Impact of glycaemic index of high carbohydrate diets on exercise energy metabolism and capacity and fasting concentration of plasma lipids in healthy physically active individuals

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ABSTRACT

The present thesis describes the impact of glycaemic index of high carbohydrate diets consumed for 5 days on exercise energy metabolism and capacity and fasting plasma lipids in healthy physically active individuals. The thesis consists of a literature review (Chapter 1), general methods (Chapter 2), four experimental chapters (Chapter 3-Chapter 6) and general discussion and conclusion (Chapter 7).

Chapter 3 presents a pilot study aimed to investigate whether high carbohydrate meals with high and low glycaemic index of foods present within meals developed by using the glycaemic index values from the published glycaemic index tables, produce significant differences in postprandial glucose response. Eight healthy active women consumed prescribed high carbohydrate diets with either high or low glycaemic index in a randomised counterbalanced order. The experimental meals which consisted of breakfast, morning snack and lunch were consumed after an overnight fast. Plasma glucose responses were measured at baseline and every 30 minutes for 300 minutes after baseline. We concluded that high carbohydrate meals with high and low glycaemic index prescribed using the glycaemic index values from the existing glycaemic index tables in the literature produced a significant difference in postprandial plasma glucose responses. Thus, for further studies high carbohydrate diets with high and low glycaemic index were developed using glycaemic index values from available glycaemic index tables.

The aim of Chapter 4 and Chapter 5 was to investigate the extent to which the glycaemic index of high carbohydrate diets consumed for 5 days reduces the rate of fat oxidation during endurance exercise and exercise capacity during running conducted in the fasted state in men and women. To determine this, 9 healthy physically active men (Chapter 4) and 9 healthy physically active women (Chapter 5) performed three treadmill runs to exhaustion at 65% $\dot{V}O_2$ max after their habitual diet, after 5 days on a high carbohydrate high glycaemic index diet, and after 5 days on high carbohydrate low glycaemic index diet, in a randomised counterbalanced order. Blood samples for the measurements of glucose, insulin, glycerol and non-esterified fatty acids, and expired air samples for the measurements of the rates of fat and carbohydrate oxidation were obtained at 15, 30, 45, 60, 75, 90 minutes and at the point of exhaustion. Running capacity was measured as time to exhaustion and distance covered. It was found that in both men and women, the extent to which high carbohydrate diets consumed for 5 days reduced the rate of fat oxidation during running in the fasted state was not influenced by the glycaemic index of the diet, and that glycaemic index of high carbohydrate diets consumed for 5 days had no impact on running capacity.

Chapter 6 aimed to investigate the impact of the consumption of high carbohydrate diets with high and low glycaemic index for 5 days on fasting plasma concentration of lipids, insulin sensitivity and biomarkers for endothelial function (i.e. intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) in physically active individuals. Fasting blood was collected from 17 healthy individuals on three occasions in a randomised counterbalanced order: after consuming habitual diet, after 5 days on high carbohydrate high glycaemic index diet and after 5 days on high

carbohydrate low glycaemic index diet. It was found that the extent to which high carbohydrate diets consumed for 5 days increases fasting plasma concentration of triglyceride and reduces the concentration of high density lipoprotein cholesterol was not influenced by the glycaemic index of the diets. It was also found that glycaemic index of high carbohydrate diets consumed for 5 days had no impact on insulin sensitivity or on biomarkers of endothelial activation.

In conclusion, consideration of the glycaemic index of high carbohydrate diets consumed by physically active healthy men and women for 5 days has no impact on insulin sensitivity and fasting concentration of plasma lipids, it does not influence the rate of fat oxidation induced by high carbohydrate diets during running conducted in the fasted state and has no influence on running capacity. Thus, when physically active individuals increase carbohydrate intake for the purposes of muscle glycogen accumulation, consideration of the glycaemic index is not important. Future studies are needed to determine whether the glycaemic index of high carbohydrate diets modify exercise energy metabolisms in top grade athletes.

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AUTHOR'S DECLARATION

I declare that the work contained in this thesis is original, and is the work of one author Sareena Hanim Hamzah except where otherwise stated. The information reported from other authors has been quoted with their names and source of publication. The relative contributions in terms of study design, data collection and analysis have been highlighted at the beginning of each research chapter.

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LIST OF ABBREVIATIONS

ACSM	American College of Sports Medicine
ADP	Adenine Diphosphate
AHA	American Heart Association
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphates
AUC	Area Under the Curve
BW	Body Weight
CAMs	Cellular Adhesion Molecules
CETP	Cholesterol Ester Transport Protein
CHD	Cardiovascular Heart Disease
CHO	Carbohydrate
CoA	Coenzyme A
EDTA	Ethylenediamine Tetra-acetic Acid
EE	Energy Expenditure
ETC	Electron Transport Chain
FABP	Fatty Acid Binding Protein
FAO	Food and Agriculture Organization
FFM	Fat Free Mass
GI	Glycaemic Index
GL	Glycaemic Load
GLUT-4	Glucose transporter type 4
HC-HGI	High Carbohydrate-High Glycaemic Index
HC-LGI	High Carbohydrate-Low Glycaemic Index

HDL	High Density Lipoprotein
HGI	High Glycaemic Index
$HOMA_{IR}$	Homeostasis Model Assessment for relative Insulin Resistance
HR	Heart Rate
HSL	Hormone Sensitive Lipase
IAUC	Incremental Area Under the Curve
ICAM-1	Inter Cellular Adhesion Molecule-1
IDL	Intermediate Density Lipoprotein
IMTG	Intramuscular Triglycerides
LDL	Low Density Lipoprotein
LGI	Low Glycaemic Index
LPL	Lipoprotein Lipase
MRS	Magnetic Resonance Spectroscopy
NADH	Nicotinamide Adenine Dinucleotide
NEFA	Non-Esterified Fatty Acids
NMES	Non-Milk Extrinsic Sugars
PAL	Physical Activity Level
P_i	inorganic phosphate
RCT	Randomised Controlled Trials
RER	Respiratory Exchange Ration
RMR	Resting Metabolic Rate
RPE	Ratings of Perceived Exertion
SD	Standard Deviation
SEM	Standard Error of the Mean
SFA	Saturated Fatty Acid

TC	Total Cholesterol
TCA	Tricarboxylic Acid
TG	Triglyceride
TMB	3,3',5,5'-tetramethylbenzidine
TRL	Triglyceride Rich Lipoprotein
VCAM-1	Vascular Cell Adhesion Molecule-1
$\dot{V}CO_2$	carbon dioxide production
VLDL	Very Low Density Lipoprotein
$\dot{V}O_2$	oxygen consumption
$\dot{V}O_2$ max	maximum oxygen consumption

Chapter 1 Introduction and Literature Review

1.1 Introduction

This chapter aims to provide evidence of the relevant scientific background of the studies contained in this thesis and to establish the theoretical basis for these studies. First of all, the concept of glycaemic index (GI) and factors influencing the GI of a food and meal are described. Then the energy metabolism and the impact of GI of high carbohydrate (CHO) diets on exercise energy metabolism and performance will be considered in relation to energy substrate availability and utilisation during endurance exercise. Finally, the impact of high CHO diets and the GI on plasma lipids and insulin sensitivity in healthy and disease state individuals will be also discussed.

