), Expired Carbon Dioxide ($\dot{V}CO_2$), respiratory exchange ratio (RER), heart rate and rating of perceived exertion RPE) during exercise in the high (HGI-T) and low glycaemic index (LGI-F) trials.

		Exercise period (min)			
Variables	Trial	15	30	45	60
$\dot{V}O_2$ ($l.min^{-1}$)	HGI-T	2.60 ± 0.11	2.65 ± 0.13	2.65 ± 0.14	2.68 ± 0.13
	LGI-F	2.63 ± 0.13	2.64 ± 0.14	2.64 ± 0.10	2.67 ± 0.15
$\dot{V}CO_2$ ($l.min^{-1}$)	HGI-T	2.50 ± 0.22	2.41 ± 0.24	2.39 ± 0.29	2.38 ± 0.27
	LGI-F	2.42 ± 0.22	2.42 ± 0.28	2.37 ± 0.21	2.35 ± 0.27
RER *	HGI-T	0.96 ± 0.01	0.91 ± 0.02	0.90 ± 0.01	0.89 ± 0.01
	LGI-F	0.93 ± 0.03	0.92 ± 0.02	0.90 ± 0.01	0.88 ± 0.02
Heart Rate *	HGI-T	129 ± 2	134 ± 2	137 ± 3	140 ± 3
	LGI-F	131 ± 4	137 ± 4	142 ± 5	142 ± 5
RPE *	HGI-T	11 ± 0	12 ± 0	12 ± 1	12 ± 1
	LGI-F	11 ± 0	12 ± 0	13 ± 1	13 ± 1

* RER, Heart Rate and RPE values changed with time ($P<0.05$); RER indicated a meal by time interaction effect ($P<0.05$).

Estimated rate of carbohydrate and fat oxidation

The ingestion of both breakfasts resulted in changes in carbohydrate and fat oxidation rates overtime ($P<0.05$) (Figure 5.5 and Figure 5.6)

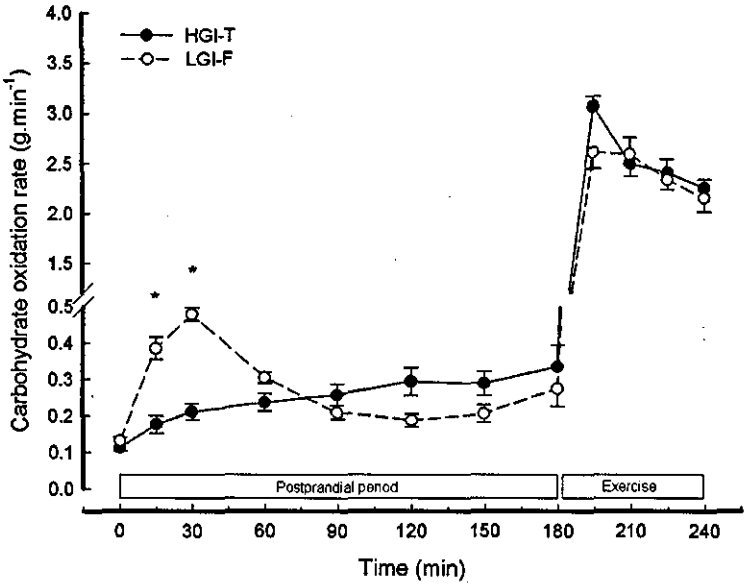


Figure 5.5 Carbohydrate oxidation rate ($g.min^{-1}$) during the 180 min postprandial period and a 60 min exercise following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-F : ○) (mean ± SEM). Meal, time and meal by time interaction effect during the postprandial period ($P<0.05$).
* HGI significantly different from the LGI trial ($P<0.05$).
Time effect only during exercise ($P<0.05$).

There was a rapid and large increase in CHO oxidation rate during the first 30 min of the postprandial period in the LGI fruit (LGI-F) compared to the HGI toast (HGI-T) trial illustrated by the statistically significant meal by time interaction effect ($P<0.05$). The HGI trial was significantly different from the LGI trial (Figure 5.5). According to the post-hoc test performed, carbohydrate oxidation rate in the LGI-F trial was significantly higher compared to the HGI-T trial at 15 min and 30 min into the postprandial period. From 30 min onwards carbohydrate oxidation rate decreased to pre HGI trial values and remained lower for the rest of the postprandial period.

Fat oxidation rate decreased rapidly during the first 30 min of the postprandial period in the LGI fruit (LGI-F) compared to the HGI toast (HGI-T) trial. There was a meal by time interaction effect ($P<0.05$). The HGI trial was significantly different from the LGI trial (Figure 5.6). The post-hoc test revealed significant difference at 30 min into the postprandial period, fat oxidation during the LGI fruit (HGI-F) trial being significantly lower compared with the HGI toast (HGI-T) trial.

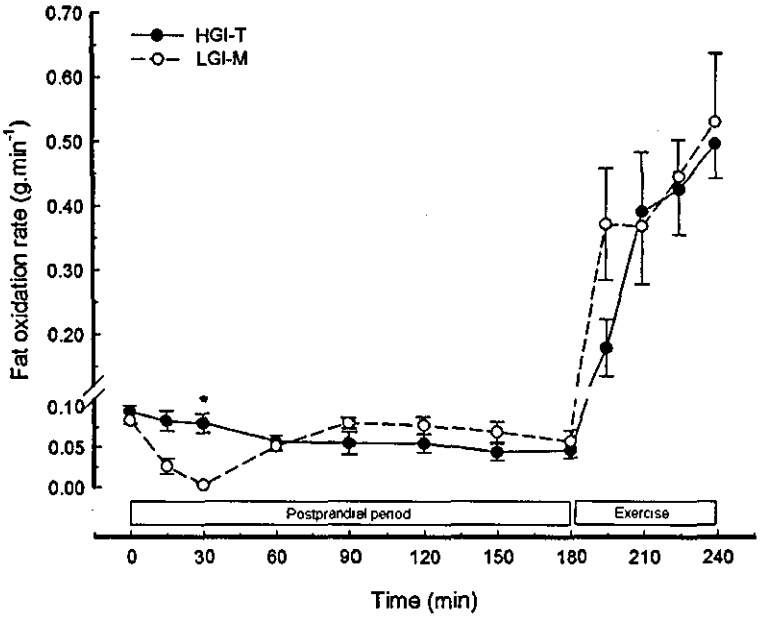


Figure 5.6 Fat oxidation rate (g.min-1) during the 180 min postprandial period and a 60 min exercise following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-F : ○) (mean ± SEM). Meal x time interaction effect ($P<0.05$)
* HGI significantly different from the LGI trial ($P<0.05$).

From 30 min onwards fat oxidation rate slowly increased just above that of the HGI-T trial. Although there was a transient change in CHO and fat oxidation rates during the postprandial period, there was no difference in the total CHO and fat oxidation between trials during the 180 min postprandial period (Table 5.6).

In order to meet the increased energy demand of exercise there was an up-regulation of CHO and fat oxidation. During the 60 min of exercise there was a decrease in carbohydrate oxidation ($P<0.05$) (Figure 5.5) and increase in fat oxidation rate with time ($P<0.05$) (Figure 5.6) in both trials. However there was no difference in substrate oxidation during exercise between the two trials (Table 5.5).

Table 5.5 Total CHO and fat oxidised in the 3 h postprandial period and during exercise (n=6; mean ± SEM)

	CHO (g)		Fat (g)	
	HGI-T	**LGI-F**	**HGI-T**	**LGI-F**
Postprandial Period	22.6 ± 2.3	24.0 ± 1.4	5.3 ± 0.8	5.2 ± 0.5
Exercise Period	139.1 ± 5.4	131.4 ± 6.7	19.1 ± 3.4	22.2 ± 4.1

Heart rate (HR) and Rating of perceived exertion (RPE)

During exercise heart rate and RPE increased with time in both trials ($P<0.05$). There was no difference in HR or RPE between the two trials.

Gut fullness and hunger

Following the ingestion of the HGI-T and LGI-F meals rating for gut fullness increased and then gradually decreased over time in both trials ($P<0.05$). Compared with the HGI trial, there was a trend for higher gut fullness ratings in the LGI trial, however this difference was not statistically significant (meal x time interaction $P=0.066$). There was no difference in the rating of gut fullness during the exercise period between the two trials (Table 5.6).

Similarly hunger ratings changed with time ($P<0.05$) decreased immediately after meal consumption and gradually increased during the 180 min postprandial period (Table 5.6). There was a trend for decreased feeling of hunger during the LGI trial however this was not statistically significant (meal x time interaction $P=0.062$). There was no difference in hunger rating in the exercise period between the two treatments.

Table 5.6. Gut fullness and hunger ratings during the postprandial period of the HGI-T and LGI-F trials (mean ± SEM).

		Postprandial Period (min)							Exercise Period (min)				
Variable	**Trial**	**Fast**	**15**	**30**	**60**	**90**	**120**	**150**	**180**	**195**	**210**	**225**	**240**
Gut fullness *	HGI-T	8 ± 0	10 ± 1	10 ± 0	10 ± 1	9 ± 1	9± 1	8 ± 0	9 ± 1	8± 0	8 ± 1	8 ± 1	8 ± 1
	LGI-F	8 ± 0	12 ± 1	11 ± 1	10 ± 1	10 ± 1	10 ± 0	9 ± 1	9 ± 0	9 ± 1	9 ± 1	8 ± 1	9 ± 1
Hunger *	HGI-T	13 ± 2	10 ± 1	9 ± 0	10 ± 1	10 ± 1	10 ± 1	11 ± 1	11 ± 2	10 ± 2	9 ± 2	9 ± 1	10 ± 1
	LGI-F	10 ± 1	8 ± 1	8 ± 0	9 ± 1	11 ± 1	11 ± 1	11 ± 1	12 ± 1	9 ± 1	11 ± 1	9 ± 1	9 ± 1

* Gut fullness ratings changed with time in both trials ($P<0.05$)

* Hunger ratings changed with time in both trials ($P<0.05$)

There was no difference in Gut fullness and Hunger rating between the two trials.

5.4 Discussion

The results from the previous study (Chapter 4) indicated that despite the lower glycaemic responses during the postprandial period following the LGI fruit breakfast (compared with the HGI toast breakfast) there were no differences in peak blood glucose concentrations.

The aim of the present study was to extend the observations made in the study described in Chapter 4 beyond the postprandial period and include measurement of metabolic responses to a 60 min sub-maximal run. The study also examined whether or not the increased fat oxidation following a LGI meal seen in athletes, ingesting 2g of CHO per kg body mass, was also evident in recreationally active people ingesting 1g of CHO per kg body mass.

The results of the present study indicate that the lower glycaemic responses during the postprandial period following the LGI fruit breakfast did result in significant changes in the rate of CHO and fat oxidation only during the 180 postprandial period. There were no statistically significant differences in the rate of substrate utilisation (Figure 5.5 and Figure 5.6) or in the total fat and CHO oxidised (Table 5.5) during the 60 min exercise . This is contrary to previous studies that have reported a higher fat oxidation rate during exercise 3 h following a consumption of LGI pre-exercise meal in men (Wu, *et al.*, 2003) and women (Stevenson, *et al.*, 2006).

Ingestion of breakfasts in both trials resulted in a rapid rise in blood glucose concentrations reaching peak values of 6.6 ± 0.6 $mmol.l^{-1}$ for the HGI-T trial and 5.9 ± 0.6 $mmol.l^{-1}$ in the LGI-F trial. (Figure 5.1). The high blood glucose peaks in the LGI-F trial was thought to be due to inclusion of tinned peaches in the breakfast. The canning process of tinned peaches would have aided the disruption of cell walls thus releasing free glucose.

Earlier studies from our laboratory reported similar values for plasma glucose and found significant difference in the peak plasma glucose for HGI (6.8 ± 0.5 $mmol.l^{-1}$) and LGI (6.2 ± 0.3 $mmol.l^{-1}$) (Wu, *et al.*, 2003) and HGI (6.89 ± 0.31 $mmol.l^{-1}$) and LGI (5.10 ± 0.4 $mmol.l^{-1}$) (Stevenson, *et al.*, 2005). One possible explanation might be the inclusion of a concentrated glucose syrup in the HGI breakfasts used in these previous studies (Lucozade Original). Another reason for the discrepancy may be the difference blood sampling sites i.e. capillary in the present studies and venous samples in previous studies (see Chapter 4).

Studies by Wu and colleagues used 2 $g.kg^{-1}$ BM of available CHO, LGI breakfasts that included apple and tinned peaches, as in the present study. Similar to our study, they found no significant difference in peak blood glucose or peak insulin values.

However they did report increased fat oxidation during exercise in the LGI compared with the HGI trial (Wu, *et al.*, 2003; Wu and Williams 2006).

In the present study, the blood glucose response to the LGI breakfast was not what is expected from a LGI food. A rapid increase to a peak followed by a fast decrease in blood glucose concentration observed in this series of studies is a characteristic response to HGI rather than LGI food.

The two breakfasts used in the present study were typical of those commonly consumed by the general public in the UK. The GI of the meal was calculated using the method described by Wolever et al (1986). The estimated GI ratio of the meals was 2:1 (72/46) and the actual measured ratio of the IAUC for blood glucose 4.4:1 It is surprising considering that in the previous study (see Chapter 4) the ratio for the IAUC of the same breakfasts was 2.7:1, still

different from 2:1. Glycaemic response and meal GI ratios matched well in our previous experiments using meals with an available CHO content of 2g.kg^{-1} BM (Stevenson, *et al.*, 2005; Wu, *et al.*, 2003; Wu, *et al.*, 2006). The exception is the study by Wee and colleagues (1999), where the ratio of 6.1:1 did not match the ratio for the meal GI and the discrepancy my be due to the composition of the LGI meal. Red lentils used in the study by Wee (1999) are classified as LGI CHO and they are broken down and absorbed slowly resulting in a slow glucose and low insulin release.

In the present study and that of Wee and colleagues (1999) the calculations of meal GI suggested by Wolever and Jenkins (1986) were not supported. One of the possibilities could be that the rapid absorption of hidden simple sugars in the LGI breakfast caused a steep rise in plasma glucose and insulin thus facilitating a faster glucose disappearance. This resulted in lower blood glucose levels thus reducing the size of the IAUC for glucose. Blood glucose responses elicited by fruit intake suggests a more rapid absorption, as blood glucose rises and falls more rapidly than after consumption for example of bread .

In the present study and contrary to expectations, carbohydrate oxidation in the LGI fruit (LGI-F) trial increased markedly in the first 90 min of the postprandial period. As peak blood glucose values were similar in the HGI toast (HGI-T) and LGI fruit (LGI-F) trial we can assume that insulin concentrations were similar as well. When carbohydrate is available as a fuel, FFA mobilisation is depressed and fat oxidation is reduced. Sidossis and colleagues (1996) found that the relative contribution of CHO and fat oxidation to energy is governed by glucose availability, rather than FFA inside the muscle cell (Sidossis, *et al.*, 1996). Hence slow glucose uptake will aid increased fat oxidation. Consumption of HGI carbohydrate foods results in

increased blood glucose concentration followed by an increased insulin demand (Foster-Powell, *et al.*, 1995; Holt, Miller and Petocz 1997). This increase also promotes the oxidation of CHO instead of fat (Febbraio, *et al.*, 2000b). Consumption of LGI foods promote fat oxidation at the expense of CHO oxidation aided by the lower blood glucose and plasma insulin levels.

During the first 90 min of the postprandial period there was a marked similarity between the RER curve and the blood lactate curve. In the LGI trial the highest RER values occurred at 30 min into the postprandial period (Figure 5.4), which coincided with the peak in blood lactate concentration (Figure 5.5). The raised RER values in the first 90 min of the postprandial period demonstrates that fructose is converted to lactate which is released in the systemic circulation. Blood lactate concentration peaked at 30 min, at 2.5 mmol.l-1 and was higher in the LGI- trial compared with the HGI ($P<0.05$). This phenomena is supported by the lack of change in oxygen consumption during the first 90 min of the postprandial period. Interestingly CHO oxidation and blood lactate concentration in the LGI trial was the highest at 30 min into the postprandial period. Therefore, the calculation of substrate oxidation for RER values may not accurately represents oxidation rates of CHO and fat because of the hyperventilation linked with the high lactate values.

The transition from rest to exercise demands an up regulation of CHO and fat metabolism in order to provide for increase energy demand. In some studies ingestion of a LGI meal (compared with ingestion of a HGI meal), was shown to aid increased fat oxidation during sub-maximal exercise (Stevenson, *et al.*, 2006; Wee, *et al.*, 1999; Wu, *et al.*, 2003; Wu, *et al.*, 2006).

According to Achten and colleagues (2002) maximal fat oxidation occurs between 55 ± 3% and 72 ± 4% of $\dot{V}O_2$max (Achten, *et al.*, 2002). However the absolute rates depends on CHO intake as previously demonstrated by several studies (Coyle, *et al.*, 1997; Febbraio, *et al.*, 1996; Horowitz, *et al.*, 1997).

In the present study the exercise intensity during the HGI toast (HGI-T) trial was 61.5 ± 1.4 % $\dot{V}O_2$max and 62.1 ± 1.4 % $\dot{V}O_2$max in the LGI fruit (LGI-F) trial. Fat oxidation rate increased ten folds from 0.05 ± 0.01 g.min^{-1} at the start of exercise (the end of the 180 min postprandial period) to 0.50 ± 0.05 g.min^{-1} at the end of exercise in the HGI-T trial. Interestingly the increase was similar in the LGI trial from 0.06 ± 0.01 g.min^{-1} at the end of the 180 min postprandial period to 0.53 ± 0.11 g.min^{-1} at the end of the 60 min run.