1.2 Glycaemic index and glycaemic load

1.2.1 Concept of glycaemic index and glycaemic load

In the past, CHO was classified as simple sugars and complex CHO based on their chemical composition. Simple sugars include the monosaccharides (glucose, fructose and galactose) and disaccharides (sucrose, maltase and lactose) and complex CHO are long polymers of glucose (starch). It has been assumed that the chemical composition could predict the plasma glucose and insulin responses following ingestion of CHO foods with simple CHO producing a rapid rise and fall in glucose and insulin while complex CHO produce a flatter response. However, it has been observed that CHO foods independent of whether they are based on simple or complex CHO elicit very individual effects on plasma glucose quite separately and

unpredictably from its chemical composition due to various factors that influence their rate of digestion and absorption (Millet et al., 1995). Thus, these lead David Jenkins and colleagues (Jenkins et al., 1981) to propose the concept of GI. It was developed to obtain a numeric physiological classification of CHO foods based on the rate of CHO digestion and absorption and to define CHO foods according to their actual postprandial glycaemic impact. Primarily, it was developed to aid individuals with diabetes and hyperlipidemia for better controlling their plasma glucose levels while following a low fat, high CHO diet.

The GI is a ranking of foods based on their actual postprandial plasma glucose response compared to a reference food, either glucose or white bread (Jenkins et al. 1981). The GI is calculated by measuring the incremental area under the plasma glucose curve following ingestion of a test food providing 50 g of CHO, compared with the area under the plasma glucose curve following an equal CHO intake from the reference food, which is set to be 100 (Jenkins et al., 1981). Capillary blood samples was taken in the fasted state and at 30 minutes intervals for 2 hours after the ingestion of CHO, with all tests being conducted on the same individual after an overnight fast (FAO, 1998). The capillary blood technique was easier to conduct, produced greater glucose concentration and less variability than venous plasma (Wolever & Bolognesi, 1996a). Thus, differences between foods were larger and easier to detect statistically using capillary plasma glucose. Since plasma glucose responses vary considerably from day-to-day within participants, it was recommended that the standard food should be repeated at least three times in each participant to obtain a representative mean response to the standard food (Brouns et al., 2005). Originally the reference food used for calculating the GI was glucose

(Jenkins et al., 1981). Now the more commonly used reference food is white bread containing 50g of CHO (Jenkins et al., 1988; Miller et al., 1995) than glucose because it is more palatable and avoids the possibility of delayed gastric emptying from the high osmolality of a glucose solution (Jenkins et al., 1984). However, 50 g CHO in white bread is more difficult to determine accurately than is 50 g glucose. For the GI data, the area under the curve (AUC) is calculated as the incremental area under the plasma glucose response curve (IAUC) (FAO, 1998) and above the fasting plasma glucose concentrations ignoring the area beneath the fasting concentrations (Brouns et al., 2005; Wolever et al., 2003). Therefore, when a plasma glucose value falls below the baseline, only the area above the fasting level is included. This can be calculated geometrically by applying the trapezoid rule as described in Wolever & Jenkins (1986).

The GI of a food depends on the rate at which the CHO is digested in the gastrointestinal tract and the rate of absorption into the bloodstream (Jenkins et al., 1982; Englyst et al., 1999) which influences the extent and duration of the rise in glucose concentration after a meal. The insulin responses are related to the postprandial glycaemic responses (Wolever & Bolognesi, 1996b). In general, quickly digested and absorbed CHO will produce a rapid increase in plasma glucose and pancreatic insulin secretion which promotes glucose uptake to counteract the rise in plasma glucose concentrations, sometimes leading to a reactive hypoglycaemia. On the other hand, CHO in a low GI (LGI) food is absorbed at a slower rate than CHO from a high GI (HGI) food, which results in reduced postprandial glycaemia and insulinemia. In essence, the GI reflects the rate of digestion and absorption of a CHO-rich food. It compares equal quantities of CHO and provides a measure of

CHO quality but not quantity. Therefore, the GI allows foods to be categorized according to having a low, moderate or high GI. CHO foods evoking the greatest responses are considered to be HGI foods, while those producing a relatively smaller response are categorised as LGI foods. A food is said to be of a high GI if it has a value of more than 70, of moderate GI if it has a value of between 56 and 69, and low GI if lower than 55 (Foster-Powell, 2002).

It is important to note that the GI allows only for a relative comparison among foods, as a prerequisite for its measurement is the intake of available CHO, which is the CHO that is absorbed via the small intestine and used in metabolism, preferably in an amount of 50g (Jenkins et al., 1981). The methodological requirement for the GI determination applies solely to the situations where food servings contain only the amount of CHO used. However, CHO foods are seldom eaten alone, but rather as a part of mixed meal. In common day-to-day situations, the amount of CHO content ingested in foods and meals varies greatly.

To overcome this problem, glycaemic load (GL) was proposed by Salmeron et al. in 1997 as the arithmetic product of GI and CHO amount to quantify the overall glycaemic effect of a portion of food (Salmeron et al., 1997). This can be estimated by multiplying the GI of the food with the amount of CHO (in grams) presents in a specified serving size of that food and dividing by 100. For example, spaghetti has a lower GI than white bread, but normal portion size of spaghetti are commonly larger than portion of a white bread. Therefore GL may or may not differ between these two CHO sources, depending on applicable GI values and portions size. GL would seem to be a much better predictor than the amount of CHO alone, because similar

glycaemic responses were observed among foods differing in available CHO by more than twofold (Brand-Miller et al., 2003). This finding is consistent with Wolever & Bolognesi (1996b) who reported that both GI and the amount of CHO are necessary to explain most of the observed variability in glycaemic response. The GL is important as the higher this is, the greater the glycaemic response and therefore the insulinemic response, as insulin is released in response to glucose entering the bloodstream. A low GL food is considered to have a GL of less than 10, whilst a high GL food is considered to have a GL of 20 or more and of medium GL food when GL is between 10 and 20 (Salmeron et al., 1997).

1.2.2 Factors influence GI

The rate of digestion and absorption of the CHO-containing foods, and therefore the GI values, are influenced by the nature of the food and the type and extent of food processing. The nature of the food includes the amount and type of dietary fibre, the presence of large amount of fat or protein, nutrient-starch interactions in CHO-containing foods such as wheat products, the ratio of amylose to amylopectin present in the raw food and antinutrients such as phytic acid, lectins and tannins.

Resistant starch and dietary fibre are undigested and not absorbed in the small intestine and therefore contribute little to postprandial glycaemia. However, a lowering glycaemic response has been found when purified extracts of fibre, particularly of the type that forms a viscous gel in water such as guar gum, are added to a test food in a sufficient quantity (Tappy et al., 1996; Wolever et al. 1991). Findings from several studies investigating the effect of fibre content on the GI of foods are not consistent. Wolever (1990) found that insoluble fibre associated with

the GI while Nuttall (1993) suggested that only the soluble fibre has an effect on postprandial glucose concentrations when fibre was added to a CHO. Other studies found no relation between GI and the fibre content of the food (Jenkins et al., 1981) or between the postprandial insulin response to and the fibre content of a food (Holt et al., 1997). In addition, GI cannot be predicted from the fibre content of a CHO-containing food or from the terms wholemeal and wholegrain for which there are no universal accepted definitions. There were small differences in the GI between brown and white rice, brown and white spaghetti, and whole-wheat and white bread although their fibre contents were quite different. For example, from the GI tables (Foster-Powell et al., 2002), the mean GI of wholemeal bread from 13 studies is 71, while the mean GI of white bread from 6 studies is 70. However, wholegrain when largely intact, have been found to lower GI (Granfeldt et al., 1995).

The addition of protein or fat to CHO-containing foods lowers the overall GI (Miller et al., 2006). Protein that is included in a meal stimulates insulin secretion and reduces postprandial glucose responses (Gannon et al., 1988; Gannon & Nuttall, 1990; Nuttall et al., 1984). Furthermore, protein-rich foods are known to increase insulin secretion without augmenting glucose concentrations (Krezowski et al., 1984; Nuttall et al., 1984). The addition of fat delays gastric emptying which decreases the rate of availability of CHO for digestion and absorption in the small intestine and therefore reduces postprandial glucose responses (Collier et al., 1984; Welch et al., 1987). Adding 5-15 g protein or fat to CHO has been shown to reduce glycaemic responses in normal participants (Owen & Wolever, 2003; Spiller et al., 1987). However, neither fat nor protein in the amounts found in most foods (except for

peanuts and most nuts) significantly alters the glycaemic responses (Wolever et al., 1994).

Polymers of glucose can occur in a branched form known as amylopectin or linear form known as amylose (Anisson & Topping, 1994). The GI is affected by the proportion of amylose to amylopectin of the CHO foods. Single strand amylose is digested and absorbed slower than branched-molecule amylopectin, therefore a high amylose: amylopectin ratio decreases the plasma glucose response of starchy foods. The GI increases when the proportion of amylopectin is higher because amylopectin is more easily hydrolysed in the gut than amylose due to their molecular structures (Vanamelsvoort & Weststrate, 1992).

CHO foods which contain phytic acid produce lower glycaemic response (Thompson et al., 1987; Trout et al., 1993). Phytic acid, which is also referred as phytate, is a non-nutrient component of seeds. It is believed to interact with amylase or protein associated with starch, delaying the digestion of starch (Thompson et al. 1987). Other non-nutrients such as lectins and amylase inhibitors may reduce the glycaemic response of foods (Wolever & Jenkins, 1986).

Extrusion, flaking, grinding, canning, storing and cooking of CHO-containing foods can affect the particle size and the integrity of the starch granules (Ocana et al., 1988) and the plant cell walls (Ellis et al., 1991), making the CHO portion more accessible to digestive enzymes (Collings et al., 1981; Wolever, 1990). The method of processing a food can greatly change the GI of the food. Large granules of starch in the CHO foods are broken down by grinding, rolling or pressing so that the amylose

or amylopectin become available for hydrolysis. The grinding of food can elicit greater glycaemic responses because ground foods increase the number of food particles and therefore the surface area, making the CHO more accessible for digestion. Ground foods also absorb larger amounts of water during cooking, allowing for increased gelatinisation and they may also empty faster from the stomach than ungrounded foods (Collings et al., 1981). Cooking of CHO foods, especially starchy foods can increase the glycaemic response for those foods (Collings et al., 1981). It appears that with cooking, such as boiling, the granules of starch rupture due to swelling, making starch more vulnerable to digestive enzymes. When the starch is then left to cool or stored for a time, it is gelatinised to which vary in structure depending on the amount of moisture, the amylose to amylopectin ratio and the time and temperature of storage (Annison & Topping, 1994). Retrogradation of starch is a crystallisation of the gel which makes it insoluble thus not amenable to hydrolysis in the small intestine. Repeated cycles of heating and cooling can further the retrogradation (Sievert et al., 1991). The GI of foods is influenced by the heat exposure, the amount of water present, and the time of cooking, (Vaaler et al., 1984; Collings et al., 1981). Thus, the more a starch-containing food is heated, moisturized, ground, or pressed, the more it will be amenable to hydrolysis and digestion, except for the portion that forms insoluble complexes. Processing methods such as extrusion, cooking, explosion, puffing and instantiation make starchy foods more readily digestible (Brand et al., 1985).

The GI values of foods must provide a reliable and consistent measure of relative plasma glucose response, and using the GI to manipulate meals and diets must produce a desired metabolic outcome. The methodology of the determination of the

GI has been discussed by numerous authors involving issues such as size of CHO load, 25 or 50 g; reference product, glucose vs. white bread; time of follow-up, 2 or 3 hours and different ways of calculation have been proposed (Wolever, 2004). The variability in the GI values reported by different laboratories for a single food are caused by numerous factors such as the nature of the CHO foods (sugar or starch); the type of sugars (fructose or sucrose); the status of starch (gelatinised or retrograded); the matrix of food (fibres, protein, fat, the amylase : amylopectin ratio, the particle size and food form); the anti-nutrients (enzyme inhibitors, lectins, tannins), differences in processing or cooking techniques (time, temperature, water content, etc), and variation between the particular species of commercial brands of a processed food. In general, published GI values represent the average of the values reported from a number of studies and a number of laboratories (Foster-Powell et al., 2002). Overall, broad agreement has been found between the relative GIs of foods, and the concept is strong enough to cope with inter-individual differences.

1.3 Energy metabolism during endurance exercise

1.3.1 Energy substrates and main metabolic pathways

CHO and fat are the main energy substrates for the continuous aerobic adenosine triphosphates (ATP) resynthesis during endurance type of exercise. CHO is stored in the body as glycogen within the skeletal muscle and in the liver. The precursor for muscle glycogen synthesis is the circulating glucose. Under resting conditions, glucose is taken up by the skeletal muscle by facilitated diffusion mediated by glucose transporter type 4 (GLUT-4) proteins in response to insulin concentration (Thorell et al., 1999) and the glucose is phosphorylated by the action of glycogen

synthase to form muscle glycogen (Friedman et al., 1991). Fat is deposited in the body as triglyceride (TG) in subcutaneous and deep visceral adipose tissue, and as intramuscular triglyceride (IMTG) in fat droplets between myofibrils close to mitochondria inside the muscle fibre (Hoppeler et al., 1985). Some fat is present in the circulation as non-esterified fatty acids (NEFA) bound to albumin or as fatty acids incorporated in TG of circulating lipoprotein particles such as chylomicrons, and very low density lipoprotein (VLDL). The precursors for IMTG formation are NEFA from the circulation, NEFA released from extramyocellular fat within muscle or NEFA released by the action of lipoprotein lipase (LPL) from plasma TG rich lipoproteins (TRL) such as chylomicrons and VLDL (Kimber et al, 2003). In non-contracting muscle, these NEFA are transported into the muscle cells via fatty acid binding proteins (FABP) or simple diffusion for a smaller portion (Hamilton & Kamp, 1999; Schaffer, 2002) where they are subsequently esterified in the sarcoplasmic reticulum to form IMTG (Sacchetti et al., 2002).

During endurance exercise, muscle glycogen and plasma glucose derived from the liver are very important substrates for ATP resynthesis. Glucose derived from glycogenolysis and from the circulation is taken up by the skeletal muscle via GLUT-4, which are translocated to the cell membrane in response to muscle contraction (Thorell et al., 1999). Glucose is then phospohorylated to glucose-6 phosphate catalysed by hexokinase and further degraded by the process of glycolysis in the sarcoplasm to form pyruvate. Pyruvate is then transported across the mitochondrial membrane by a specific carrier protein. Inside the mitochondria, pyruvate is converted to acetyl coenzyme A (CoA) and enters the tricarboxylic acid (TCA) cycle, which is a series of reactions to generate hydrogen atoms. The

hydrogen atoms are carried by the reduced coenzymes (nicotinamide adenine dinucleotide; NADH) for their subsequent passage to the electron transport chain (ETC). The ETC, with the presence of oxygen, accepts the reducing equivalents (NADH) to generate the proton motive force, which provides the chemical energy used to synthesise ATP from inorganic phosphate (P_i) and adenosine diphosphates (ADP) in the process of oxidative phosphorylation. As exercise continues, glycogen utilisation in the muscle declines due to reduced muscle glycogen content and glucose availability.