Previous studies from our laboratory reported significantly higher fat oxidation rates during a 60 min run (65% of $\dot{V}O_2$max) following a 3 h postprandial period following the consumption of a LGI meal compared with that of a HGI meal (Stevenson, *et al.*, 2006; Wu, *et al.*, 2003). The CHO content of the meals were 2 g.kg^{-1}BM, double the quantity of the CHO intake from the present study. Carbohydrate and fat oxidised during the 60 min exercise was about 75% of that from our previous study using a meal with an available CHO content of 2 g.kg^{-1} BM. However fat oxidation was higher in the present study following a smaller available CHO intake of 1g.kg^{-1} BM. It has been reported that when a LGI breakfast is consumed 3 h prior to exercise, less CHO is synthesized as muscle glycogen than when a HGI breakfast is consumed (Wee, *et al.*, 2005).

During the HGI trial, RER and blood lactate concentration changed less than in the LGI trial. Although there was a tendency for higher RER values in the

HGI compared with LGI trial at 15 min into the run, there was no difference in RER during exercise during the two trials (Figure 5.4).

Consistent with our previous studies, there was an increase in satiety and decreased feeling of hunger in the LGI compared to the HGI trials ($P<0.05$). However unlike our previous studies there were no differences reported between trials for gut fullness and hunger. Both HGI and LGI carbohydrate foods have an impact on gut fullness and satiety. The lack of difference observed in hunger could be explained by the similar peak blood glucose concentrations in the early part of the postprandial period, signalling available glucose to the brain and depressing the sensation of hunger.

In order to supply equal amount of CHO in the two breakfasts, the size of the LGI breakfast was 4 times larger than the HGI breakfast (663 ± 23 g and 167 ± 6 g respectively). It has been reported that mechanical dissention of the stomach can reduced the degree of hunger and increase a concomitant feeling of fullness, but the effect was only apparent during distension with volumes of 600 ml and 800 ml (appreciative 600 – 800g) of food. (Oesch, et al., 2006). In a previous study a $2g.kg^{-1}BM$ carbohydrate intake via a breakfast size of 582g for HGI and 898g for LGI food (for a 70kg man) resulted in a significant difference in gut fullness. Therefore the lack of difference in peak blood glucose observed in the present study might explain the lack of difference in hunger rating and the relatively small sizes of breakfasts (<600g) could explain the lack of difference in gut fullness rating between the two trials.

The results of this study show that the effects of a HGI and LGI breakfasts does not always have the expected outcome. Blood glucose responses in the HGI toast (HGI-T) and LGI fruit breakfast (LGI-F) followed the same

pattern as in the previous study (Chapter 4). The rapid rise and fall in blood glucose concentration seen in the LGI trial, more of a characteristic of HGI food, explains the increase in carbohydrate oxidation in the first hour of the postprandial period in the LGI trial and the lack of difference in fat and carbohydrate oxidation during the 60 min sub-maximal exercise between the two trials. These results may be the consequence of free glucose being released during the canning process of the tinned peaches. Therefore, even though the fruit breakfasts by definition was LGI, its metabolic response was one expected from a HGI food explaining the lack of difference in substrate oxidation during exercise between the two trials. Although the shapes of the blood glucose curves were different and there was a transient change in carbohydrate and fat oxidation rates during the postprandial period but no differences in total CHO and fat oxidation during the 180 min period. The CHO content of 1g.kg^{-1} in the present breakfast might have been too small to provide a serious metabolic challenge similar to the CHO content of 2 g.kg^{-1} in breakfasts used in previous studies. Also the HGI and LGI breakfasts less than 600g does not seem to lead to differences in gut fullness in male participants.

CHAPTER 6

The influence of high and low glycaemic index carbohydrate breakfast on blood glucose response and substrate metabolism during rest and exercise in recreationally active women

6.1 Introduction

The previous chapter presented and discussed the metabolic responses of recreationally active males to the HGI and LGI fruit breakfast. Surprisingly there is only a small amount of information available on females despite the fact that women are more likely to switch from a HGI to a LGI diet in order to loose weight.

Several studies reported differences in glucose metabolism between men and women. Basu and colleagues (2006) observed that following the ingestion of a mixed CHO meal young women had a greater postprandial glucose and insulin concentrations compared with young men (Basu, Dalla Man, Campioni, Basu, Klee, Toffolo, Cobelli and Rizza 2006). Horton (2006) and colleagues have shown sex differences in glucose kinetics during sub-maximal exercise (Horton, Grunwald, Lavely and Donahoo 2006) while others studies reported sex differences for the relative utilisation of carbohydrates and lipids as sources of fuel (Knechtle, Muller, Willmann, Kotteck, Eser and Knecht 2004; Tarnopolsky, *et al.*, 1995; Venables, Achten and Jeukendrup 2005). However there were also studies that did not find sex differences in substrate oxidation during exercise (Mittendorfer, *et al.*, 2002; Roepstorff, *et al.*, 2002). At present there is insufficient evidence to warrant extrapolation of research findings from males to females.

Therefore the aim of this study was to extend the assessment of the influences of HGI and LGI breakfast in active men described in the previous study (Chapter 5) to active female participants.

6.2 Methods

Participants

Six, healthy and recreationally active females (aged 27.0 ± 1.3 y, height: 1.68 ± 0.03 m; weight: 65.0 ± 4.1 kg; BMI: 23.0 ± 0.8 $kg.m^2$; $\dot{V}O_2$ max: 49.0 ± 2.3 $ml.kg^{-1}.min^{-1}$) took part in the study and completed both trials in a randomised cross-over design. At the time of the study, all participants were involved in recreational running three to four times per week. The women were pre-menopausal, four eumenorrheic (3 on contraceptive pill), and two amenorrheic. To control for the menstrual cycle eumenorrheic women took part in two trials within one week in the early follicular phase of their cycle (to avoid naturally elevated and fluctuating blood glucose levels in the lutheal phase of the cycle) None of the participants had a history of diabetes mellitus.

Test meals

Test meals were the same as for male participants in the previous chapter (Chapter 5), consisting of a HGI toast and jam and LGI fruit and yogurt breakfast (Table 3.2). Information on the test breakfasts and standard evening meal for this study are included in Table 6.1.

Main trial

All the procedures used in this study were identical to those described in the previous chapter (Chapter 5).

Statistical analysis

Similarly statistical analysis of the data followed the same method as described in Chapter 3 (General Methods).

Table 6.1 Energy and macronutrient intake for the test meals and pre trial evening meal (mean ± SEM) (n = 6)

Variable	Code	Quantity (g)	Energy (kcal)	CHO (g)	Fat (g)	Protein (g)	Meal GI	Meal GL
Test Meal	HGI-T	137 ± 9	325 ± 20	65.0 ± 4.1	2.7 ± 0.2	10.0 ± 0.6	72 ± 0	47 ± 3
	LGI-F	544 ± 34	336 ± 21	65.0 ± 4.1	3.4 ± 0.2	9.9 ± 0.6	36 ± 0	23 ± 1
Evening Meal	EM	425 ± 27	671 ± 42	97.4 ± 6.1	18.2 ± 1.1	28.9 ±1.8	35 ± 0	34 ± 2

6.3 Results

Blood Glucose

Fasting blood glucose values were similar in both trials: 4.5 ± 0.2 mmol.l^{-1} before HGI-T compared to 4.6 ± 0.2 mmol.l^{-1} before the LGI-F trial. Following the consumption of breakfast there was a rapid increase in blood glucose to a peak at 15 min of the postprandial period in both trials. Peak blood glucose concentration was 7.9 ± 0.6 mmol.l^{-1} for the HGI-T and 6.9 ± 0.5 mmol.l^{-1} in the LGI-F trial. Due to the large between-participant variation (especially in the HGI trial) this difference was not statistically significant at the 0.05 level (P=0.099). (Figure 6.1).

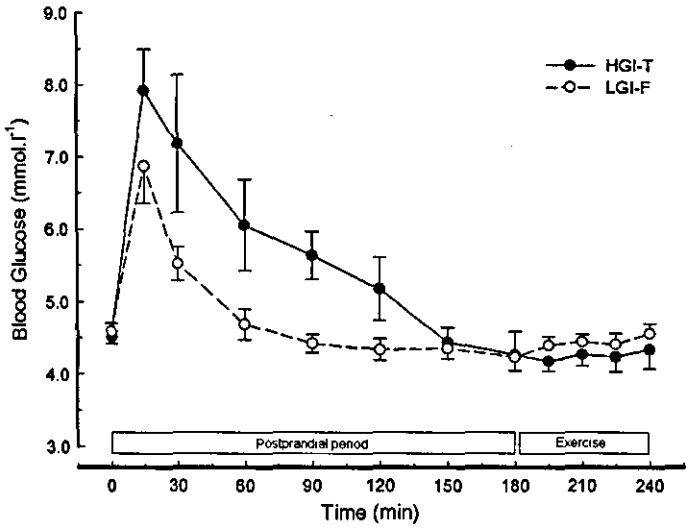


Figure 6.1 Blood glucose concentrations (mmol.l^{-1}) during the 180 min postprandial period following the ingestion of a HGI toast (HGI-T: •) and a LGI fruit (LGI-F: ○) breakfast (mean ± SEM). There was a meal effect (P<0.05), time effect (P<0.05) and a meal x time interaction effect (P<0.05).

From 15 min onwards, blood glucose concentrations rapidly decreased with time (P<0.05), falling to fasting values at 90 min of the postprandial period in the LGI fruit (LGI-F) trial compared with reaching fasting values at 150 min in the HGI toast (HGI-T) trial. Blood glucose concentration during the 180 min postprandial period was higher following the HGI compared with LGI breakfast (meal effect P<0.05) (Figure 6.1). During exercise the average blood

glucose concentration was 4.3 ± 0.2 mmol.l^{-1} in the HGI-T trial compared with 4.5 ± 0.1 mmol.l^{-1} in the LGI-F trial. There was no difference in blood glucose concentration between the two trials.

The glycaemic response following the HGI-T breakfast was 3.3 times larger compared with that following the LGI-F breakfast (217.8 ± 49.6 mmol.l^{-1} versus 66.0 ± 14.6 mmol.l^{-1} respectively)(P<0.05) (Figure 6.2).

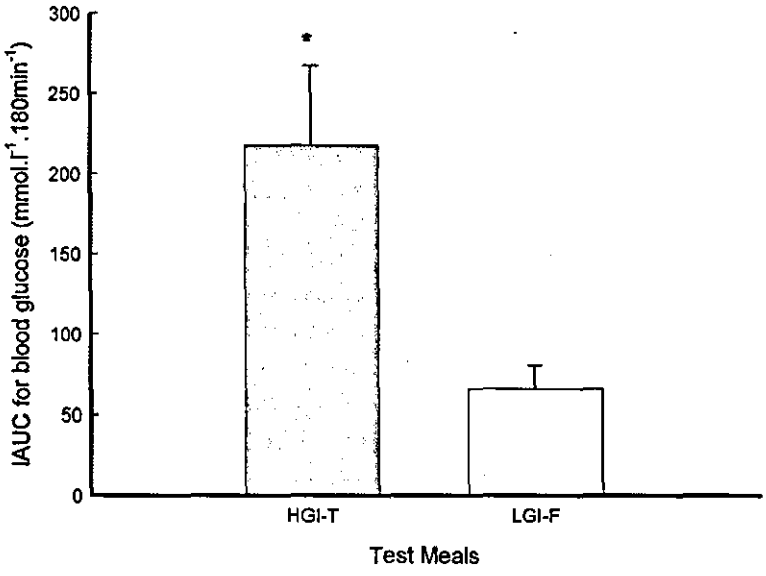


Figure 6.2. The incremental area under the blood glucose curve (mmol.l^{-1}.180min) following the ingestion of a high glycaemic (HGI-T) and low glycaemic index meal (LGI-F) for the 180 min postprandial period (mean ± SEM).
* HGI significantly different from the LGI trial (P<0.05).

Table 6.2 Incremental area under the blood glucose curve (mmol.l^{-1}.180 min).

		180 min		120min	
Breakfasts	Meal GI	IAUC (mmol.l^{-1}.min)	% lower than HGI-T	IAUC (mmol.l^{-1}.min)	% lower than HGI-T
HGI-T(toast)	72	218 ± 50*		202 ± 50*	
LGI-F (fruit)	36	66 ± 15	70	64 ± 15	68

* HGI significantly different from LGI (P<0.05)

The IAUC over the first 120 min resulted in a 3.2 fold difference in glycaemic response to the HGI-T compared to the LGI-F breakfast (Table 6.2).

Blood Lactate

Fasting blood lactate values were the same, 0.9 ± 0.1 mmol.l^{-1} in both trials. Postprandial blood lactate concentration during the LGI-F trial was higher compared with HGI-T trial (P<0.05). Following the consumption of the HGI-T breakfast blood lactate concentration increased and reached a peak value of 1.6 ± 0.3 mmol.l^{-1} at 60 min into the postprandial period . Following the consumption of the LGI-F breakfast the peak blood lactate concentration of 3.1 ± 0.2 mmol.l^{-1} occurred at 30 min into the postprandial period (Figure 6.3).

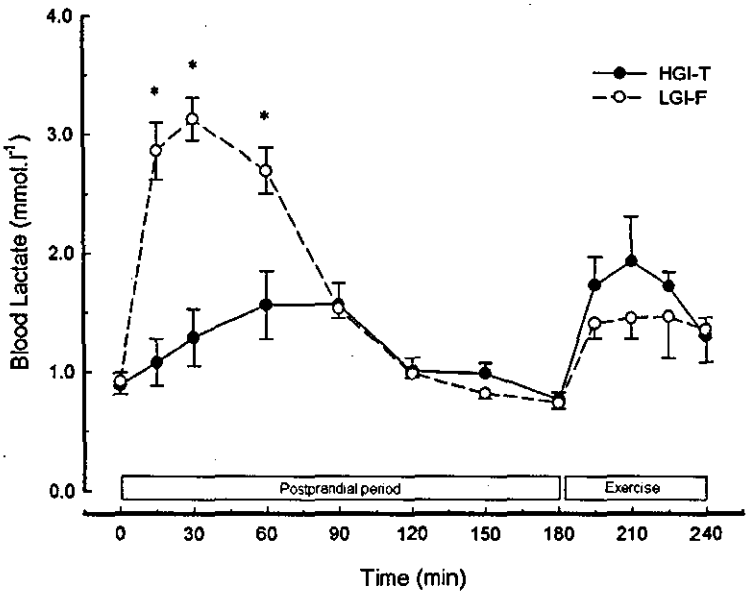


Figure 6.3 Blood lactate concentrations (mmol.l^{-1}) during the 180 min postprandial period and the 60 min exercise period following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-F : ○) (mean ± SEM)
* HGI significantly different from the LGI trial (P<0.05).

Blood lactate concentration changed with time in both trials. There was a meal, time and meal by time interaction (P<0.05). Furthermore the largest variation was found at 15, 30 and 60 min of the postprandial period (P<0.05). The concentrations of this metabolite returned to near fasting values by the

end of the 180 min postprandial period. There was no difference in this metabolite between trials during the 60 min exercise period.

Respiratory Exchange Ratio

Pre-prandial RER values were the same in both trials: 0.76 ± 0.02 for the HGI-T compared with 0.76 ± 0.03 for the LGI-F trial. In the postprandial period RER values were higher for the first 90min of the LGI-F trial compared with the HGI-T trial. For the remainder of the postprandial period RER values in the LGI trial fell and remained below the values for the HGI trial ($P<0.05$) (Figure 6.4). There was a significant meal, time and meal by time interaction effect ($P<0.05$) during the postprandial period with the largest variation occurring at 15 and 30 min.

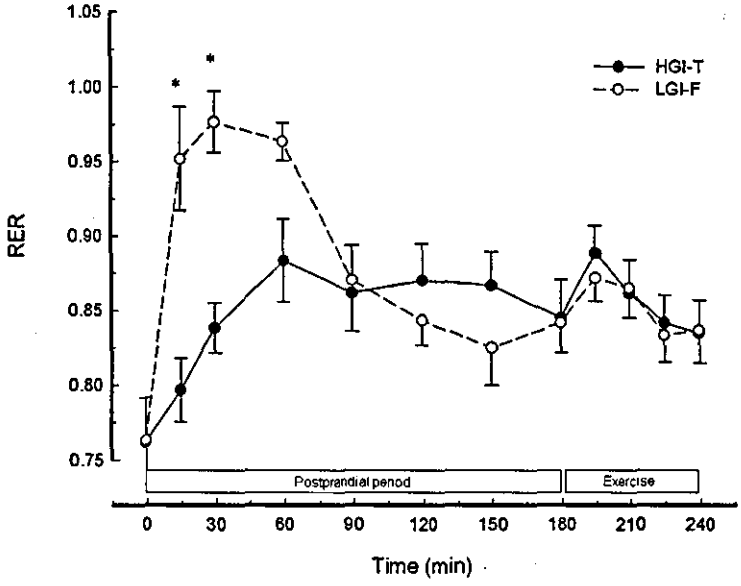


Figure 6.4. Respiratory exchange ratio (RER) during the 180 min postprandial period and the 60 min exercise period following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-F : ○) (mean ± SEM). Time and meal by time effect during the postprandial period ($P<0.05$). Time effect only during exercise ($P<0.05$).
* HGI significantly different from the LGI trial ($P<0.05$).