NEFA derived from the adipose tissue is the main energy substrate during low intensity exercise while both IMTG and plasma NEFA are important fuels during moderate intensity exercise. Muscle contraction and catecholamine activate hormone sensitive lipase (HSL) (Watt et al., 2003a; Langfort et al., 2000) in the skeletal muscle and adipose tissue to hydrolyse the TG molecules into its components, three molecules of NEFA and one molecule of glycerol from one molecule of TG. This process of breaking down TG is known as lipolysis. HSL is a neutral lipase that cleaves the first and second fatty acids from the glycerol backbone. NEFA also can be derived from the TG core of circulating chylomicron and VLDL, which are hydrolysed by LPL. NEFA are taken up by the muscle cells by the FABP and then are activated via binding with CoA and converted to fatty acyl-CoA. Fatty acyl-CoA molecules bind with carnitine to form fatty acyl carnitine compound and are transported across the mitochondrial membrane while bound with carnitine. Inside the mitochondria, the carnitine is removed and fatty acyl-CoA molecules are metabolized in the β-oxidation pathway, a process of sequential removal of 2 carbon units from the fatty acid chain. The production of NADH and acetyl-CoA then enter

the TCA cycle for subsequent passage to the ETC allowing oxidative phosphorylation to regenerate ATP from ADP and P_i. Figure 1.1 presents the schematic overview of energy production from CHO and fat in skeletal muscle.

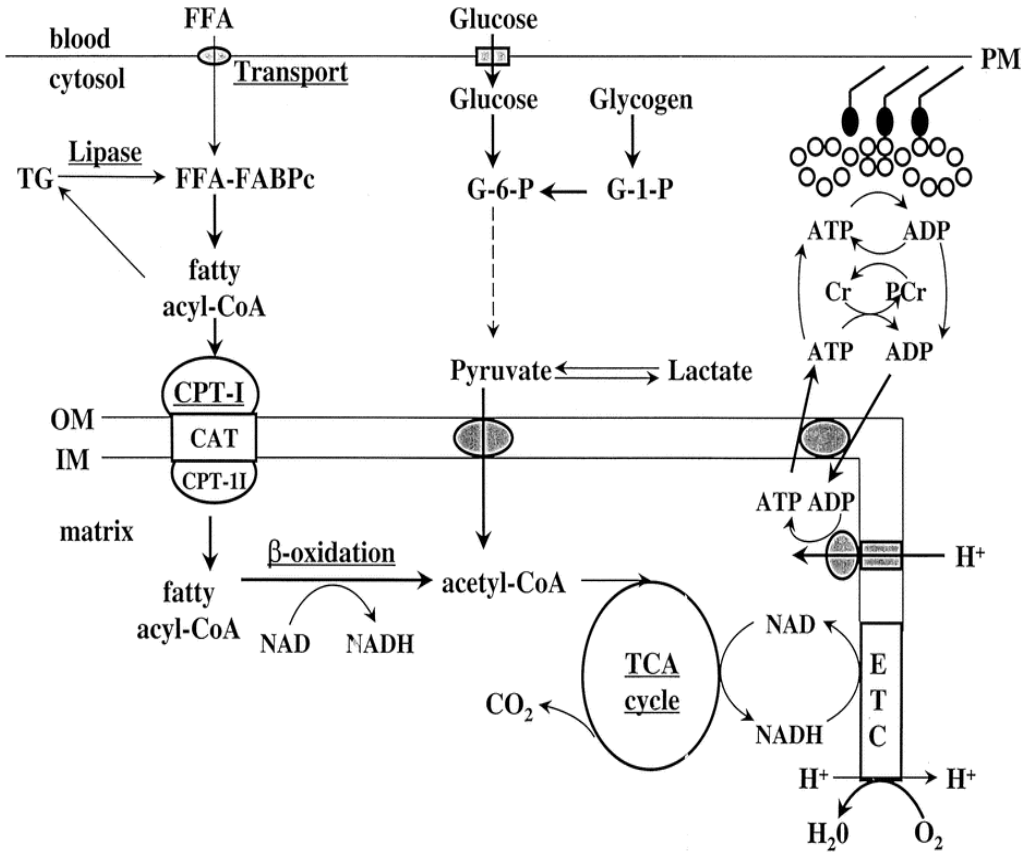


Figure 1.1 Schematic overview of energy production from CHO and fat in skeletal muscle. PM, plasma membrane; OM, IM, outer and inner mitochondrial membranes; NEFA, free fatty acid; FABPc, cytoplasmic fatty acid binding protein; TG, triacylglycerol; CoA, coenzyme A; CPT I, II, carnitine palmitoyltransferase I and II; CAT, carnitine-acylcarnitine translocase; NAD, NADH, oxidised and reduced nicotinamide adenine dinucleotide; G-6-P, G-1-P, glucose 6- and 1-phosphate; PCr, phosphocreatine; Cr, creatine; TCA, tricarboxylic acid; ETC, electron transport chain. *Adapted from Spriet (2002).*

During endurance exercise, both CHO and fat energy pathways are up-regulated to meet the increase in energy demand. In 1963, Randle et al. proposed the glucose-fatty acid cycle to explain the reciprocal relationship between CHO and fat metabolism where the increase in NEFA availability resulted in increased fat metabolism and inhibition of carbohydrate metabolism (Randle et al., 1963). During exercise, a fat-induced shift in CHO metabolism involves the enzymes that play key roles in regulating CHO metabolism and oxidation. For example, accumulation of acetyl-CoA inhibit pyruvate dehydrogenase, accumulation of citrate inhibit phosphofructokinase, and accumulation of glucose-6-phosphate reduce hexokinase activity which then inhibit CHO metabolism with increasing availability and oxidation of NEFA (Jeukendrup, 2002). Although there is some evidence for the existence of the glucose-fatty acid cycle at rest and during low-intensity exercise, it cannot explain substrate use at moderate to high exercise intensities. More recently, evidence has accumulated that an increase in CHO metabolism might regulate fat metabolism (Coyle et al., 1997; Sidossis et al., 1996, 1999). CHO-induced down regulation of fat metabolism and oxidation involve transport of long chain fatty acids into the sarcoplasm (fatty acid translocase CD36), release of fatty acids from IMTG (HSL) and transport of NEFA across the mitochondria membrane (carnitine palmitoyl transferase complex) (Jeukendrup, 2002). For example, the accumulation of acetyl-CoA that results in acetylation of the carnitine pool, reducing the free carnitine concentration and thus could theoretically reduce NEFA transport into the mitochondria. The proportions of energy from CHO and fat during endurance exercise are influenced by several factors, which are briefly discussed in this section of this chapter.

1.3.2 Impact of intensity and duration of exercise

During exercise the proportion of CHO and fat oxidised as well as rate of fat and CHO oxidation depends on the exercise intensity (Achten et al., 2002, Romijn et al., 1993; Sidossis et al., 1997). Study of endurance trained men after an overnight fast had found that plasma NEFA decreased whilst glucose tissue uptake and muscle glycogen oxidation increased as the intensity of exercise increased (Romijn et al., 1993). In this study, stable tracers and indirect calorimetry were used to evaluate the regulation of endogenous fat and glucose metabolism during exercise intensities of 25% $\dot{V}O_2$ max (low), 65% $\dot{V}O_2$ max (moderate) and 85% $\dot{V}O_2$ max (high). It was found that plasma NEFA and fat oxidation was highest during exercise at 25% $\dot{V}O_2$ max, and then declined progressively as exercise intensity exercise increased to 65% $\dot{V}O_2$ max and 85% $\dot{V}O_2$ max. Lipolysis of IMTG was not stimulated during low intensity exercise. However, IMTG was stimulated when exercising at 65% $\dot{V}O_2$ max but did not increase when exercise intensity was increased to 85% $\dot{V}O_2$ max. During exercise at 65% $\dot{V}O_2$ max, lipolysis in the peripheral adipocytes and of the IMTG stores contributed equally to fat oxidation. Figure 1.2 shows the contribution of plasma glucose and plasma NEFA, muscle glycogen and IMTG stores to energy expenditure during 30 minutes of exercise at 25, 65 and 85% of $\dot{V}O_2$ max.

The decline in plasma NEFA with increasing exercise intensity is counterbalanced by increase in plasma glucose concentration. NEFA and glucose taken up by the tissues during exercise are almost completely oxidised (Coggan et al., 1990), thus these results indicated a progressive shift from plasma NEFA oxidation to plasma glucose oxidation with increasing intensity. It has been shown that muscle glycogen become

the most important substrate when the exercise intensity increases above 50% $\dot{V}O_2$ max (Romijn et al., 1993; van Loon et al., 2001). Several studies have indicated that this limitation in fat oxidative capacity is mainly due to the rate of long chain NEFA transport over the mitochondrial membrane (Coyle et al. 1997, Jeukendrup, 2002). In a more recent study, Birnbaum et al. (2008) compared the energy substrate utilisation during low intensity (30% $\dot{V}O_2$ max) and high intensity (70% $\dot{V}O_2$ max) exercise in ten college-aged men. Results showed that during exercise at 30% $\dot{V}O_2$ max, the energy derived from fat oxidation was 60.5% and the remaining 39.5% was derived from CHO oxidation. While during the 70% $\dot{V}O_2$ max session, 90.1% of the energy was derived from CHO oxidation compared to 9.9% from fat oxidation.

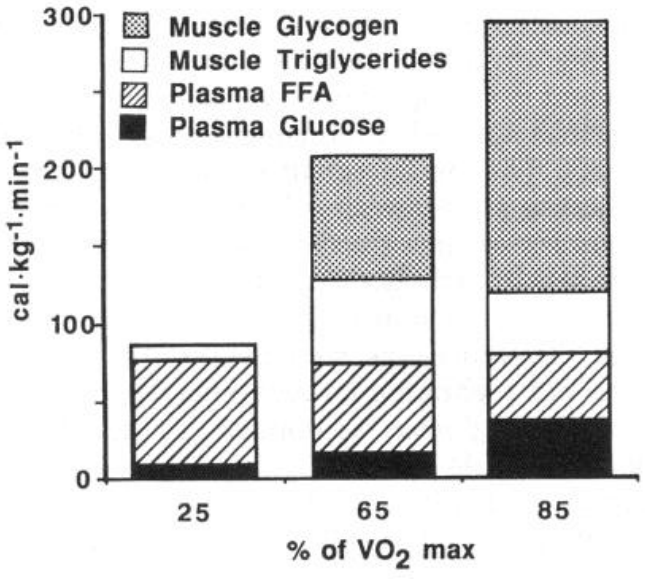


Figure 1.2 Contribution of plasma glucose and plasma NEFA, muscle glycogen and IMTG stores to energy expenditure during 30 minutes of exercise at 25, 65 and 85% of $\dot{V}O_2$ max. *Adapted from Romijn et al. (1993).*

Achten & Jeukendrup (2003) determined the exercise intensity which elicits the maximum rate of fat oxidation. Endurance trained men performed three graded exercise test to exhaustion on a cycle ergometer on one occasion after an overnight fast. Results showed that maximal fat oxidation rates of 0.52 ± 0.15 g/min were reached at exercise intensity of 63% $\dot{V}O_2$ max. However, there was a relatively large inter-individual variation in the rate of maximal fat oxidation within the group of this study. Romijn et al. (1993) also showed that endurance trained men have their highest fat oxidation rate at 65% $\dot{V}O_2$ max and when the same protocol was performed on endurance trained women, a similar maximal rate of fat oxidation as in men was found in women (Romijn et al., 2000). Other findings reported that endurance trained men (Knechtle et al., 2004) and women (Astorino, 2000; Knechtle et al., 2004) had their highest fat oxidation at 75% $\dot{V}O_2$ peak.

The mode of exercise can also affect fat oxidation. Achten et al. (2003) demonstrated that maximal fat oxidation rates were significantly higher during running compared with cycling (0.65 versus 0.47 g/min, respectively) in moderately trained men. The intensity, which elicited maximal fat oxidation, was not significantly different between the cycle ergometer and treadmill test (62.1 versus 59.2 % $\dot{V}O_2$ max, respectively). Knechthe et al. (2004) found that at 65% $\dot{V}O_2$ peak, both men and women showed a significantly higher maximal fat oxidation for running compared to cycling.

Total fat oxidation has been shown to increase and CHO oxidation decrease as the duration of exercise increased (Romijn et al., 1993). During exercise for 2 hours at

65% $\dot{V}O_2$ max there was a progressive increase in plasma NEFA and glucose availability over time (Romijn et al., 1993). This increased fat oxidation is likely to be caused by a reduction in muscle glycogen stores towards the later stages of prolonged exercise. There is evidence to suggest that muscle glycogen contribution to energy production decreases over time during exercise of moderate to high intensity (Romijn et al., 1993). It is thought that the development of fatigue occurs when the muscle glycogen concentrations reach very low levels and CHO oxidation rates cannot be maintained at a sufficient rate to maintain ATP resynthesis. Watt et al. (2003b) investigated IMTG utilisation during 4 hours of moderate intensity exercise measured by muscle biopsies. They found a decrease in IMTG content during the first 2 hours of exercise and an increase in plasma NEFA concentration and its rate of oxidation during the latter stages of prolonged exercise.

1.3.3 Impact of training status

Training status has also been recognised as an important factor modifying substrate use. It has been reported that training induced an increase in GLUT-4 protein content, and therefore glucose uptake, in the glycogen depleted state during intense exercise after 3 weeks of endurance training (Kristiansen et al., 2000). Numerous exercise studies have confirmed that training induce increases in fat oxidative capacity (Coggan et al., 1995; Kiens et al., 1993; Martin et al., 1993, Phillips et al., 1996). Training studies applying either labelled fatty acids tracer or muscle biopsies taken before and after exercise had demonstrated a substantial increased in IMTG utilisation during moderate-intensity exercise after 1-3 months of training (Martin et al. 1993; Philips et al., 1996; Schrauwen-Hindering et al., 2006). Cross-sectional studies comparing substrate use between trained and untrained participants have

reported similar results (Klein et al., 1994; Sidossis et al. 1998). Therefore, the increase in total fat oxidation after training is mainly due to the increased IMTG and thus the capacity to utilize IMTG during exercise is related to training status (Schrauwen-Hindering et al. 2003). A recent study investigated the effect of consistent training in the fasted state, versus training in the fed state, on muscle metabolism and substrate selection during fasted exercise (Van Proeyen, 2010). This study showed that training in the fasted state increased IMTG breakdown during a 2-hour constant-load exercise bout in the fasted state at ~65% pre-training $\dot{V}O_2$ max while training in fed state (CHO were ingested before and during exercise) did not affect IMTG breakdown. This was related to the more evident increase in muscular oxidative capacity during training in the fasted state. In addition, training in the fasted state prevented the development exercise-induced drop in plasma glucose concentration during fasting exercise (Van Proeyen, 2010).