During exercise there was a steady decline in RER values ($P<0.05$) in both trials however there was no difference between the two trials. A summary of the RER values can be found in Table 6.3 and Table 6.4.

Estimated rate of carbohydrate and fat oxidation

Fasting resting oxygen uptake was 0.20 ± 0.01 $l.min^{-1}$ for the HGI-T and 0.22 ± 0.01 $l.min^{-1}$ for the LGI-F trial, there was no difference between trials (Table 6.3). Following the ingestion of breakfast there was a small but significant change in oxygen uptake with time in both trials ($P<0.05$). The meal by time interaction was significant during the postprandial as well as the exercise period ($P<0.05$). A summary of oxygen uptake and carbon dioxide values can be found in Table 6.3 and Table 6.4.

Table 6.3 Oxygen Uptake ($\dot{V}O_2$), Expired Carbon Dioxide ($\dot{V}CO_2$) and respiratory exchange ratio (RER) during the high (HGI-T) and low glycaemic index (LGI-F) trials.

			Postprandial period (min)						
Variable	**Meal**	**Fast**	**15**	**30**	**60**	**90**	**120**	**150**	**180**
$\dot{V}O_2$ ($l.min^{-1}$)	HGI-T	0.20 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.23 ± 0.02	0.24 ± 0.02
	LGI-F	0.22 ± 0.01	0.28 ± 0.01	0.29± 0.01	0.27 ± 0.01	0.26 ± 0.02	0.24 ± 0.01	0.24 ± 0.02	0.25 ± 0.02
$\dot{V}CO_2$ ($l.min^{-1}$)	HGI-T	0.15 ± 0.01	0.19 ± 0.00	0.21 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.20 ± 0.02	0.21 ± 0.02
	LGI-F	0.16 ± 0.01	0.26 ± 0.01	0.28± 0.01	0.26 ± 0.01	0.23 ± 0.02	0.21 ± 0.01	0.20± 0.02	0.21 ± 0.02
RER	HGI-T	0.76 ± 0.02	0.80 ± 0.02#	0.84 ± 0.02#	0.88 ± 0.03	0.86 ± 0.03	0.87 ± 0.02	0.87 ± 0.02	0.85 ± 0.03
	LGI-F	0.76 ± 0.03	0.95 ± 0.03	0.98± 0.02	0.96 ± 0.01	0.87 ± 0.03	0.84 ± 0.02	0.83 ± 0.02	0.84 ± 0.02

* $\dot{V}O_2$, $\dot{V}CO_2$ and RER values changed with time ($P<0.05$) and all showed a meal by time interaction effect ($P<0.05$)

HGI-T significantly different from LGI-F ($P<0.05$)

Table 6.4 Oxygen Uptake ($\dot{V}O_2$), Expired Carbon Dioxide ($\dot{V}CO_2$), respiratory exchange ratio (RER), heart rate and rating of perceived exertion (RPE) during exercise in the high (HGI-T) and low glycaemic index (LGI-F) trials.

		Exercise period (min)			
Variables	**Trial**	**15**	**30**	**45**	**60**
$\dot{V}O_2$ ($l.min^{-1}$)*	HGI-T	1.97 ± 0.12	2.02 ± 0.08	1.97 ± 0.10	2.00 ± 0.10
	LGI-F	2.00 ± 0.08	1.98 ± 0.08	2.04 ± 0.09	2.01 ± 0.08
$\dot{V}CO_2$ ($l.min^{-1}$)*	HGI-T	1.75 ± 0.12	1.74 ± 0.09	1.66 ± 0.11	1.68 ± 0.11
	LGI-F	1.74 ± 0.07	1.71 ± 0.07	1.70 ± 0.08	1.68 ± 0.06
RER*	HGI-T	0.89 ± 0.02	0.86 ± 0.02	0.84 ± 0.02	0.84 ± 0.02
	LGI-F	0.87 ± 0.02	0.87 ± 0.02	0.83 ± 0.02	0.84 ± 0.02
Heart Rate*	HGI-T	145 ± 5	150 ± 5	153 ± 6	154 ± 6
	LGI-F	148 ± 5	153 ± 7	154 ± 8	155 ± 8
RPE	HGI-T	10 ± 1	10 ± 1	10 ± 1	10 ± 1
	LGI-F	10 ± 1	10 ± 1	11 ± 1	11 ± 1

* $\dot{V}O_2$, $\dot{V}CO_2$, RER and heart rate changed with time ($P<0.05$)

There was no difference in the rates of carbohydrate or fat oxidation in the fasted state. However, following both breakfasts carbohydrate oxidation rate changed significantly with time during the 180 min postprandial period in the LGI-F and HGI-T trial ($P<0.05$) (Figure 6.5 and Figure 6.6).

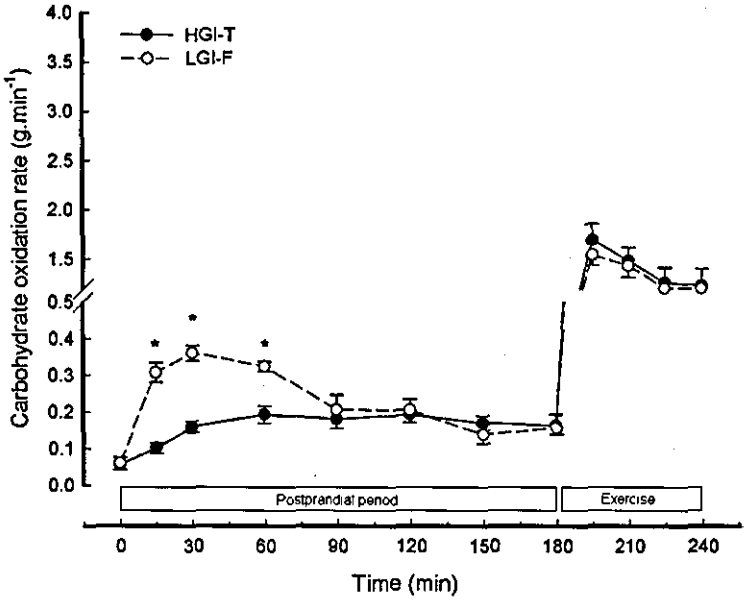


Figure 6.5 Carbohydrate oxidation rate ($g.min^{-1}$) during the 180 min postprandial period and a 60 min exercise following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-F : ○) (mean ± SEM) Meal, time and meal by time interaction effect during the postprandial period ($P<0.05$). Time effect only during exercise ($P<0.05$). * HGI significantly different from the LGI trial ($P<0.05$).

There was a meal by time interaction for CHO oxidation rates during the postprandial period ($P<0.05$), HGI-T > LGI-F. The post-hoc test showed the largest variations at 15, 30 and 60 min of the postprandial period ($P<0.05$). There was 28% more CHO oxidized during the postprandial period during the LGI-F trial (20.6 ± 1.7 g) compared with the HGI-T trial (14.9 ± 1.5 g) ($P<0.05$) (Table 6.6).

Fat oxidation showed a meal by time effect ($P<0.05$) with the highest variation occurring at 15, 30 and 60 min of the postprandial period. LGI-F trial was significantly different from HGI-T trial ($P<0.05$).

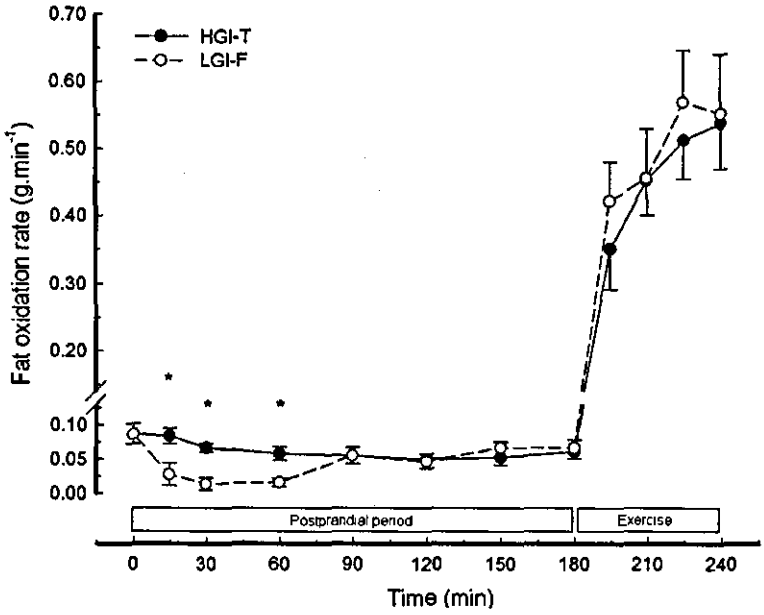


Figure 6.6 Fat oxidation rate ($g.min^{-1}$) following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-F : ○) (mean ± SEM) during the 180 min postprandial period and the 60 min exercise period. * HGI significantly different from the LGI trial ($P<0.05$).

Fat oxidation in the postprandial period was 26% less in the LGI-F trial (3.9 ± 0.6 g) compared with the HGI-T trial (5.3 ± 0.6 g in) ($P=0.054$).

Table 6.5 Total CHO and fat oxidised in the 3 h postprandial period and during exercise (n=6; mean ± SEM)

	CHO (g)		Fat (g)	
	HGI-T	**LGI-F**	**HGI-T**	**LGI-F**
Postprandial	14.9 ± 1.5*	20.6 ± 1.7	5.3 ± 0.7	3.9 ± 0.6
Exercise	77.1 ± 8.1	73.0 ± 5.7	24.2 ± 3.0	26.3 ± 3.7

* HGI-T different from LGI-F
There was a strong trend towards higher fat oxidation during exercise in the LGI-F trial ($P=0.054$)

During exercise the rate of CHO oxidation decreased and the rate of fat oxidation increased with time ($P<0.05$), however there was no difference in substrate oxidation rates between treatments (Figure 6.3 and Figure 6.4). The estimated total CHO oxidised was 5% lower in the LGI-F trial (73.0 ± 5.7g) compared with the HGI-T trial (77.1 ± 8.1g). This small difference was not significant at the $P=0.05$ level.

During exercise there was a strong trend for higher fat oxidation (P=0.054) (Table 6.5). Total fat oxidised was 8%higher in the LGI-F trial (26.3 ± 3.7g) compared with the HGI-T trial (24.2 ± 3.0g).

Heart Rate (HR) and Rating of Perceived Exertion (RPE)

During exercise there was an increase in heart rate with time in both trials ($P<0.05$). There was no differences in heart rates between trials. There was no difference in RPE between the two trials (Table 6.4).

Gut fullness and hunger

Following the ingestion of the HGI-T and LGI-F meals, ratings for gut fullness peaked at 15 min into the postprandial period and then gradually decreased over time. There was a meal, time and meal by time interaction effect ($P<0.05$). Participants reported higher ratings of gut fullness following the LGI-F compared with the HGI-T meal. There was a strong trend towards an increased sensation of gut fullness in the LGI trial compared with HGI at 60min ($P=0.056$) and 90 min ($P=0.052$)of the postprandial period). During exercise there was only a significant meal effect ($P<0.05$), ratings of gut fullness were higher in the LGI-F compared with HGI-T trial.

Participant's ratings of hunger changed with time after the consumption of both meals. There was a time effect and meal effect during the postprandial

($P<0.05$) and exercise period. ($P<0.05$). Participants felt less hungry in the LGI-F compared with the HGI-T trial ($P<0.05$).

Table 6.6 Gut fullness and hunger ratings during the postprandial period of the HGI-T and LGI-F trials (mean ± SEM).

		Postprandial Period (min)							Exercise Period (min)				
Variable	**Trial**	**Fast**	**15**	**30**	**60**	**90**	**120**	**150**	**180**	**195**	**210**	**225**	**240**
Gut fullness *	HGI-T	9 ± 1	12 ± 1	11 ± 1	10 ± 1	9 ± 1	10± 1	9 ± 1	8 ± 1	8± 1	9 ± 1	8 ± 1	9 ± 1
	LGI-F	8 ± 1	16 ± 1	15 ± 1	14 ± 1	12 ± 1	11 ± 1	11 ± 1	10 ± 2	10 ± 1	10 ± 1	10 ± 1	10 ± 1
Hunger *	HGI-T	14 ± 1	9 ± 1	9 ± 1	10 ± 1	10 ± 1	11 ± 1	11 ± 1	13 ± 2	13 ± 2	13 ± 2	11 ± 2	12 ± 2
	LGI-F	11 ± 1	7 ± 0	7 ± 0	7 ± 1	8 ± 1	9 ± 1	10 ± 1	12 ± 2	11 ± 2	12 ± 2	11 ± 2	11 ± 2

* Gut fullness: meal effect during the postprandial and exercise period ($P<0.05$); Meal by time interaction effect in the postprandial period ($P<0.05$). Variation was the largest but not significant at 60 min ($P=0.056$) and 90 min ($P=(0.052)$ into the postprandial period.

* Hunger ratings: meal and time effect during the postprandial period only ($P<0.05$).

6.4 Discussion

Chapter 5 presented and discussed the metabolic responses of recreationally active males to the HGI toast (HGI-T) and LGI fruit (LGI-F) breakfast. Although differences were observed in the rate of CHO and fat oxidation during the postprandial period between the two trials, there were no differences during exercise.

The aim of this chapter was to examine whether women have a similar metabolic response to the HGI toast (HGI-T) and LGI fruit (LGI-F) breakfast as men.

The present study was conducted on female members of the general population. Four of the female participants were eumenorrheic and two amenorrheic. Three out of the four eumenorrheic women used oral contraceptives. Several oral contraceptive intervention trials have investigated the effects of GI on health and disease risk(Liu, Willett, Stampfer, Hu, Franz, Sampson, Hennekens and Manson 2000; Schulze, *et al.,* 2004; Sloth, Krog-Mikkelsen, Flint, Tetens, Bjorck, Vinoy, Elmstahl, Astrup, Lang and Raben 2004) and its effect in altering glucose metabolism and insulin sensitivity at rest and during exercise. The results are inconclusive hence present study did not control the use of contraceptive pill.

The results from the present study indicate that, similar to men, there were no differences in substrate utilisation between the LGI fruit and HGI toast trials during the 60 min of exercise in women.

Following ingestion of the test meal the peak blood glucose value of 7.9 ± 0.6 $mmol.l^{-1}$ from the HGI-T trial was similar to the peak plasma glucose value of 7.5 ± 0.7 $mmol.l^{-1}$ from a previous study using female participants (Stevenson,

et al., 2006). However the peak of 6.9 ± 0.5 mmol.l^{-1} from the LGI fruit trial in the present study is higher than that 5.9 ± 0.2 mmol.l^{-1} from previous study on women (Stevenson, *et al.*, 2006). The difference might be attributed to the difference in blood sampling methods (see Chapter 4). There was no statistically significant difference in the peak blood glucose values between trials for female participants as was the case for male participants (Chapter 5).

In the postprandial period there was a rapid decrease in blood glucose concentration from 15 min onward in the LGI-F trial reaching near fasting values at 60 min. In contrast the fall in blood glucose concentration was slower in the HGI-T trial reaching near fasting values only at 150 min into the postprandial period. The rapid decrease to near fasting levels occurred earlier in the study of Stevenson and colleagues (2006) than in the present study (i.e. at 30 min in the LGI and 60min HGI trial) (Stevenson, *et al.*, 2006). One of the possible explanation could be that the breakfast containing 2g.kg^{-1}BM CHO induced a larger insulin response compared with ingestion of 1g.kg^{-1}BM CHO the present study, thus aiding an increased removal of blood glucose from the systemic circulation (insulin was not measured in this study).

Following the consumption of the LGI-F breakfast the peak blood lactate concentration of 3.1 ± 0.2 mmol.l^{-1} occurred at 30 min into the postprandial period and was 2.8 times higher than that of the HGI-T trial (P<0.05) (Figure 6.3). The differences in postprandial blood lactate was similar to the ones observed in the previous study.

During the first 90 min of the postprandial period there was a marked similarity between the RER curve and the blood lactate curve in the LGI-F (Figure 6.8) and HGI-T trial (Figure 6.9). The relationship between excess lactate production and RER was discussed in Chapter 5.

Several studies have reported higher rates of fat oxidation during exercise following the consumption of a LGI meal in women (Stevenson, *et al.*, 2006; Thomas, *et al.*, 1991; Wee, *et al.*, 1999; Wu, *et al.*, 2003). The relative exercise intensity was 63.8 ± 1.1 % of $\dot{V}O_2$ max in the HGI-T and 64.6 ± 0.9 % $\dot{V}O_2$ max in the LGI-F trial. These intensities were chosen to elicit maximal fat oxidation.

In the present study total carbohydrate oxidation during exercise was 77.1 ± 8.1 g in the HGI-T and 73.0 ± 5.7 g in the LGI-F trial. In a study by Stevenson and colleagues (2006) consumption of a breakfast with an available CHO content of 2 $g.kg^{-1}$ BM elicited a higher total CHO oxidation of 101.5 ± 12.0 g in the HGI trial (Stevenson, *et al.*, 2006) compared with 77.1 ± 8.1 g in the present study. The difference was due to a higher CHO intake and a the different composition of the meal. Added glucose syrup (Lucozade Original) provided readily available CHO. Interestingly in the LGI trials the total CHO oxidation were similar following the breakfasts containing 1 g or 2 g of available CHO per kg BM (73.0 ± 5.7 g in the present study compared with 70.5 ± 10.2 g) (Stevenson, *et al.*, 2006). It is interesting that despite of a 50 % increase in CHO intake total fat oxidation was similar in the LGI trial of the two studies.