1.3.4 Impact of Gender

There are several differences between men and women with regards to substrate utilisation during exercise. Tarnopolsky et al. (1995) demonstrated that only endurance trained men but not women increased their muscle glycogen concentration in response to an increase in dietary CHO intake (from 58 to 75% of energy) consumed for 4 days. Men increased muscle glycogen concentration by 41% in response to the dietary manipulation and had a corresponding increase in performance time during an 85% $\dot{V}O_2$ peak trial, whereas women did not increase glycogen concentration or performance time. Women oxidised significantly more lipid and less CHO and protein compared with the men during exercise at

75% $\dot{V}O_2$ peak. The study found that the relative CHO intake expressed per kg free fat mass (FFM) per day, for men was increased from 7.7 to 9.6 g/kg FFM/day and for women from 5.9 to 7.9 g/kg FFM/day, when they increased their CHO intake from 58 to 75% of energy intake. Thus by increasing recommended CHO intake estimated as percentage of total energy intake, women did not achieve the recommended daily CHO intake expressed per kg FFM i.e. >10 g/FFM/day (Burke & Hawley, 2006; Bussau et al., 2002) thus might explain why muscle glycogen supercompensation was not achieve for women in this study (Tarnopolsky et al., 1995).

It has also been reported that in aerobically trained women, the magnitude of increase in muscle glycogen content in response to a high dietary CHO (78% of energy) compared with a moderate CHO (48% of energy) intake was smaller than previously observed in men (Walker et al., 2000). In the study by Tarnopolsky et al. (1995) the absence of supercompensation of muscle glycogen in women was explained by lower CHO intake relative to FFM in women than men, therefore a study of James et al. (2001) re-examined this issue using participants fed comparable amounts of CHO. Endurance-trained women and men ingested 12 g CHO/kg FFM/day in conjunction with the cessation of their daily physical training. A 3-day exposure to this diet resulted in a marked rise in muscle glycogen levels in both the men and women. This study concluded that women athletes have the capacity to accumulate supranormal levels of muscle glycogen, and that when exercise-trained men and women are fed comparable amounts of CHO relative to FFM, there is no gender-related difference in their ability to accumulate supranormal levels of muscle glycogen (James et al., 2001).

Another study suggested that women during CHO loading can achieve recommended relative CHO intake only when they also increase daily energy intake (Tarnopolsky et al., 2001). This study reported that during glycogen loading in women, CHO intake provided 75% of energy and dietary energy intake was increased by 30%. Daily CHO intake expressed as per kg of body weight (BW) and per kg FFM was 8 g/kg BW/day and 10 g/kg FFM/day, respectively and resulted in muscle glycogen supercompensation. This finding that women who attempt to increase muscle glycogen stores should be particularly attentive to both total energy intake and CHO intake relative to FFM is supported by other studies (James et al., 2001; Sedlock, 2008). Thus, it seems that in trained women as well as in trained men, muscle glycogen loading is achievable when CHO intake is increased to recommended values relative to FFM (Burke & Hawley, 2006; Bussau et al., 2002).

Women oxidise more fat and less CHO compared with men during endurance exercise (Knechtle et al., 2004; Venables et al., 2005; Wallis et al., 2006; Tarnopolsky et al., 2008). The higher in fat oxidation is associated with higher IMTG content (Devries et al., 2007; Hoeg et al., 2009; Roepstorff et al. 2006; Tarnopolsky et al., 2007) and IMTG utilisation (Roepstorff et al., 2006; Steffensen et al., 2002) as well as greater adipocyte lipolysis (Tarnopolsky et al., 2008) in women than men. Previous evidence indicated that in women, in which a combination of an isotope tracer technique and indirect calorimetry was used, the additional source of fatty acids oxidised during exercise was provided by IMTG (Horowitz & Klein 2000; Romijn et al. 2000).

1.3.5 Impact of pre-exercise content of muscle glycogen and IMTG

Pre-exercise muscle substrate concentration which is mainly modified by CHO intake is an influential factor in the source of substrate utilisation during exercise. Increasing muscle glycogen content before competition and replenishment of glycogen stores after prolonged exercise is achieved by the consumption of high CHO diet. It has been recommended that for achieving the required levels of muscle glycogen during training and competition athletes should consume 8-12 g of CHO per kg BW (Brown, 2002; Bussau et al., 2002; Burke & Hawley, 2006). For example, in a study of Bussau et al. (2002), eight endurance trained athletes consumed 10 g CHO/kg BW/day for 3 days while remaining physically inactive. Muscle biopsies taken after day 1 and day 3 on a high CHO diet showed that muscle glycogen content increased significantly from pre loading levels after only 1 day, and remained stable afterwards despite another 2 days of CHO rich diet. Thus, a high CHO intake increased muscle glycogen content and reached maximal storage levels after only 1 day on a high CHO diet provided the athletes remained inactive. The ingestion of a high CHO diet increases the availability of glucose and the secretion of pancreatic insulin which enhances GLUT-4 concentrations and glycogen synthase activities for muscle glycogen synthesis. The greater pre-exercise muscle glycogen concentration leads to a greater reliance on muscle glycogen during exercise which enhances performance in endurance events exceeding 90 minutes (Brewer et al., 1988; Hargreaves et al., 2004; Rauch et al., 1995). However, the greater rates of glycogenolysis and glycogen depletion (Arkinstall et al., 2004; Hargreaves et al., 1995; Wojtaszewski et al., 2003) reduces the availability and contribution of fat towards exercise energy expenditure (Brewer et al., 1988; Coyle et al., 2001) and,

thus may attenuate the enhancement of exercise performance expected from a high CHO intake.

The reduced lipolysis during exercise after a high CHO diet was related to reduced availability of IMTG and reduced oxidation of IMTG (Coyle et al., 2001: Johnson et al., 2003). It has been shown that a high CHO diet substantially decreased IMTG storage (Coyle et al., 2001, Johnson et al., 2003). In a study by Johnson (2003), vastus lateralis IMTG content was assessed by H-1-magnetic resonance spectroscopy (MRS) before and after 3 hours time trial cycling bouts. High CHO (9.3 g/kg BW/day) and low CHO (0.6 g/kg BW/day) diets were consumed by the highly trained cyclist in a double-blind, randomised crossover design after the exercise bout. The results showed that the resting IMTG content was significantly lower after a high CHO diet than a low CHO diet and that the IMTG content was reduced during exercise by 57 and 64%, respectively. Thus, reduced IMTG content after a high CHO diet reduces its utilisation during exercise and increases reliance on CHO oxidation. Similar results were observed in a study by Coyle et al. (2001) where a high CHO diet reduced whole body lipolysis, total fat oxidation, and non-plasma NEFA oxidation during exercise in the fasted state associated with a reduced concentration of IMTG.