During the 180 min postprandial period in the present study total fat oxidation was similar in the two trials (24.2 ± 3.0 g for the HGI-T trial and 26.3 ± 3.7 g for the LGI-F trial). Interestingly the total fat oxidised (24.2 ± 3.0 g) in the HGI-T trial of the present study was 2.9 times higher than the total amount of fat oxidised (8.3 ± 2.2 g) in the HGI trial of Stevenson and colleagues (Stevenson, *et al.*, 2006). This finding may point to the preferential use of readily available glucose in the form of Lucozade original, which in turn would have increased plasma insulin concentration and depressed fat

mobilization and oxidation (Stevenson, *et al.*, 2006). Total fat oxidation (26.3 ± 3.7 g) for the LGI-F trial in the present study was 1.4 times higher compared with the study of Stevenson and colleagues 2006 (Stevenson, *et al.*, 2006) and it can be explained by the reduced CHO content of the test breakfast (1g.kg^{-1}BM versus 2g.kg^{-1}.BM). In the present study total energy expenditure during the 180 min postprandial period was 107 ± 5 kcal in the HGI-T compared with 118 ± 7 kcal in the LGI-F trial. Total energy expenditure during the 60 min run was 527 ± 29 kcal in the HGI-T compared with 529 ± 21 kcal in the LGI-F trial and were not different.

There was an increase in satiety and decreased feeling of hunger in the LGI compared to the HGI trials (P<0.05). Unlike the male data from the previous study, the female data demonstrated a meal, time and a meal by time interaction effect in the rate of gut fullness during the postprandial period. In a previous study using female participants and a breakfast with an available CHO content of 2g.kg^{-1} BM, reported differences during the postprandial period but no differences in gut fullness rating during exercise (Stevenson, *et al.*, 2006).

Hunger ratings in females had shown a meal and time effect during the postprandial period but only a meal effect during exercise (P<0.05), LGI < HGI (Table 6.6).

Summary

The data from the present study indicate that despite the lower glycaemic responses during the postprandial period following the LGI breakfast, no differences in substrate oxidation were observed during the subsequent 60 min of exercise in female participants. These findings are similar to the results from the previous study using recreationally active men (Chapter 5).

CHAPTER 7

The influence of high and low glycaemic index carbohydrate breakfast on blood glucose and plasma insulin as well as substrate oxidation during rest and exercise in recreationally active women

7.1 Introduction

Results from the previous two studies (Chapter 5 and Chapter 6) illustrated that consumption of LGI as well as HGI breakfasts can result in high peak blood glucose values. Considering the high postprandial peak blood glucose values the assumption was made that plasma insulin concentration would have been elevated accordingly which in turn resulted in a faster glucose uptake by tissues, hence a reduction in circulating blood glucose concentration.

Therefore the aim of this study was to examine the glycaemic and insulinaemic responses to a HGI and a LGI pre-exercise meals in active women during the postprandial period and subsequent 60 min of sub-maximal exercise . In this study we choose to assess the metabolic responses to toast (HGI-T) and a muesli breakfast (LGI-M) previously used in Study 1 (Chapter 4).

7.2 Methods

Participants

Nine, healthy recreationally active females took part in the study and completed both trials (age: 30.4 ± 3.2 y, height: 1.69 ± 0.0 m, weight: 55.0 ± 0.9 kg, BMI: 19.4 ± 0.5 $kg.m^2$, $\dot{V}O_2$ max: 48.4 ± 1.5 $ml.kg^{-1}.min^{-1}$. At the time of the study, all participants were involved in recreational running three to four times per week. None of the participants had a history of diabetes

mellitus or any other endocrine disorders. All female participants were pre-menopausal and five took oral contraceptives (for more than six month). Both trials were completed when the women were in the follicular phase of their menstrual cycle.

Test meals

In Study 1 demonstrated the different glycaemic responses to four carbohydrate breakfasts. There was no statistically significant difference between the glycaemic response to the HGI and LGI cereal breakfasts (cornflakes and muesli). For study 2 and study 3 we have chosen breakfasts with the largest difference in glycaemic response
(as measured by the IAUC) (Chapter 4 (Figure 4.3).
The two breakfasts used in this study were HGI toast (HGI-T) and LGI muesli (LGI-M) previously used as test meals in study 1 (Chapter 4). The two test meals were chosen because they completed the comparison of the original test breakfasts (Chapter 4). The HGI meal was based on lightly toasted white bread and jam (calculated GI **72**, calculated GL **51**) and the LGI breakfasts was based on muesli, apple and milk (calculated GI **48**, calculated GL **34**) (see Method section, Chapter 3, Table 3.2). Test meals contained 1g.kg^{-1} BM available CHO and were prepared on site, just before the trial and were randomly assigned to the participants. The mean energy and macronutrient contents of the breakfasts consumed by participants of this study are contained in Table 7.1.

Table 7.1 Energy and macronutrient intake for the test meals and pre trial evening meal (mean ± SEM) (n = 9)

Variable	Code	Quantity (g)	Energy (kcal)	CHO (g)	Fat (g)	Protein (g)	Meal GI	Meal GL
Test Meal	HGI-T	116 ± 2	275 ± 5	55.0 ± 1.0	2.3 ± 0.0	8.5 ± 0.2	72 ± 0	40 ± 1
	LGI-M	324 ± 6	299 ± 5	55.0 ± 1.0	3.0 ± 0.1	12.0 ± 0.2	48 ± 0	26 ± 0
Evening Meal	EM	425 ± 27	671 ± 42	97.4 ± 6.1	18.2 ± 1.1	28.9 ±1.8	35 ± 0	34 ± 2

Pre-test food intake

Participants were asked to carefully record their food intake for the day before the first trial and repeat the same food intake the day before the second trial. Food records were analysed for energy macro and micro- nutrient intake (CompEat 4.0, Nutrition Systems, UK). Due to the lifestyle/family commitment of participants it was not possible to issue a prescribed evening meal as in the previous two studies, however all participants repeated the same food intake in both trials.

Main trial and statistical analysis

The experimental protocol, preliminary tests and statistical analysis were the same as described in the General Methods section (see Chapter 3). As an extension of our previous studies we measured plasma insulin. In addition we have also compared two types of rating scales used in the measurement of perceived gut fullness (Appendix A5) and hunger (Appendix A6). The two scales were the adapted Borg scale (Borg 1973) and a visual analogue scale (VAS) (Appendix A7).

7.3 Results

Blood glucose

Peak blood glucose concentration occurred at 15 min into the postprandial period in both trials and were 8.1 ± 0.2 mmol.l^{-1} (HGI-T) and 7.8 ± 0.3 mmol.l^{-1} (LGI-M) (Figure 7.1). From 15 min onwards there was a rapid decrease in blood glucose concentrations in the LGI-M trial reaching fasting values at 120 min compared with 180 min in the HGI-T trial of the postprandial period. There was a significant meal by time interaction ($P<0.05$) (Figure 7.1). The post hoc tests did not show statistically significant differences at any particular time point in the trials.

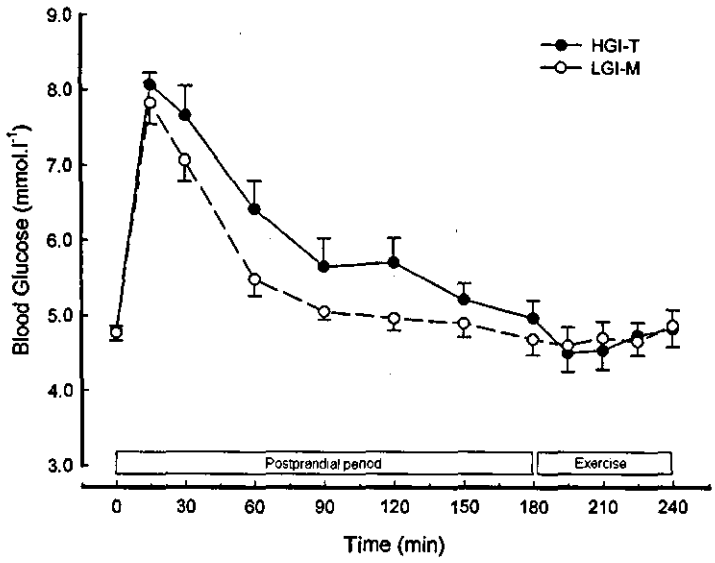


Figure 7.1. Blood glucose concentrations (mmol.l^{-1}) during the 180 min postprandial period and the 60 min exercise period following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-M : ○) (mean ± SEM). There was a meal by time interaction effect HGI > LGI (P<0.05).

Blood glucose concentrations remained between 4.5 and 4.9mmol.l^{-1} during the 60 min exercise in both trials. There was no difference in blood glucose concentration between trials during the 60 min exercise.

Table 7.2 Incremental area under the blood glucose curve .

Breakfasts	Meal GI	IAUC (mmol.l^{-1}.180 min)	% lower than HGI-T	IAUC (mmol.l^{-1}.120 min)	% lower than HGI-T
HGI-T (toast)	72	235 ± 35*		204 ± 28*	
LGI-M (muesli)	48	144 ± 19	43	144 ± 19	29

* HGI significantly different from LGI (P<0.05)

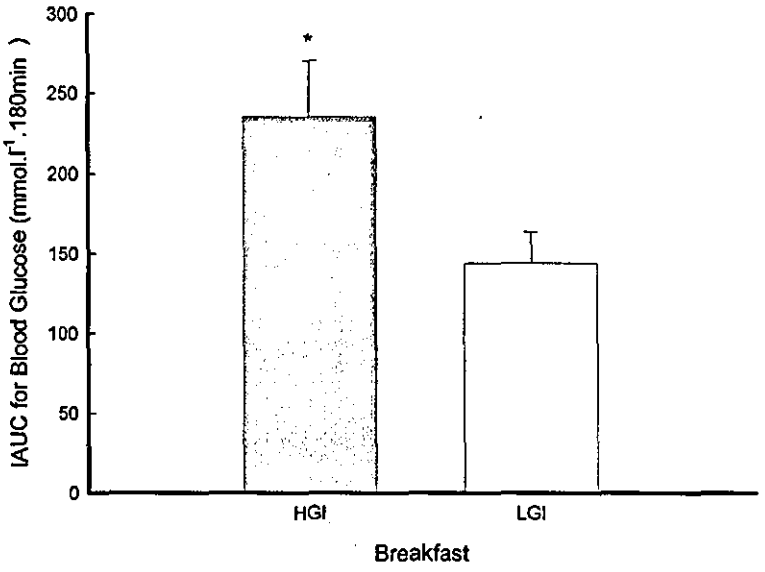


Figure 7.2. Incremental area under the blood glucose curve (mmol.l^{-1}.180min) for the 180 min postprandial period following the ingestion of a high glycaemic (HGI-T) and low glycaemic index meal (LGI-M) (mean ± SEM).
* HGI significantly different from the LGI trial (P<0.05).

The Incremental Area Under the Curve (IAUC) for glucose was larger in the HGI-T (235 ± 35 mmol.l^{-1}.180min) compared with the LGI-M (144 ± 19 mmol.l^{-1}.180min) trial (P<0.05) (Figure 7.2) (Table 7.2).

Plasma insulin

Plasma insulin concentration peaked at 15 min into the postprandial period in both trials. The peak insulin concentration was 35.1 ± 3.6 mU.l^{-1} in the HGI-T trial and 45.2 ± 3.5 mU.l^{-1} LG-M trial (Figure 7.3). This difference was not statistically significant. From 15 min onwards there was a more rapid decline in plasma insulin concentrations in the LGI-M trial compared with the HGI-T trial. There was also a significant meal by time interaction (P<0.05). The largest variation in plasma insulin concentration was found 90 min into the postprandial period (P<0.05) (Figure 7.3). Plasma insulin concentrations fell

below fasting values during exercise and remained close to ~2.1 $mU.l^{-1}$ in both trials.

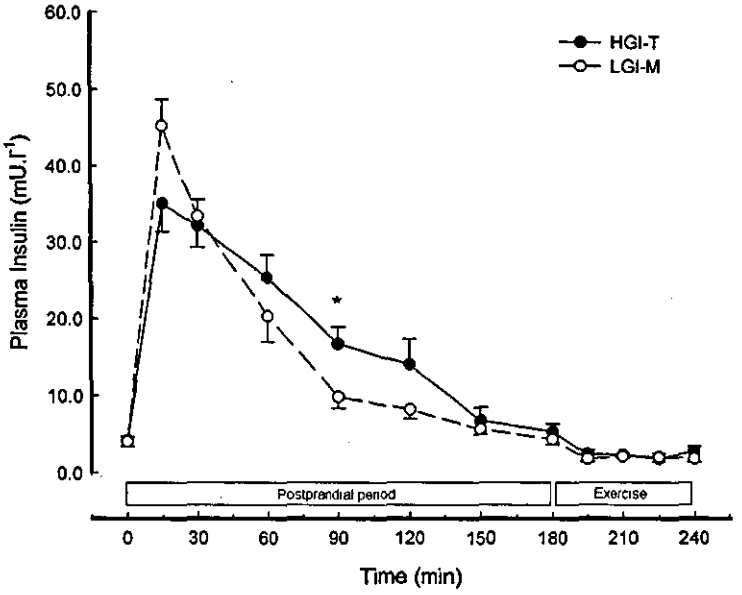


Figure 7.3. Plasma insulin concentrations ($mU.l^{-1}$) during the 180 min postprandial period and the 60 min exercise period following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-M : ○) (mean ± SEM). There was a meal by time interaction effect in the postprandial period.
* HGI different from LGI trial ($P<0.05$).

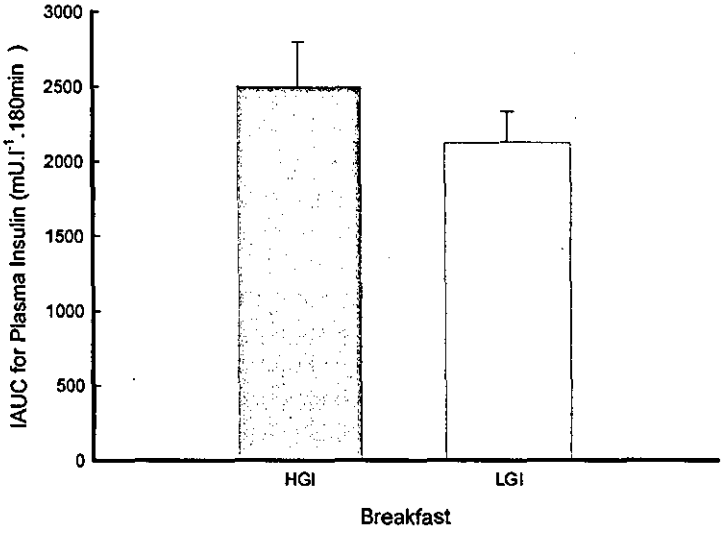


Figure 7.4. Incremental area under the plasma insulin curve ($mU.l^{-1}.180min$) for the 180 min postprandial period following the ingestion of a high glycaemic (HGI-T) and low glycaemic index meal (LGI-M) (mean ± SEM).

The Incremental Area Under the Curve (IAUC) for insulin was 2498 ± 299 $mU.l^{-1}$.180 min in the HGI-T trial, compared with 2122 ± 204 $mU.l^{-1}$.180 min in the LGI-M trial. The difference was not statistically significant (Figure 7.4) (Table 7.3).

Table 7.3 Incremental Area Under the Blood Glucose and Plasma Insulin curve for the 180 min postprandial period.

	IAUC for Blood Glucose ($mmol.l^{-1}$)	IAUC for Plasma Insulin ($mU.l^{-1}$)
HGI-T	235 ± 35 *	2498 ± 299
LGI-M	144 ± 19	2122 ± 204

* HGI-T significantly different from LGI-M ($P<0.05$)

Blood lactate

During the postprandial period blood lactate concentration increased more rapidly and to a higher value in the LGI-M compared with the HGI-T trial. Blood lactate concentration was the highest at 60min into the postprandial period during the HGI-T and 30 min during the LGI-M trial (1.36 ± 0.08 $mmol.l^{-1}$ and 1.96 ± 0.14 $mmol.l^{-1}$ respectively). There was a meal by time interaction ($P<0.05$) (Figure 7.5), the largest variation occurring at 15min and 30 min of the postprandial period ($P<0.05$) (Figure 7.5). However blood lactate concentrations were similar from 90 min onwards in the two trials and returned to fasting values by the end of the 180 min postprandial period. There was no difference in blood lactate concentrations between trials during the 60 min exercise.