Several studies have reported that a high CHO recovery diet increases post-exercise muscle glycogen repletion (Burke et al. 1993; Kiens et al. 1990; Tsintzas et al. 2003) but reduces the post-exercise resynthesis of IMTG (Coyle et al. 2001, Decombaz et al., 2000; 2001). Decombaz et al. (2001), applied the MRS technique reported that a high CHO diet (70% of total energy) consumed within 32 hours following a 2-hour

run at 50% $\dot{V}O_2$ max reduced IMTG stores by 17% when compared to the pre-exercise values. Starling et al. (1997) showed that a high CHO diet ingested during 24 hours recovery after a 2-hour cycling at 65% $\dot{V}O_2$ max resulted in lower IMTG concentrations than a high fat diet. The reduction in IMTG concentration after a high CHO diet might be the result of a high concentration of insulin which due to its inhibitory action on HSL and muscle LPL reduces NEFA availability for IMTG synthesis (Roberts et al., 1988). Failure to replenish muscle stores in the post-exercise period can be detrimental to subsequent exercise performance (Achten et al., 2004).

1.3.6 Impact of fed versus fasting states

Muscle glycogen content is enhanced by the ingestion of CHO meal of 2.5-4.0 g CHO/kg BW 3-4 hours before exercise and improves subsequent exercise performance (Chryssanthopoulos et al., 2002; Schabort et al. 1999). However, the ingestion of CHO before exercise inhibits lipolysis and fat oxidation during subsequent exercise (Coyle et al. 1997; Horowitz et al., 1997). Although the increased plasma insulin following CHO ingestion (Coyle et al., 1997) in the hour before exercise inhibits lipolysis (Horowitz et al., 1997), there is no convincing evidence that this is always associated with impaired exercise performance. Interventions to increase fat availability before exercise have been shown to reduce CHO utilisation during exercise, but do not appear to have ergogenic benefits (Hargreaves, 2004). On the other hand, a study by Pitsiladis & Maughan (1999) suggested that increasing fat availability immediately before exercise by acute fat feeding and heparin infusion could improve endurance exercise in a cool environment in well-trained individuals. Time to exhaustion increased from 118.2 ±

12.4 min on the CHO trial to 127.9 ± 12.1 min on the fat trial. Although no difference in the respiratory exchange ratio (RER) was found between trials, there was an earlier reduction in RER and an earlier rise in rating of perceived exertion (RPE) observed on the fat trial. However these findings were not significant. No difference in total CHO oxidation was found between trials (Pitsiladis & Maughan (1999).

Increased plasma insulin at the onset of exercise caused by a high CHO intake a few hours before exercise reduced plasma glucose and plasma NEFA and increased reliance on muscle glycogen compared with exercise in the fasted state (Coyle et al., 1985; Heath et al. 1983). However, it has been shown that plasma insulin returned to fasting basal levels 2-3 hours following a CHO meal in lean endurance trained athletes (Heath et al. 1983). Coyle et al. (1985) showed that pre-exercise feedings altered substrate availability and utilisation during exercise that was begun after plasma insulin returned to fasting basal levels. In this study, 7 endurance trained cyclists performed 105 minutes of exercise at 70% $\dot{V}O_2$ max following either a high CHO (2 g/kg BW) meal 4 hours prior to exercise or a 16 hours fast, in randomised counterbalance order. They found that the pre-exercise meal produced a transient elevation of plasma insulin and plasma glucose, which returned to fasting basal levels prior to the initiation of exercise. The high CHO meal increased glycogen content within the vastus lateralis by 42% at the beginning of exercise. During the first hour of exercise, plasma glucose, plasma NEFA and glycerol were lower and the rate of CHO oxidation was higher in the fed state compared with exercise when participants were fasted. There were no significant differences on the measured parameters in both fed and fasted states after 105 minutes of exercise. Although

plasma insulin had returned to fasting basal levels before the beginning of exercise, the plasma glucose concentration was still declined during the first hour of exercise. These lowering effects after a high CHO meal was suggested not to be mediated by the action of insulin but rather caused by the persistent effect of insulin on suppressing lipolysis in adipocytes.

There is evidence that exercise in the fasted state increase IMTG utilisation than exercise postprandially in conjunction with CHO intake (De Bock et al., 2005). In this study, 9 young healthy volunteers participated in two experimental sessions in which they performed 2 hours of constant load cycling at 75% $\dot{V}O_2$ max followed by 4 hours of controlled recovery in each session. On one occasion they exercised after an overnight fast and on the other they received CHO before (150g) and during (1 g/kg BW/hour) exercise. In both conditions, participants ingested 5 g of CHO/BW during recovery. They observed that exercise-induced net glycogen breakdown was similar during exercise in the fasted and CHO-fed stated. However, IMTG breakdown was evident during prolonged submaximal exercise only in the fasted state. In addition, exercise in the fasted state enhanced the post-exercise insulin response to glucose ingestion which is likely to contribute to stimulation of post-exercise muscle glycogen resynthesis (De Bock et al., 2005).

1.4 Impact of GI of a single meal and of diet on exercise energy substrates utilisation and performance

1.4.1 Impact of GI of pre-exercise meals

There have been many studies investigating whether consideration of foods with LGI can be used as a strategy to prevent the reduction in fat oxidation induced by high CHO and thus improve athletic performance. The majority of investigations have looked at the impact of a single meal's GI consumed prior to exercise. Several studies have suggested that lipid availability and fat oxidation during endurance exercise conducted under conditions of a high CHO intake might be influenced by the GI. A higher rate of fat oxidation and a better maintenance of plasma glucose concentrations during subsequent exercise were reported after ingesting a single LGI CHO food compared with a single HGI CHO food (DeMarco et al., 1999; Febbraio & Stewart, 1996; Sparks et al., 1998; Thomas et al., 1991). These studies carried out on single GI food are in agreement with some recent studies carried out on mixed GI meal (Stevenson et al., 2006; Wee et al., 1999, 2005; Wu et al. 2003; Wu & Williams, 2006).

Wee et al. (1999) observed an increase in plasma NEFA and fat oxidation during a run to exhaustion at 70% $\dot{V}O_2$ max after ingesting a LGI breakfast compared with a HGI breakfast. Wu et al. (2003) showed that pre-exercise CHO meal (2 g CHO/kg BW) with LGI resulted in higher rates of fat oxidation than the HGI during 60 minutes subsequent running at 65% $\dot{V}O_2$ max . Similar findings were reported when the same study design was used on women (Stevenson et al., 2006). On the other hand, using the needle biopsy technique, muscle glycogen concentration was increased by 15% after a HGI meal but no change was observed after a LGI meal (Wee et al., 2005). It is most likely that the rapidly digested and absorbed foods in the HGI meal supplied the necessary glucose to the blood and muscle for glycogen synthesis within the 3 hours postprandial period. During the subsequent 30 minutes

of running on the treadmill at 71% $\dot{V}O_2$ max, the muscle glycogen utilisation during exercise was greater in the HGI trial compared with the LGI trial (Wee et al., 2005). Most of these studies showed a greater incremental area under the plasma glucose and insulin responses curve after the HGI meal than those of LGI meal during the 2-3 hour postprandial period (Stevenson et al., 2005a; Wee et al., 1999, 2005; Wu et al., 2003). The rapid increase in plasma glucose concentration following a HGI meal induced an increase in plasma insulin concentration (Wolever & Bolognesi, 1996a). A large release of insulin rapidly reduced the plasma glucose concentration by both increasing cellular absorption of glucose and by reducing hepatic output due to inhibition of gluconeogenesis and glycolysis (Camacho et al., 2004), which may cause hypoglycaemia in the 2-3 hour postprandial period. In addition, increases in insulin concentration after HGI meal reduced the rate of adipose tissue lipolysis by inhibiting HSL and hence the availability of circulating NEFA , which reduced the uptake of fatty acids by muscle cells and thus diminished fat oxidation (Coyle et al.. 1997; Sidossis et al., 1998). In contrast to a HGI meal, the insulin response to a LGI meal is much lower and therefore HSL suppression is attenuated, which leads to better maintenance of the circulating NEFA concentration and enables a greater rate of fat oxidation during subsequent exercise (Stevenson et al., 2006; Wee et al., 2005; Wu et al., 2003; Wu & Williams, 2006).