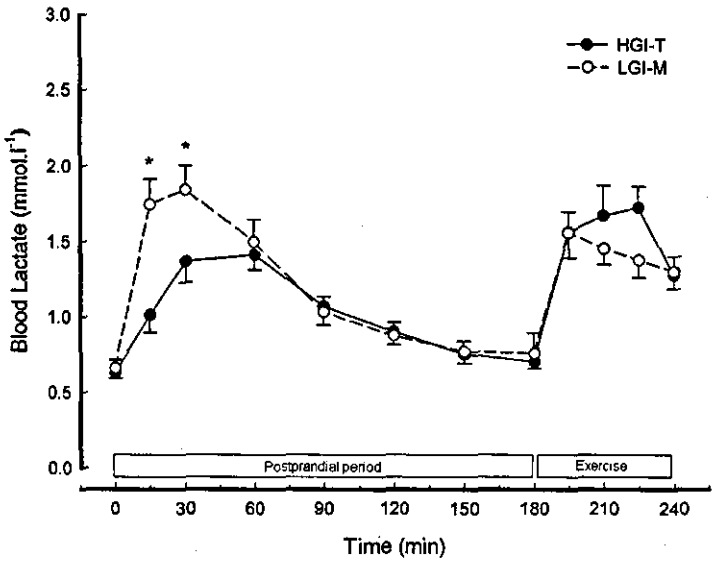


Figure 7.5 Blood lactate concentrations (mmol.l^{-1}) during the 180 min postprandial period and the 60 min exercise period following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-M: ○) (mean ± SEM). Meal, time and meal by time interaction during the postprandial period (P<0.05).
* HGI significantly different from the LGI trial (P<0.05).

Respiratory Exchange Ratio

Fasting RER value was 0.81 ± 0.01 in the HGI-T compared with 0.80 ± 0.01 in the LGI-M trial. Postprandial RER values changed with time (P<0.05) and there was a meal by time interaction effect (P<0.05) (Figure 7.5) (Table 7.4). The highest RER value was observed at 30 min into the postprandial period of the LGI and 120 min of the HGI trial. The post-hoc analysis showed that the largest variation occurred 15 min into the postprandial period (P<0.05). During exercise there was a steady decrease in RER values with time (P<0.05) but there was no difference between the two trials (Figure 7.5) (Table 7.4).

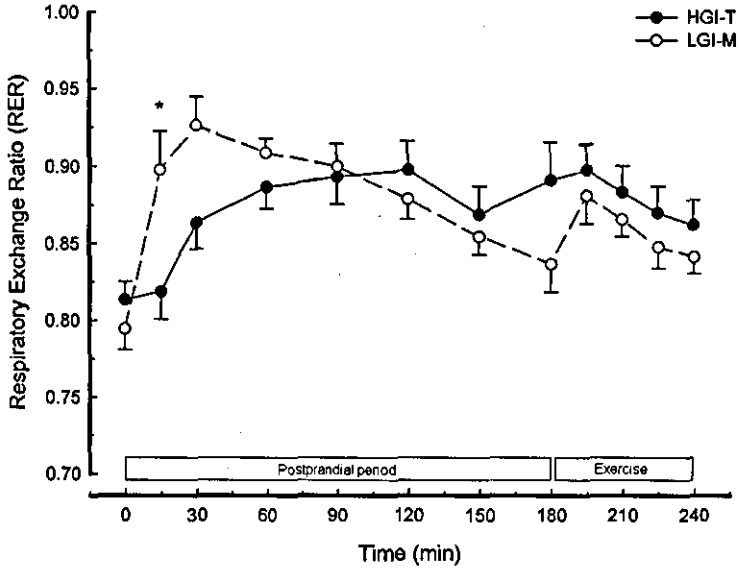


Figure 7.6. Respiratory exchange ratio (RER) during the 180 min postprandial period and the 60 min exercise period following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-M : ○) (mean ± SEM). Time and meal by time effect during the postprandial period ($P<0.05$). Time effect only during exercise ($P<0.05$).
* HGI significantly different from the LGI trial ($P<0.05$).

Estimated rate of carbohydrate and fat oxidation

There was a transient change with time in the rate of CHO and fat oxidation during the postprandial period ($P<0.05$). There was a significant, again transient, meal effect ($P<0.05$) and meal by time interaction effect for fat ($P<0.05$) and CHO oxidation rates ($P<0.05$). Fat oxidation rate was lower and CHO oxidation higher in the first hour of the postprandial period during the LGI-M trial compared to the HGI-T trial (Figure 7.7 and 7.8). A post-hoc analysis of the data showed small but significant difference in CHO oxidation rates at 15 min and 30 min into the postprandial period ($P<0.05$) (LGI>HGI).

Table 7.4 Oxygen Uptake ($\dot{V}O_2$), Expired Carbon Dioxide ($\dot{V}CO_2$) and respiratory exchange ratio (RER) at rest, during the high (HGI-T) and low glycaemic index (LGI-M) trials.

			Postprandial period (min)						
Variable	**Meal**	**Fast**	**15**	**30**	**60**	**90**	**120**	**150**	**180**
$\dot{V}O_2$ ($l.min^{-1}$)*	HGI-T	0.20 ± 0.00	0.23 ± 0.01	0.23 ± 0.01	0.24 ± 0.00	0.23 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
	LGI-M	0.21 ± 0.01	0.24 ± 0.01	0.24± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.01
$\dot{V}CO_2$ ($l.min^{-1}$)*	HGI-T	0.16 ± 0.00	0.23 ± 0.01	0.23 ± 0.01	0.24 ± 0.00	0.23 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
	LGI-M	0.17 ± 0.01	0.22 ± 0.01#	0.22± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.20± 0.01	0.19 ± 0.01
RER*	HGI-T	0.81 ± 0.01	0.82 ± 0.02	0.86 ± 0.02	0.89 ± 0.01	0.89 ± 0.02	0.90 ± 0.02	0.87 ± 0.02	0.89 ± 0.02
	LGI-M	0.80 ± 0.01	0.90 ± 0.02#	0.93± 0.02	0.91 ± 0.01	0.90 ± 0.01	0.88 ± 0.01	0.85 ± 0.01	0.84 ± 0.02

* $\dot{V}O_2$ Time effect ($P<0.05$); $\dot{V}CO_2$ Meal, time and meal by time interaction effect ($P<0.05$); RER Time and meal by time interaction effect ($P<0.05$).
# HGI-T significantly different from LGI-M ($P<0.05$)

Table 7.5 Oxygen Uptake ($\dot{V}O_2$), Expired Carbon Dioxide ($\dot{V}CO_2$), respiratory exchange ratio (RER), heart rate and rating of perceived exertion RPE) during exercise in the high (HGI-T) and low glycaemic index (LGI-M) trials.

		Exercise period (min)			
Variables	**Trial**	**15**	**30**	**45**	**60**
$\dot{V}O_2$ ($l.min^{-1}$)*	HGI-T	1.66 ± 0.08	1.64 ± 0.07	1.66 ± 0.07	1.68 ± 0.08
	LGI-M	1.64 ± 0.07	1.67 ± 0.08	1.66 ± 0.08	1.66 ± 0.08
$\dot{V}CO_2$ ($l.min^{-1}$)*	HGI-T	1.49 ± 0.07	1.45 ± 0.07	1.44 ± 0.07	1.45 ± 0.07
	LGI-M	1.45 ± 0.08	1.45 ± 0.08	1.41 ± 0.08	1.40 ± 0.07
RER*	HGI-T	0.90 ± 0.02	0.88 ± 0.02	0.87 ± 0.02	0.86 ± 0.02
	LGI-M	0.88 ± 0.02	0.87 ± 0.01	0.85 ± 0.01	0.84 ± 0.01
Heart Rate*	HGI-T	149 ± 5	151 ± 5	152 ± 5	153 ± 5
	LGI-M	150 ± 5	152 ± 5	155 ± 4	157 ± 4
RPE	HGI-T	10 ± 1	10 ± 1	10 ± 1	10 ± 1
	LGI-M	11 ± 1	11 ± 1	11 ± 1	11 ± 0

* $\dot{V}O_2$, $\dot{V}CO_2$, RER and heart rate changed with time ($P<0.05$)

Fat oxidation during exercise increased and CHO oxidation decreased with time in both trials ($P<0.05$). There was no difference in the rate of substrate oxidation during the 60 min of exercise (Figure 7.7 and Figure 7.8).

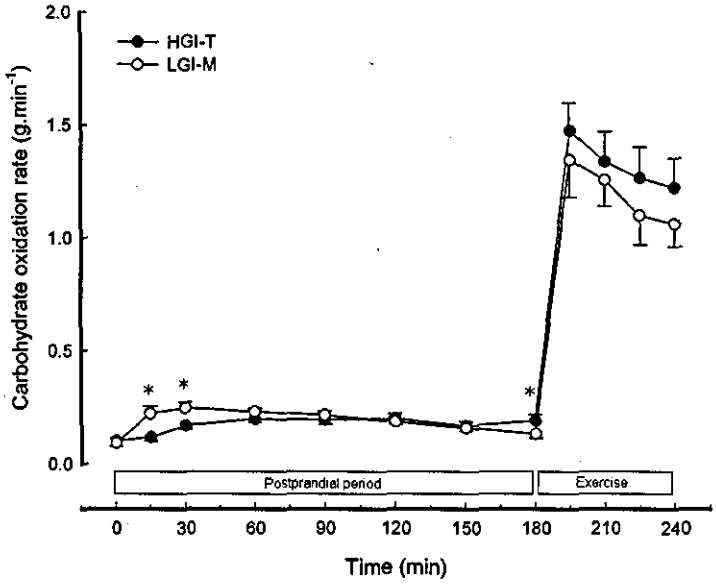


Figure 7.7. Carbohydrate oxidation rate ($g.min^{-1}$) during the 180 min postprandial period and the 60 min exercise period following the ingestion of a high glycaemic (HGI-T: •) and low glycaemic index meal (LGI-M : ○) (mean ± SEM). Meal, time and meal by time interaction effect during the postprandial period ($P<0.05$). Time effect only during exercise ($P<0.05$).
* HGI significantly different from the LGI trial ($P<0.05$).

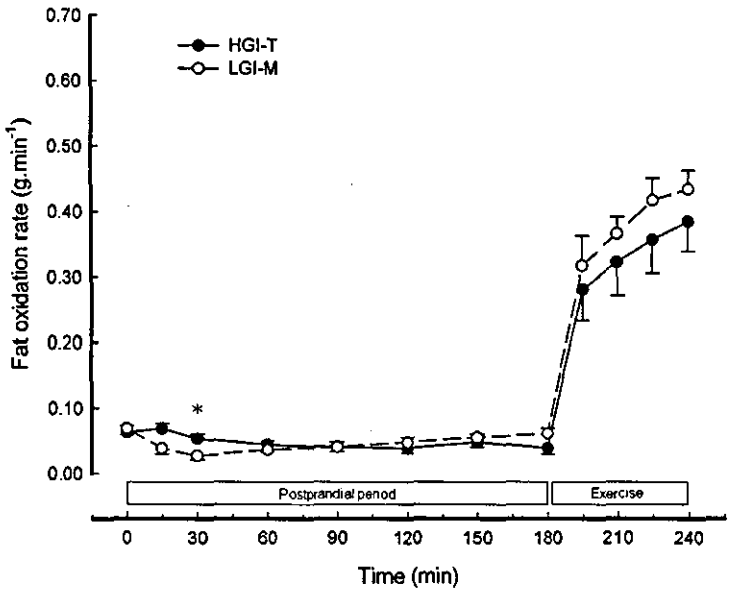


Figure 7.8. Fat oxidation rate (g.min-^{1}) during the 180 min postprandial period and the 60 min exercise period following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-M: ○) (mean ± SEM). Time and meal by time interaction effect during the postprandial period (P<0.05). Time effect only during exercise (P<0.05).
* HGI significantly different from the LGI trial (P<0.05).

Heart Rate and Rating of perceived exertion (RPE)

Heart rate increased with time during exercise in both trials (P<0.05) However there was no difference between trials (Table 7.5).

The ratings of perceived exertion (RPE) stayed the same during the 60 min run in both trials and there was no difference between trials. (Table 7.5).

Perceived gut fullness and hunger

Following the ingestion of the HGI-T and LGI-M meals rating for gut fullness reached similar peak values at 15 min in both trials, then gradually decreased over time (P<0.05). There was no statistically significant difference in the gut fullness ratings between the two trials. (Figure 7.9) (Table 7.6).

Following the consumption of breakfast, hunger ratings rapidly decreased in the first 15 min following food consumption, after which they steadily increased with time reaching near fasting values at 180 min into the postprandial period ($P<0.05$). There was no difference in hunger ratings between the two trials (Figure 7.10) (Table 7.6).

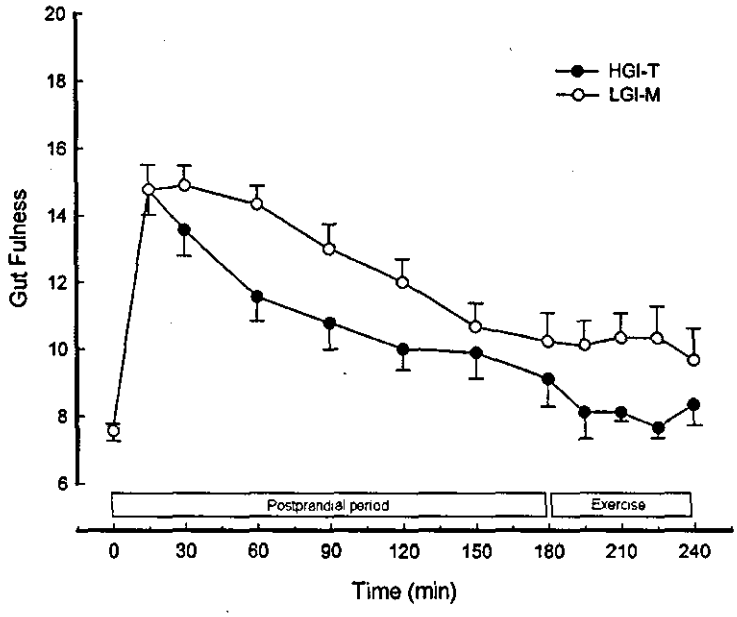


Figure 7.9. Gut Fullness ratings during the 180 min postprandial period and the 60 min exercise period following the ingestion of a high glycaemic (HGI-T: •) and low glycaemic index meal (LGI-M: ○) (mean ± SEM). Time effect only during the postprandial period ($P<0.05$). Meal effect during exercise ($P<0.05$).

Hunger ratings decreased in the first 15 min of exercise in both trials however they remained lower in the LGI muesli (LGI-M) trial compared with the HGI toast (HGI-T) trial for the remainder of the exercise period. There was a significant meal effect ($P<0.05$) (HGI>LGI) during the 60 min exercise period.

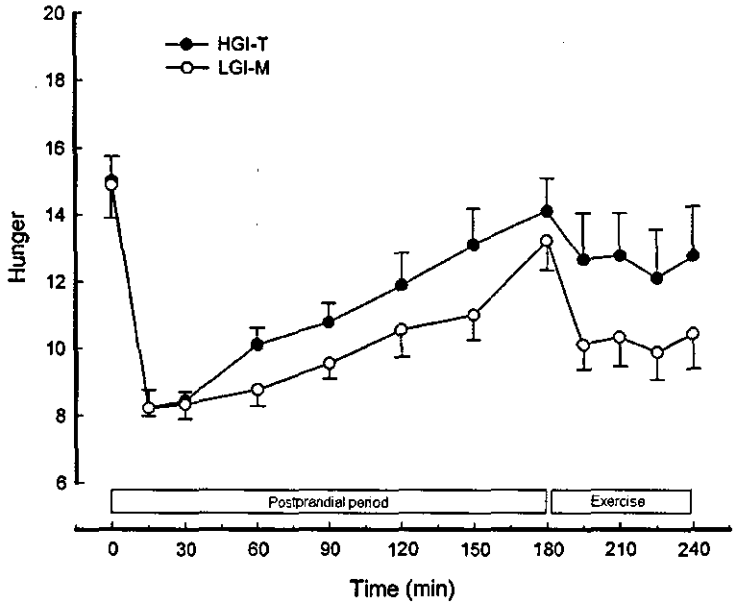


Figure 7.10. Hunger ratings during the 180 min postprandial period and the 60 min exercise period following the ingestion of a high glycaemic (HGI-T: ●) and low glycaemic index meal (LGI-M: ○) (mean ± SEM). Time effect only during the postprandial period ($P<0.05$). Meal effect during exercise ($P<0.05$).

Table 7.6 Gut fullness and hunger ratings during the postprandial period of the HGI-T and LGI-M trials (mean ± SEM).

		Postprandial Period (min)								Exercise Period (min)			
Variable	**Trial**	**Fast**	**15**	**30**	**60**	**90**	**120**	**150**	**180**	**195**	**210**	**225**	**240**
Gut fullness *	HGI-T	8 ± 0	15 ± 1	14 ± 1	12 ± 1	11 ± 1	10± 1	10 ± 1	9 ± 1	8± 1	8 ± 0	8 ± 0	8 ± 1
	LGI-M	8 ± 0	15 ± 1	15 ± 1	14 ± 1	13 ± 1	12 ± 1	11 ± 1	10 ± 1	10 ± 1	10 ± 1	10 ± 1	10 ± 1
Hunger *	HGI-T	15 ± 1	8 ± 0	8 ± 0	10 ± 1	11 ± 1	12 ± 1	13 ± 1	14 ± 1	13 ± 1	13 ± 1	12 ± 1	13 ± 1
	LGI-M	15 ± 1	8 ± 1	8 ± 0	9 ± 0	10 ± 0	11 ± 1	11 ± 1	13 ± 1	10 ± 1	10 ± 1	10 ± 1	10 ± 1

* Gut fullness: Time effect during the postprandial period ($P<0.05$); Meal effect during exercise ($P<0.05$) (HGI<LGI).
* Hunger ratings: Time effect during the postprandial period ($P<0.05$); Meal effect during exercise ($P<0.05$) (HGI>LGI).

Comparison of modified Borg scale and Visual Analogue Scale (VAS) for Perceived Gut Fullness and Hunger

In addition to the adapted Borg scale, gut fullness and hunger were also assessed using the Visual Analogue Scale (0-100 scale). For comparison purposes the original values were recalculated by converting the Borg scale into a 0-100 scale.

Results obtained using the VAS scale followed the same trend as ratings obtained with the adapted Borg scale (Figure 7.11 and Figure 7.12). Large individual variations were seen in both the perception of gut fullness and hunger.

Both scales showed changes with time in both trials. Comparing the data on the same 0-100 scale the VAS scores appeared higher than the ones obtained using the adapted Borgh scale, however there were no significant differences between the two measurements.

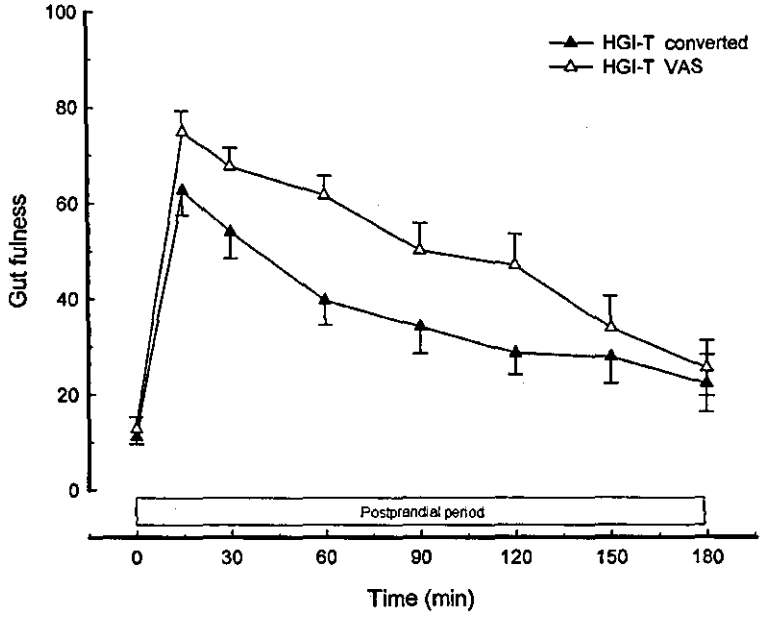


Figure 7.11. Comparison of Gut fullness converted Borg scale (HGI-T: ▲) and VAS scale (HGI-T : Δ) during the 180 min postprandial period following the ingestion of a high glycaemic index meal (mean ± SEM). Time effect ($P<0.05$).

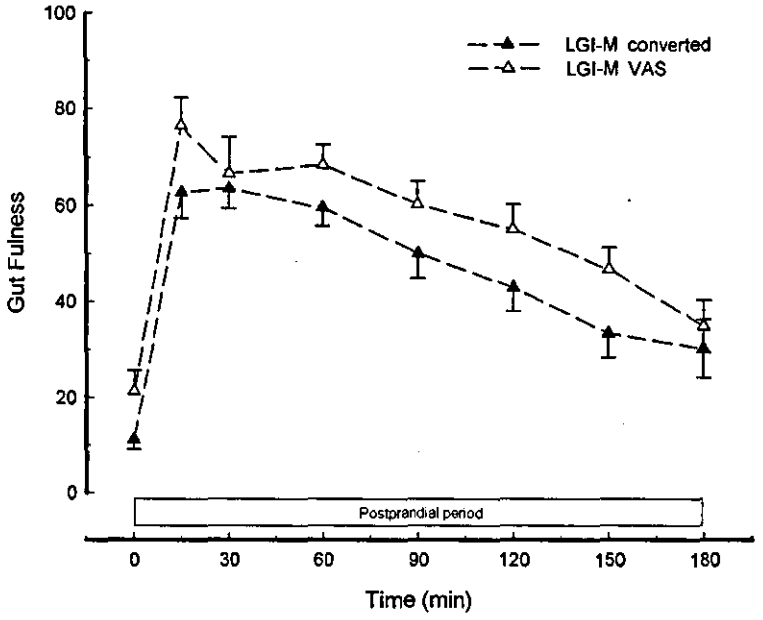


Figure 7.12. Comparison of Gut fullness converted Borg scale (LGI-M: ▲) and VAS scale (LGI-M : △) during the 180 min postprandial period following the ingestion of a low glycaemic index meal (mean ± SEM). Time effect ($P<0.05$).

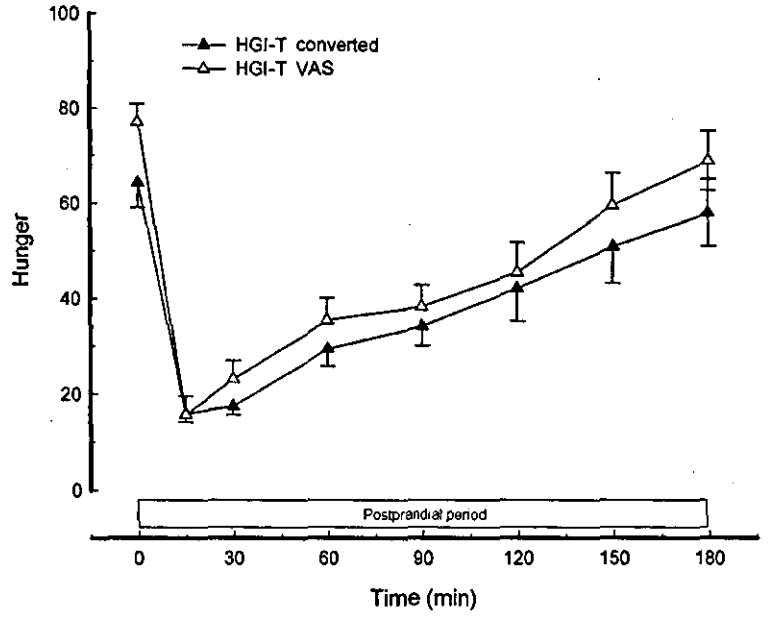


Figure 7.13. Comparison of Hunger ratings from the converted Borg scale (HGI-T: ▲) and VAS scale (HGI-T : △) during the 180 min postprandial period following the ingestion of a high glycaemic index meal (mean ± SEM). Time effect ($P<0.05$).

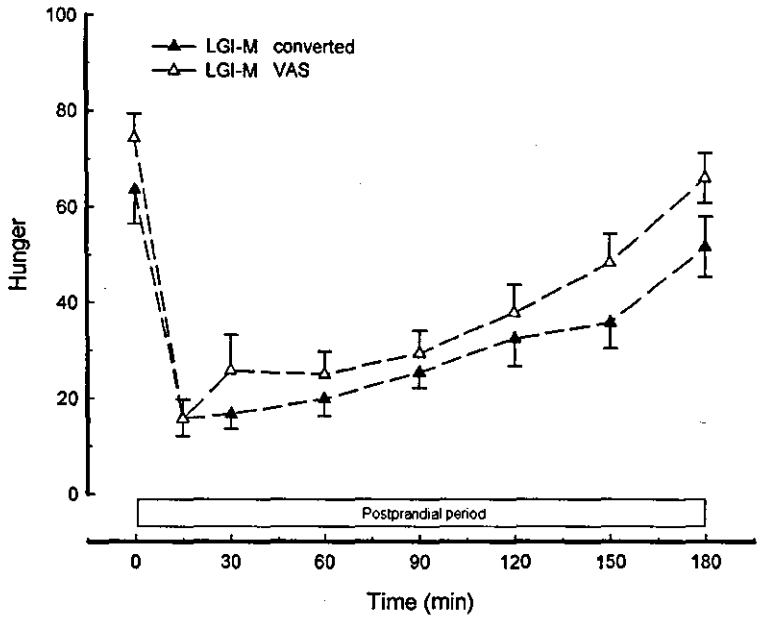


Figure 7.14. Comparison of Hunger ratings from the converted Borg scale (LGI-M: ▲) and VAS scale (LGI-M : △) during the 180 min postprandial period following the ingestion of a low glycaemic index meal (mean ± SEM). Time effect ($P<0.05$).

7.4 Discussion

The aim of this study was to extend our previous observations on the glycaemic response to the HGI and a LGI carbohydrate breakfast to include changes in insulin.

The main findings of the study was that while there was a significant difference in the glycaemic response (as defined by the IAUC) between the HGI-T and LGI-M trial (Figure 7.2) ($P<0.05$) there was no significant difference in the insulinaemic response between the two trials (Figure 7.4).

As in the last two studies the peak blood glucose values in the LGI trial were again higher than expected. The shape of blood glucose curve following the

LGI-T breakfast closely followed that of the HGI-M breakfast. Peak blood glucose concentration of 8.1 ± 0.2 mmol.l^{-1} in the HGI-T trial from the present study compared well with 7.9 ± 0.6 mmol.l^{-1} in the HGI-T trial from our previous study using female participants (see Study 2, Chapter 6). However the peak blood glucose of 7.8 ± 0.3 mmol.l^{-1} from the LGI muesli (LGI-M) trial in the present study, was higher than the peak blood glucose of 6.9 ± 0.5 mmol.l^{-1} following the fruit and yoghurt breakfast (LGI-F trial) (see Study 2, Chapter 6). At this stage we can only assume that differences were due to the effect of a higher or even earlier peak insulin concentration in the LGI-F trial and possibly influenced by the fructose content of the LGI-F breakfast.

In contrast, previous studies from our laboratory did find significant difference in the peak plasma glucose concentration. Stevenson and colleagues recorded peak blood glucose concentrations of 7.5 ± 0.7 mmol.l^{-1} for the HGI trial and 5.9 ± 0.2 mmol.l^{-1} for the LGI trial in female participants (Stevenson, *et al.*, 2006). Similarly Wu and colleagues recorded peak blood glucose concentration of 6.8 ±0.2 mmol.l^{-1} (HGI trial) and 6.2 ± 0.3 mmol.l^{-1} (LGI trial) for male participants (Wu, *et al.*, 2003). Both of the above studies used breakfasts with an available CHO content of 2g.kg^{-1}BM designed for highly trained athletes.

As mentioned in the previous chapter Shenk and colleagues (2003) suggested that the difference in the glycaemic response to the HGI and LGI meals lies in the rate of glucose removal from the systemic circulation (Schenk, *et al.*, 2003). In the present study there was only a 4% non-significant difference between peak blood glucose concentration (8.1 ± 0.2 mmol.l^{-1} for HGI-T and 7.8 ± 0.3 mmol.l^{-1} for the LGI-M trial). Peak plasma insulin concentrations showed a larger (23%) difference (35.1 ± 3.6 mIU.l^{-1} HGI-T trial and 45.2 ±

3.5 mIU.l^{-1} for the LGI-M trial). This difference was not statistically significant despite of the fact that 7 out of the 9 participants had a higher peak insulin concentrations in the LGI-M compared with the HGI-T trial. The higher plasma insulin peak in the LGI-M trial could be attributed to the milk included in the LGI-M breakfast. It is recognised that milk has a strong insulinotropic effect (Nilsson, *et al.*, 2004) and milk protein has been shown to stimulate β-cell function (van Loon, *et al.*, 2000). Another point worth considering is that although Alpen muesli, according to Englyst and colleagues (2003) had more slowly available glucose compared with toast, it also had a higher amount of available free sugar (Englyst, *et al.*, 2003). This 'hidden' sugar in the breakfast cereal might have been the cause of the higher postprandial insulin peak . Peak plasma insulin concentration of 45.2 ± 3.5 mIU.l^{-1} (LGI-M trial) and 35.1 ± 3.6 mIU.l^{-1} (HGI-T trial) reported in the present study were elicited following ingestion of an average of 55 g of CHO. Shenk and colleagues (2003) reported peak insulin concentration of 20.4 ± 4.5 mU.l^{-1} in the bran cereal trial (BC) and 16.6 ± 2.1 mU.l^{-1} in cornflakes (CF) trial following the ingestion of 50 g of CHO (Schenk, *et al.*, 2003). Although the amount of ingested CHO was nearly the same as in the present study the responses in insulin concentration were very different.

In the present study the IAUC for blood glucose was 1.6 times higher in the HGI-T trial then the LGI-M trial (235 ± 35 mmol.l^{-1}.180min versus 144 ± 19 mmol.l^{-1}.180min) (P<0.05) (Figure 7.2) (Table 7.2). In the study by Shenk and colleagues (2003) the IAUC for blood glucose was 2.3 times higher in the cornflakes compared with the bran flakes trial (192 ± 3.8 mmo.l^{-1}.180min^{-1} and 85.7 ± 12.1 mmo.l^{-1}.180min respectively (Schenk, *et al.*, 2003).

Although there were significant differences between glycaemic responses of HGI and LGI breakfasts in both studies there were no difference in the

insulinaemic responses between HGI and the LGI trials. The IAUC for insulin in the present study was 2498 ± 299 $mU.l^{-1}$.180min in the HGI-T trial, compared with 2122 ± 204 $mU.l^{-1}.180min^{-1}$ in the LGI-M trial (Figure 7.4) (Table 7.2). These values were 36% and 28% higher than reported by Shenk and colleagues (1602 ± 139 $mU.l^{-1}$.180min for cornflakes trial compared with 1523.3 ± 155.7 $mU.l^{-1}$.180min for the All-Bran trial) (Schenk, *et al.*, 2003) in spite of the similarity in CHO intake of (55 ± 1 g in the present study compared with 50 g in the study by Schenk and colleagues (2003)).

The above differences could be due to variation in blood glucose concentration between sampling sites. In Shenk's study insulin was measured from a venous plasma sample compared with arterialised plasma sample in the present study. The insulin concentration in venous blood is determined not only by the insulin release from the pancreas but also by insulin extraction in tissues drained by the venous blood. Thus a low insulin concentration in venous blood in response to a CHO meal may theoretically be due to either a low secretion or due to an exaggerated peripheral extraction or a combination of these two processes.

Difference could also be due to methods of measuring insulin, i.e. ELISA in the present study compared with Radio Immuno Assay (RIA) used in Shenk's study (2003).

The marked early rise in insulin concentration in the LGI compared with the HGI trial in both studies was transient and occurred in the first 30 min of the postprandial period. This early insulin response increased plasma glucose removal from the systemic circulation reducing postprandial hyperglycaemia.

Breakfasts in previous as well as the present study consisted of various carbohydrate foods. According to Cummings and colleagues each of the component foods could have contributed to different physiological responses (Cummings, Roberfroid, Andersson, Barth, Ferro-Luzzi, Ghoos, Gibney, Hermonsen, James, Korver, Lairon, Pascal and Voragen 1997). Starch is considered to be digested slowly resulting in a modest glycaemic response. However the extent to which starch is digested and absorbed depends on the source and degree of processing (Englyst, *et al.,* 1986). Simple sugars like glucose and sucrose are rapidly absorbed. Breakfast cereals possibly containing hidden sugars could raise blood glucose and insulin concentrations. Englyst and colleagues measured the rapidly available glucose (RAG) in carbohydrate foods and found that this correlates highly with the foods GI ($r=0.981$, $P<0.0001$) (Englyst, *et al.,* 2003) For example 50g of available CHO from cornflakes contained 45.7 g of RAG compared with only 38.8 g of RAG for Alpen. However slowly available glucose (SAG) was higher in Alpen than cornflakes (3.2 g compared with 1.8 g per 50 g available CHO) According to Englyst (2003) a high SAG content identifies low GI foods that are rich in slowly released carbohydrates. However, free sugar glucose was 2.6 g in cornflakes and 8.8 g in Alpen as measured by Englyst and colleagues (Englyst, *et al.,* 2003). This would probably cause an early rise in postprandial blood glucose and insulin concentration.

The present study as well as the one by Schenk (2003) and colleagues and also Wu and colleagues (2003) reported early rises in insulin concentrations in the LGI compared with the HGI trials (Schenk, *et al.,* 2003; Wu, *et al.,* 2003). The rise in insulin concentrations were transient, and occurred in the first 30 min of the postprandial periods. In the study by Schenk (2003) this was followed by an increase in plasma glucose removal from the systemic circulation which reduced postprandial hyperglycaemia.

The increased rate of plasma glucose removal might explain the lack of or presence of significant difference in blood glucose peaks seen in previous studies. It might also partially explain the large variation in the IAUC for plasma glucose seen in the general literature.

Comparing the HGI trial of the present study with that of Stevenson and colleagues (2006) we found that for a 2 fold increase in the amount of ingested CHO there was a 5 fold increase in the peak serum insulin, i.e. a larger CHO intake resulted in an increased insulin release. The ingested glucose in the form of a glucose syrup (Lucozade original) was part of the HGI breakfasts in the study by Stevenson and colleagues (Stevenson, *et al.*, 2006). This readily available glucose increased blood glucose and consequently plasma insulin concentration.

In the same study a 2 fold increase in the amount of available carbohydrate in the LGI meal was mirrored by a 2.7 fold increase in peak insulin value. An enhanced glucose uptake resulted in a higher CHO oxidation and a decreased fat oxidation (Stevenson, *et al.*, 2006). In the present study fat oxidation rates in the LGI trial were similar to those reported by Stevenson at al (2006), however fat oxidation in the HGI trial was not (Stevenson, *et al.*, 2006). One explanation for this difference may be that the larger insulin response to the HGI meal depressed fat metabolism to a greater extent in the Stevenson et al (2006) study than in the present study.