1.4.2 Impact of GI meals during recovery from prolonged exercise

A number of studies have reported that GI plays a significant role in exercise recovery (Burke et al., 1993; Stevenson et al., 2005b; Stevenson et al., 2009; Trenell et al., 2008). Burke et al. (1993) reported that after glycogen depleting exercise, a high CHO (10 g/kg BW) with HGI diet consumed over a recovery period of 24 hours

resulted a 35% increase in muscle glycogen storage than in the isocaloric high CHO with LGI diet. The greater accumulation of muscle glycogen following the HGI diet was a result of the greater insulinemic response favouring muscle glycogen resynthesis (Burke et al., 1993; Stevenson et al.,2005a; Van Den Bergh, 1996). However, another study found no differences in muscle glycogen storage between HGI and LGI diets when consumed during 20 hour of recovery (Kiens et al., 1990). However, in this study the diets were described interchangeably as simple (HGI) and complex (LGI) CHO.

As mentioned earlier, high CHO recovery diet reduces the post-exercise resynthesis of IMTG (Decombaz et al., 2001, Starling et al., 1997). Thus, during recovery from exercise when meals are consumed every 4 hours, a LGI diet may favour IMTG resynthesis over a HGI diet. However, when intervals between meal consumption are greater than 4 hours, IMTG synthesis may be enhanced more after a HGI meal than a LGI meal. This may be explained by the plasma NEFA concentration rebound being higher after a HGI meal than after a LGI meal due to a quicker return of insulin to basal values and release of higher concentration of catecholamine after a HGI meal than after a LGI meal (Wolever et al.,1995). Indeed, greater concentration of plasma NEFA have been reported the morning after a HGI evening meal compared to a LGI evening meal (Stevenson et al., 2008), which would suggests that when there are long periods between meals such as overnight, a HGI meal may benefit IMTG synthesis.

In the earlier study, using a muscle biopsy technique, a moderate intake of CHO (47% of energy) of HGI diet consumed for 30 days resulted in a higher IMTG

concentration compared to LGI diet (Kiens & Richter, 1996).When a higher CHO (8-10 g/kg BW) intake was consumed, by using the MRS technique, HGI resulted in a greater IMTG accumulation after the 24 hour recovery period (Kiens & Richter, 1996; Stevenson et al., 2009; Trenell et al., 2008). Three recent studies have investigated the impact of the GI of high CHO (8 g/kg BW) diets during 24 h recovery between bouts of prolonged strenuous exercise (90 minutes cycle at 70% $\dot{V}O_2$ max) on substrate availability and utilisation (Stevenson et al., 2005b, 2009; Trenell et al., 2009). These investigations found that the LGI diet resulted in greater availability of plasma NEFA (Stevenson et al., 2005b,2009; Trenell et al., 2008) and a higher rate of fat oxidation (Stevenson et al., 2005b) than the HGI diet during the subsequent exercise conducted in the fasted state. The reduction in plasma NEFA availability has been shown to increase reliance on IMTG as a fuel source during exercise (Stellingwerff et al., 2007). The differences in IMTG utilisation between HGI and LGI diets may be also influenced by the pre-exercise substrate availability (Stellingwerff et al., 2007). However, the reduction of the muscle glycogen pool during the subsequent exercise was not influenced by the GI of the high CHO recovery diets (Stevenson et al., 2009).

1.4.3 Impact of GI meals consumed for several days before endurance exercise

As previously mentioned, the majority of investigations into the effect of the GI on exercise substrate utilisation and performance or capacity have only studied the effect of a single meal ingested prior to exercise or mixed meals after the recovery from exercise. Only a few studies have investigated the impact of the GI while on high CHO diets for several days prior to endurance exercise. It is possible that metabolic adaptations may occur after an extended period on a LGI or HGI diet.

There is evidence that muscle glycogen accumulation and the availability of fasting NEFA are influenced by the GI of moderate CHO diet (47% of energy) consumed for 30 days in men. Kiens & Ritcher (1996) showed that muscle glycogen and IMTG concentration were increased by 14% and 22%, respectively, with the HGI compared with the LGI diet. In this study, plasma NEFA was observed to be higher in the LGI diet compared to the isocaloric HGI diet during postprandial period measured after 30 days i.e. at the end of the intervention trial. On the other hand, there was no impact of the quality of high CHO (70% of energy) diets consumed for 3 days on time to complete treadmill run to exhaustion (Kiens et al., 1990). In this study the diets were described interchangeably as simple (HGI) and complex (LGI) CHO.

Only recently has the impact of high CHO diets with contrasting GI consumed for several days on exercise energy metabolism been determined (Chen et al., 2008). In this study, 9 male runners completed three different standardised preloaded treadmill performance runs. In randomised counterbalanced order, the procedures involved a 3 days CHO loading with different GI and GL meals after a 1hour exhaustive run (a 30 min run at 80% $\dot{V}O_2$ max followed by a further 30 min run at 70% $\dot{V}O_2$ max to reduce the muscle glycogen) followed by a standardised preloaded exercise protocol i.e. 1 hour preloaded constant run at 70% $\dot{V}O_2$ max (T1) and a 10 km time trial (TT). After a glycogen depletion exercise was performed, the participants consumed high CHO diets (73% of energy) with either a HGI (GI 80, GL 553) or a LGI (GI 36, GL 249), and a low CHO diet consisting of 31% of energy from CHO (GI 70, GL 227) for 3 days. On the trial day, the participants consumed the test breakfast and plasma glucose concentration was measured throughout the 2 hours postprandial period at specific time points. After the 2 hours resting period, the participants performed T1

run followed by TT and expired air samples were collected at 20 minute intervals during T1, at 2.5 km intervals during TT and at 1 hour intervals during the 2-hours recovery. Blood samples were collected at each time point in the pre-meal, before and every 20 minutes during T1, 5 km interval during TT and the first and second hour post-exercise.

They found that there were no differences in fat and CHO oxidation between the two high CHO trials during the 2 hours postprandial and T1. However the total fat oxidation was lower in the two high CHO trials than the low CHO trial during the 2 hours postprandial period and T1. The results also showed that the 2 hours IAUC for plasma glucose after the breakfast was 1.5 times larger in the high CHO HGI trial than the high CHO LGI and low CHO trials. Higher plasma glucose levels were observed in both high CHO trials during