The results of the present study suggest that although some foods are classified as LGI based on the 'area under the glucose curve', metabolically they might behave closer to what is expected from a HGI CHO food. An early rise in insulin concentration seems to reduce postprandial hyperglycaemia and hence the GI of a meal. As a consequence food items or

meals might be classed as LGI , defined by the size of the IAUC, when the reduced area under the curve might be due to the more rapid glucose clearance, especially during the first 30 min of the postprandial period (Schenk, *et al.*, 2003). If the reduction in blood glucose concentration is indeed due to the higher and earlier plasma insulin levels it is hard to see some of the claimed health benefits. For individuals with reduced insulin sensitivity or those already having type 2 diabetes a so called low GI meal might not be beneficial, but the contrary, could lead to an unexpected hyperglycaemia and hyperinsulinaemia.

Low glycaemic index foods have been shown to promote fat oxidation at the expense of CHO oxidation (Stevenson, *et al.*, 2006; Wu, *et al.*, 2003). It has also been suggested that LGI food might promote weight loss by promoting the feeling of satiety (Brand-Miller, *et al.*, 2002). Previous studies from our laboratory have shown an increase in satiety following the consumption of LGI compared with HGI food. In the present study there were no differences in fat and carbohydrate oxidation between trials, neither in some of the important blood parameters.

Carbohydrates effect satiety and this varies according to their composition. Studies using pre-load found that both HGI and LGI carbohydrate foods suppress appetite and food intake, but they follow different time courses. High-glycemic carbohydrates suppress food intake more effectively in the short-term (1-3 hour) compared with LGI carbohydrate foods that suppress food intake over longer periods of time (4-6 hour) (Bornet, *et al.*, 2007).

Although there were no differences in gut fullness and hunger during the postprandial period in the present study, there was a significant difference during exercise. Gut fullness ratings were higher and hunger ratings lower in

the LGI-M compared with the HGI-T trial. This finding agrees with finding from the study on women in Chapter 6 of this thesis. However it is contrary to findings of Stevenson and colleagues (2006) where there was a significant difference between trials during the postprandial period but not during exercise (Stevenson, *et al.*, 2006).

Ingestion of HGI carbohydrate food results in a rapid increase in the occupancy of glucoreceptors in the gut which will produce a surge in pre-absorptive satiety signals. As the glucose is transported from the gut lumen into the bloodstream these satiety signals will decrease relatively quickly. The postprandial rise in blood glucose concentrations have also been associated with a slowing of gastric emptying (Schvarcz, Palmer, Aman, Horowitz, Stridsberg and Berne 1997), which in turn would also contribute to a feeling of gut fullness and short-term feeling of satiety (Bergmann, Chassany, Petit, Triki, Caulin and Segrestaa 1992). As the amount of ingested CHO in the present study was only 50% that used in the study by Stevenson and colleagues (2006) it is expected that it was probably absorbed faster. The LGI muesli breakfast, contained more slowly available glucose that contributed to the increased satiety during exercise compared with the HGI toast trial.

Over the years we have successfully used the modified Borg scale to measure gut fullness and hunger rating. However it was pointed out that by having a numbered scale with anchor points, our method could / will influence the participants' ratings of gut fullness and hunger. We found no evidence of this, the two methods of measurements correlated well. Participants preferred the numbered scale with anchor points.

In summary, the muesli breakfast used in this study, calculated as a LGI breakfast, has shown characteristics of a HGI breakfast by a sharp rise and

fast decline in blood glucose concentrations and a trend for a higher peak insulin concentration. High glucose and insulin concentrations in both trials would have inhibited FFA release and transport. This explains the lack of difference in fat oxidation between the two treatments. These findings also highlight the need to consider the shape of the postprandial blood glucose and plasma insulin curve as well as the IAUC when examining the glycaemic and insulinaemic effect of a meal. More frequent blood sampling in the first 30 min of the postprandial period would give a better picture of the blood glucose and insulin response to food intake. Further studies are needed to investigate the effect of the early rise in insulin concentrations on blood glucose concentrations and the glycaemic response (IAUC) of the ingested meal during rest and exercise.

CHAPTER 8

General Discussion

The aim of this thesis was to examine the metabolic responses to high and low GI carbohydrate breakfasts using commonly consumed foods. The hypothesis was that eating a low GI breakfast will promote a greater rate of fat oxidation during subsequent exercise when compared with consuming a high GI breakfast.

In all studies blood glucose concentrations were followed during a 180 min resting postprandial period. In three subsequent studies the male and female participants also completed a 60 min treadmill run at 65% $\dot{V}O_2$ max in order to try to identify any differences in the rates of fat and carbohydrate oxidation following the different pre-exercise meals. In all the studies reported in this thesis the participants consumed the equivalent of 1g.kg^{-1} BM available CHO, using commonly consumed breakfast foods.

8.1 Main findings

The main findings of the study were that while the ingestion of the LGI breakfasts resulted in a low glycaemic response (as measured by the IAUC) the peak blood glucose values were similar to the those obtained following the ingestion of the HGI breakfasts. High peak blood glucose concentrations in the LGI trials were followed by a rapid fall towards fasting values. These responses resembled those that are characteristic of the responses to HGI meals rather than LGI meals. In the study in which plasma insulin concentrations were determined i.e. Chapter 7, there was a trend for higher concentrations in the LGI than in the HGI trial. Therefore, it is not surprising that there was no significant difference in substrate oxidation during the 60 min exercise between the HGI and LGI trials in men or women.

8.2 Validity of calculation of mixed meals

The commonly held view is that the consumption of LGI carbohydrate foods results in a slow release of glucose that does not reach the same peak values as produced following the consumption of HGI foods and that the glucose concentrations fall away to base line values more slowly. This view is based largely on the results from studies that have used single foods, such as lentils, rather than mixed meals containing different macronutrients. The unexpected responses to the LGI mixed meals used in the present studies raises the question about calculated GI values for the mixed meals.

The validity of the GI values for mixed meals has been questioned in several studies (Coulston, *et al.*, 1984a; Coulston, *et al.*, 1984b; Hollenbeck, *et al.*, 1988). Previous studies from our laboratory have shown that the ratio between the glycaemic response to HGI and LGI meals closely matches the ratio of their calculated GI values (Stevenson, *et al.*, 2006; Wu, *et al.*, 2003). However, in Study 1 (Chapter 4) of the 6 possible pairing of breakfasts only 3 demonstrated a close relationship between measured and calculated GI responses; none of them included the HGI toast (HGI-T) breakfast (Table 4.2). The current data suggests that the discrepancies between the ratio of the calculated breakfast GI and measured glycaemic ratio are due to the high IAUC in the HGI toast trial. The recommended blood sampling interval and measurements of glycaemic response in GI testing is 120 min even though the ingested CHO will have an effect on glycaemia and insulinaemia for a further 2-3 hrs. Even when the areas under the blood glucose curves are calculated over 2 rather than 3 hours the same results for the ratios were obtained.

Wolever and Jenkins (1986) tested the glycaemic responses to four mixed meal containing 45 g CHO. The correlation coefficient for the meal GIs and IAUC for the meals was 0.987 ($p < 0.02$) and the y-intercept was virtually 0 (-

4.4 ± 26) (Wolever, et al., 1986). Similar good correlation, 0.914, $P<0.05$, was found in Study 1 of the present series, however the intercept varied between -123 and + 96. Furthermore it was expected that the ratio of measured glycaemic response would match the calculated GI ratios for the particular breakfasts. However, some ratios appeared to be matched while others were not. For example, the calculated ratio for the HGI-toast and LGI fruit breakfasts was 2:1 (72/36) compared with the measured minimum of 2: 1.7 and maximum 2 : 6. The measured glycaemic responses in the present study only partly confirm Wolever's method of estimating the GI of mixed meals (Wolever, et al., 1986).

8.3 Glycaemic load of carbohydrate meals (1g v 2g of CHO)

The present study has provided some insights into CHO oxidation following the ingestion of 1 g compared with 2 g of available CHO per kg BM from previous studies. In Study 3 (Chapter 6) total carbohydrate oxidation during exercise was 77.1 ± 8.1 g in the HGI-T and 73.0 ± 5.7 g in the LGI-F trial. Consumption of a breakfast with an available CHO content of 2 $g.kg^{-1}$ BM that was used in a previous study elicited a higher total CHO oxidation of 101.5 ± 12.0 g in the HGI trial compared with the present study. Interestingly in the LGI trials the total CHO oxidation were similar following the breakfasts containing 1 g or 2 g of available CHO per kg BM (73.0 ± 5.7 g in the present study compared with 70.5 ± 10.2 g (Stevenson, *et al.*, 2006). The greater CHO content of the breakfast in the previous study was mainly provided by glucose syrup (Lucozade Original) (Stevenson, *et al.*, 2006). The larger amount of CHO oxidised was probably due to the readily available glucose from the glucose syrup solution.

8.4 Readily available glucose (RAG)

An important characteristic of CHO foods is the amount of rapidly available glucose (RAG). The rate at which glucose is released from a food can influence the magnitude of peak glucose and plasma insulin concentrations. Englyst and colleagues found RAG in starchy foods correlates well with their glycaemic response (Englyst, et al., 2003). In vitro analysis has shown that for example while 50g of available CHO from cornflakes contains 45.7 g of RAG, Muesli (Alpen)only contains 38.8 g. However, Muesli (Alpen) contains 8.8 g of free sugar compared with just 2.6 g from cornflakes. This free sugar could have been responsible for an early increase and a higher peak insulin concentration in the LGI muesli trial (Chapter 7). However, the higher peak insulin was transient and there was no difference in the insulinaemic responses for 180 min in the two trials. Blood glucose and insulin levels can be effected by free sugar content of a mixed meal. Unfortunately, most food-processing techniques employed in the manufacture of cereal products and bakery products tend to result in the disruption of the food matrix and the gelatinisation of starch granules. Readily digestible and high in RAG they generally behave like HGI foods. The same disruption of cell walls also occurs in the canning process of fruit (medium to LGI). The challenge is to process food with minimal disruption of structure in order to produce slow release CHO foods.

8.5 The effect of peak blood glucose on substrate oxidation

Peak blood glucose concentrations were similar during all the trials reported in this thesis. There was no difference in the insulinaemic response to the HGI toast and LGI muesli breakfast, so it has been assumed that the same may be true for the HGI toast and LGI fruit breakfasts. Hence there was no difference in CHO and fat oxidation rates during exercise between the LGI and HGI trials in men or women. It is interesting that despite of a 50 %

increase in CHO intake and higher insulinaemia in the study by Stevenson and colleagues (2006), total fat oxidation was similar in the LGI trial of the two studies (1 $g.kg^{-1}$ versus 2 $g.kg^{-1}$ BM CHO intake). This suggest that there was a delay in the absorption of the larger amount of CHO. Part of the CHO in Stevenson's study came from apple juice which contain fructose that does not contribute to the elevation of plasma insulin.

8.6 Conclusions

The results of the studies presented in this thesis do not support the hypothesis that eating a low GI breakfasts of commonly consumed foods leads to a greater rate of fat oxidation during subsequent sub-maximal exercise when compared to consuming high GI breakfasts. Furthermore it was noted that the 'expected' slow release of glucose into the systemic circulation that has been traditionally associated with low GI foods was not observed with any of the low GI meals used in this study.

8.7 Limitations of the studies

It is important to mention some of the limitation of these studies. Wolever and colleagues demonstrated that it is the amount of available CHO in a meal that provides 90% of the variation in the glycaemic response. Similar to previous studies from our laboratory the amount of available CHO of the HGI and LGI breakfast was standardised and on this occasion it was set to 1$g.kg.^{-1}$ BM. Consequently participants had to consume different amounts of food (Table 3.2). For example, in the trials containing the LGI fruit (LGI-F) breakfast, the participants consumed 4 times more food than in the HGI trial. In the LGI muesli (LGI-M) breakfast they consumed 2.8 times more food than in HGI toast (HGI-T) breakfast. These larger meals will have had a marked effect on gut fullness and hunger responses (Oesch, *et al,* 2006). If the comparison of glycaemic responses were based on total CHO content of

breakfasts, then the participants on a LGI trial would have consumed less available CHO, because of the higher fibre content of these foods. Similarly participants consuming breakfast ad-libitum would probably not eat enough of the LGI CHO food as they would feel fuller earlier. Using the same 'serving size' for participants in these studies would produce misleading results unless the participants were of the same body size but this impractical for ongoing experiments. Finally it must be acknowledged that the studies reported in the thesis may be slightly 'under-powered' and a larger number of participants may have revealed significant differences in the rate of fat oxidation during exercise. This having be said it appears from the results that it would require the recruitment of a significant number of additional participants which would have made the studies logistically and financially impossible to complete.

8.8 Direction for future research

Further studies needed to clarify what is the main effect of GI on metabolism. It is clear from the studies reported in this thesis that the IAUC on its own does not necessarily provide information about the fate of blood glucose during the postprandial period i.e. provides no information about the blood glucose peaks or the shape of the curve. It would be also beneficial, although probably hard to achieve in every day life, to choose mixed meals based on their RAG content. This might be a better way of assessing the glycaemic impact of food items compared with just their GI values.

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Appendices

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

Influence of low and high glycaemic carbohydrate breakfast meals with different glycaemic indices on substrate utilisation during subsequent exercise in men and 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, Stevenson 2005).

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 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 and Stewart 1996, Wee et al. 1999, Febbraio et al. 2000, Wu et al. 2003, Stevenson 2005.

The majority of the research carried out on glycaemic index and fat oxidation used food portions equivalent to 50g (available carbohydrate, thus not taking into account participants bodyweight, their carbohydrate or energy requirement. Previous studies from our laboratory have used HGI and LGI meals containing 2.5g/kg and 2.0g/kg available carbohydrate thus matching the energy and carbohydrate requirements of our subjects, namely athletes.

In our last study we have investigate the glycemic responses to HGI and LGI meals containing 1g/kg body mass available carbohydrate during rest and subsequent exercise in recreationally

active men and women. The next two studies will be a continuation of the above, using different meals normally used in every day life.

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

Title Mrs Surname Nute Forename Maria

Department School of Sport and Exercise Sciences

Email address M.L.Nute@lboro.ac.uk

Personal experience of proposed procedures and/or methodologies.

Trained in the collection and analysis of blood samples, expired air samples and heart rate data.

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.

5. Proposed start and finish date and duration of project

Start date February 2007 Finish date May 2007 Duration 4 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 an MPhil project.

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** [x]

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 (eg 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 (containing 1g/kg available carbohydrate) on the metabolic responses to a subsequent exercise in men and 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_2max 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 3 -7 days 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. Before breakfast, a finger tip 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 glycaemic index or low glycaemic index breakfast. Following the consumption of breakfast participants 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. Hunger and gut fullness ratings will be recorded throughout the trial. At the end of the 3 h postprandial period, participants will complete a warm up of 5min at 60% VO_2max and then will run for 60min at 65% VO_2max on a motorised treadmill. At the end of the run, a final blood sample will be collected and then participants body mass will be obtained. At the end of the experiment participants will be offered lunch after which they will be free to leave the laboratoryAppendix A1 - EAC 2-Research Proposal- GI-Breakfast study-Draft 1.

B) MEASUREMENTS TO BE TAKEN

PRELIMINARY TRIALS

$VO_{2\,max}$ Test – An incremental treadmill test for the determination of VO_{2max} (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).

Test run at 65% $VO_{2\,max}$ – A 40 min sub maximal run.

MAIN TRIAL

Participants will weigh themselves in minimal clothing 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 15minutes.

Expired air samples will be collected before breakfast and at 15, 30, 60, 90, 120, 150 and 180 minutes after breakfast and every 15 minutes throughout the exercise period using the Douglas Bag technique.

Finger tip blood samples will be taken at the same time points as expired air samples.

The participants gut fullness and hunger ratings will be recorded at 15, 30, 60, 90, 120, 150 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:

Involves taking bodily samples	Yes	x	No	
Involves procedures which are physically invasive (including the collection of body secretions by physically invasive methods)	Yes	x	No	
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	Yes	x	No	
Involves intake of compounds additional to daily diet, or other dietary manipulation / supplementation	Yes	x	No	
Involves pharmaceutical drugs (please refer to published guidelines)	Yes		No	x
Involves testing new equipment	Yes		No	x
Involves procedures which may cause embarrassment to participants	Yes		No	x
Involves collection of personal and/or potentially sensitive data	Yes		No	x
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)	Yes		No	x
Involves use of hazardous materials (please refer to published guidelines)	Yes		No	x
Assists/alters the process of conception in any way	Yes		No	x
Involves methods of contraception	Yes		No	x
Involves genetic engineering	Yes		No	x

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 from a finger tip 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 VO_{2max} 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 for group one will be recreationally active men and eumenorrheic women who include running as part of their training programmes. Ages will range from 18-65 years.

Number of participants to be recruited: 10 men and 10 women

How will participants be selected? Please outline inclusion/exclusion criteria to be used.
Participants in group will be volunteers from the student and general population. All subjects must satisfy the above criteria and will be screened via the generic health questionnaire 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** [x]

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 weight in the strictest privacy. At least one male and one female investigators will be present at all times during testing.

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

The determination of VO_{2max} 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 60minutes 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 finger tip may cause minor bruising but good practice minimises the risk.

17. 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**		**No**	x
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:

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

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

20. How will participants be informed of their right to withdraw from the study?

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**		**No**	x

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

Participants Information Sheet

STUDY TITLE: Influence of low and high glycaemic index pre-exercise carbohydrate meal on fat metabolism in men and women.

LOCATION: The Sport and Exercise Nutrition Research Laboratory situated in the Brockington Building (B.0.10.)

INVESTIGATOR: Maria Nute

SUPERVISOR: Professor Clyde Williams

Aim of Study

The aim of the present study is to compare the effect of the ingestion of High Glycaemic Index (HGI) and Low Glycaemic Index (LGI) breakfasts on fat metabolism during rest and subsequent exercise in men and women.

PRELIMINARY TESTS

You will be asked to complete preliminary tests in the week preceding the first main trial.

VO_2-Speed Test

This is a sixteen-minute, continuous, sub-maximal treadmill run which determines the relationship between running speed and oxygen uptake.

You will be asked to run for four minutes at four different sub maximal speeds. Each subsequent four minutes is 1-1.5 kilometre an hour faster than the previous. During the final minute of each four-minute block expired air samples will be collected in a Douglas bag via a lightweight valve and mouthpiece. Heart rate will be recorded by short range telemetry from a strap placed on the chest (Polar, Finland).

Maximal Oxygen Uptake (VO_{2max}) Test

This is a test which determines the ability of your body to take oxygen from the air, deliver it to your working muscles. In this test you will be asked to run to volitional fatigue (usually between 8 to 12 minutes) at a constant sub-maximal speed on the treadmill. The treadmill gradient will initially be set at 3.5 % and will increase by 2.5 % every three minutes. Expired air samples will be obtained during the last minute of each stage. When you feel that you can only maintain the required speed for one more minute then a final expired air sample will be obtained and the treadmill will be stopped. Recovery from this test takes 10-15 minutes.

Familiarisation Run

On a separate visit to the laboratory you will be asked to run at a constant sub-maximal speed (corresponding to 65% of your VO_{2max}) for forty five minutes. This exercise will familiarise you with all the collection procedures that will occur in the main trial and will also help to establish the correct running speed for your main trials.

MAIN TRIALS

Following the preliminary tests you will be asked to attend the laboratory at 8:00 am in the morning in a fasted state (10-12 hr overnight) on two occasions. Following a short rest an expired air sample a finger tip blood sample will be collected. Following the collection of a urine sample you will be asked to weigh yourself (wearing minimal clothing) in the strictest privacy. Following these preliminaries you will be provided with your high carbohydrate breakfast. All breakfasts will be served with a cup of tea with milk, without sugar.

Once the breakfast has been consumed, you will be asked to rest for 3 hours. Expired air and blood samples will be taken at 15, 30, 60, 90, 120, 150 and 180 minutes post meal. Hunger and Gut fullness ratings will be recorded during the gas sampling periods.

At the end of the third hour you will be asked to change into your running kit and following a five min warm up, complete a 60 min run on a motorised treadmill at a speed equivalent to 65% of your maximal oxygen uptake. Heart rate will be monitored throughout the run (see above). Expired air samples, will be collected and your Rating of Perceived Exertion (RPE) will be recorded every 15 minutes throughout exercise. Finger tip blood samples will be taken from at the same time points as expired air samples.

The final blood sample will be taken at the end of the 60 min run, after which you will weigh yourself. Shower and changing facilities will be available for your use, please bring a towel with you. Before leaving the laboratory you will be served a sandwich lunch with a hot or cold drink.

Requirements

You will be provided with a food diary and asked to weigh and record your food intake the day before your first trial (this will include meals and all drinks and snacks). You will be asked to accurately replicate this diet on the days before your next trials. **This is very important.**

You must arrive at the laboratory on the morning of each trial in a fasted state, having not eaten anything since 10 pm the evening before. Please do not eat or drink anything in the morning except water.

You will also be asked to refrain from alcohol, heavy exercise, the day prior to each trial.

Possible Risks/Discomforts

Physical Challenge – The VO_{2max} test will challenge you but it is to volitional fatigue therefore you will stop running when you find the challenge intolerable. The fatigue experienced will be similar to that of routine training and competition.

Blood samples will be taken from a finger tip. Your might experience some soreness during the next 10-24 hours.

You are free to stop at any time if you experience considerable discomfort or you do not wish to continue with the experiment.

Contacts:

Maria Nute	M.L.Nute@lboro.ac.uk	Tel:
Prof C. Williams	C.Williams@lboro.ac.uk	Tel:

Influence of low and high glycaemic index carbohydrate pre-exercise meal on fat metabolism in men and women.

INFORMED CONSENT FORM

(to be completed after Participant Information Sheet has been read)

The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethical Advisory Committee.

I have read and understood the information sheet and this consent form.

I have had an opportunity to ask questions about my participation.

I understand that I am under no obligation to take part in the study.

I understand that I have the right to withdraw from this study at any stage for any reason, and that I will not be required to explain my reasons for withdrawing.

I understand that all the information I provide will be treated in strict confidence.

I agree to participate in this study.

Your name _____

Your signature _____

Signature of investigator _____

Date _____

HEALTH SCREEN FOR STUDY VOLUNTEERS **Name** .....

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

Please complete this brief questionnaire to confirm fitness to participate:

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

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

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

Influence of high and low glycaemic index carbohydrate pre-exercise meal on fat metabolism in men and women.

Pre test 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:

_____

_____

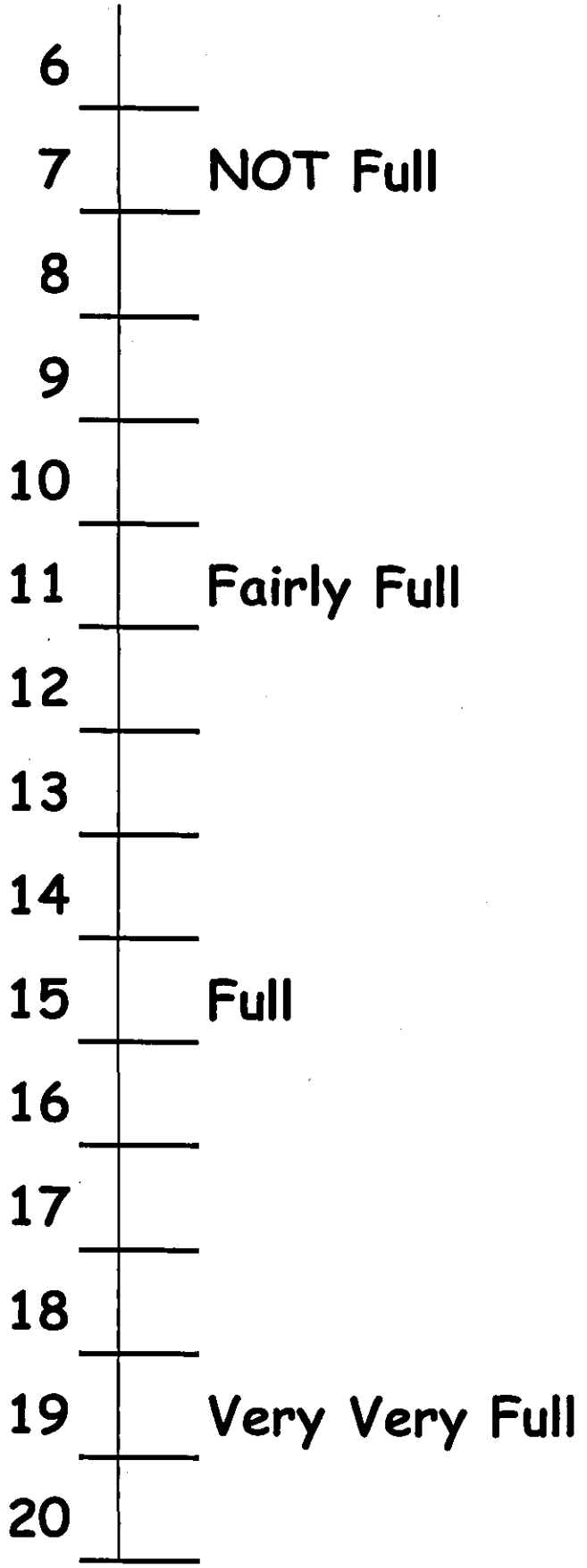
_____

_____

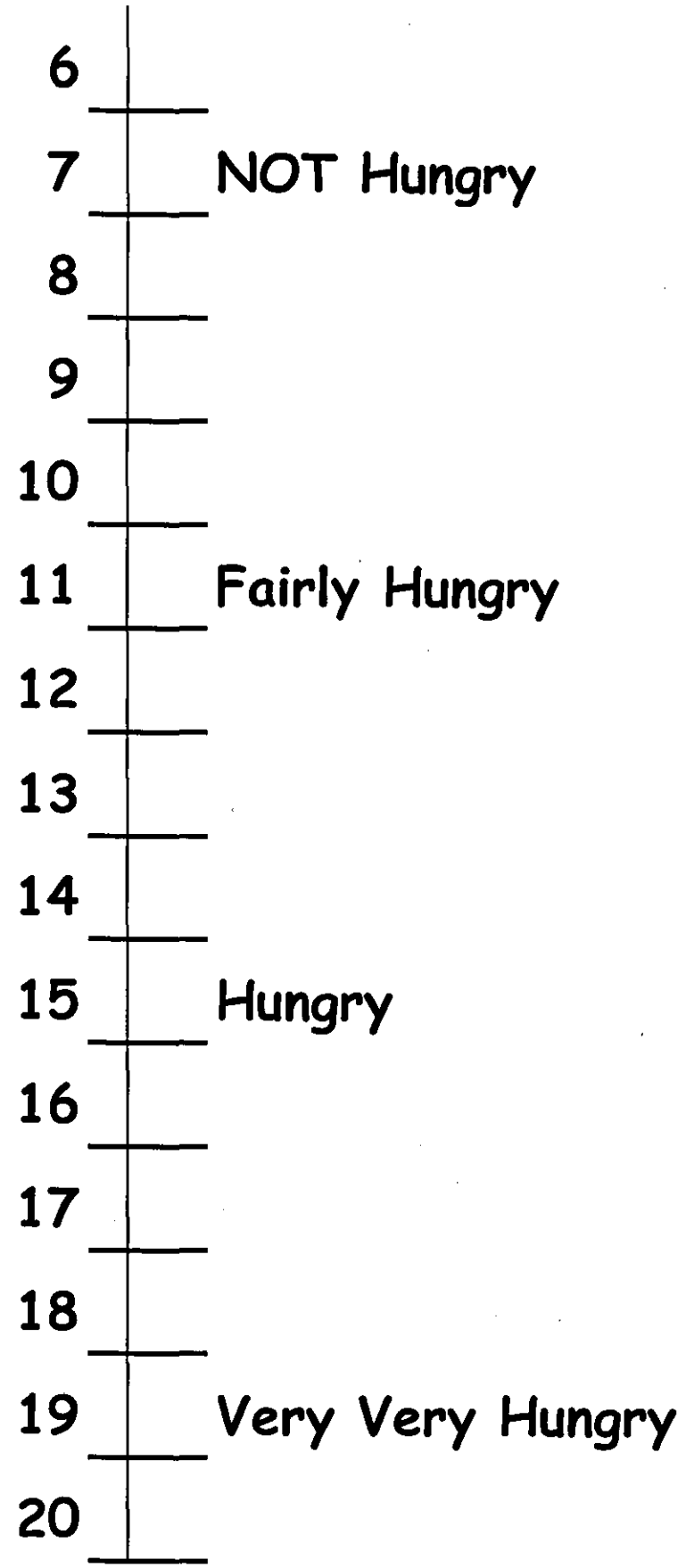
Do you want to take part in today's experiments? YES ☐ NO ☐ .

Signature:_____ Date:_____

Gut Fullness Scale



Hunger Scale



Visual Analogue Scale (VAS)

Name:_____ **Date:**_____

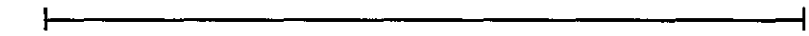
VAS No:_____

Please make a vertical mark through the horizontal line to show how you feel at the moment. Left and right extremes represent a minimum and maximum value.

How full do you feel?

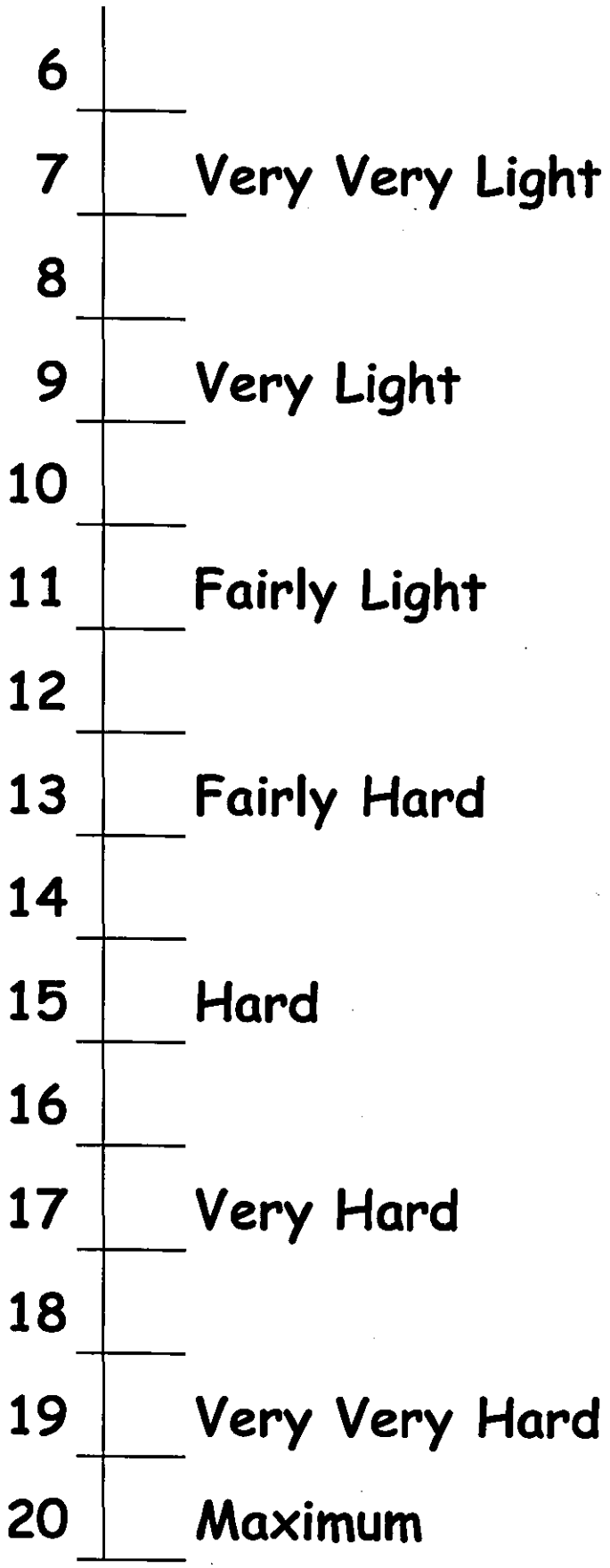
not at all extremely

How hungry do you feel?



not at all exrtemely

Rate Of Perceived Exertion



INSTRUCTION FOR USING THE FOOD DIARY

Everything that you eat and drink the day before your test date should be weighted and the weight and type of food or drink recorded.

For solid foods, the food should be placed on the scale on a plate or container. The plate or container must be weighted empty first and the scales can then be zeroed. Each item of food can then be added to the plate and weighted individually, returning the scales to zero between each item.

eg.	Plate	150g zero scale.
	Roast chicken	100g zero scale.
	Potato	150g zero scale.
	Gravy	30g zero scale.

For drinks, a cup or glass must first be weighed and then the scale can be returned to zero and the drink added. Please remember to record separately the weight of tea, milk and sugar put into a drink.

Do not forget to weight and record second helpings and between meal snacks.

Any leftovers (eg. apple cores) should also be weighted and recorded in the leftovers column.

Eating Out – Most people eat foods away from home each day, please do not forget to record these. Take your diary and scales with you where ever it is possible. If this is too inconvenient just record the type of food eaten with an estimated weight – but please say when a weight has been estimated.

Most snack foods will have the weight of the food on the packet so they do no need weighing if you eat the whole packet yourself.

Names and descriptions of foods should be as detailed as possible, including the brand name and any other information available.

eg. Cheese – is insufficient information.
Cheese, cheddar (Shape reduced fat) – is sufficient information.

Start a new page in your diary for each day, and record each item on a separate line. Record the time of day in the first column of each line.

eg. 10.30 am Mcvities Digestive Biscuits (2) 50g
one banana – medium
one cup of coffee, medium, with semi-skimmed milk, no sugar

The space provided at the food of each page for general comments is for you to give any further information about your diet and your training/activity for that day.

eg. Steady run, morning 1 hour.
Missed lunch. Low on fluid intake during the day, too busy.

Please try to be as accurate as possible and try to choose a fairly typical week to record. For instance do not record a week when you are on holiday or when you are ill, if you feel that this is would alter your normal diet or activities.

Day Month Year

Day.....day Date: / / Day Order:

Please use a separate line for each item eaten; write in weight of plate; leave a line between different 'plate' entries.

A	B		C	D	E	F	*Office Use*
Time	Food eaten		Brand name of each item (except fresh food)	Full description of each item including: -whether fresh, frozen, dried, canned -cooked: boiled, grilled, fried, roasted. -what type of fat food fried in	Weight Served	Weight of Leftovers	Actual Weight
am/pm	home	away			(gms)	(gms)	(gms)

GENERAL COMMENTS: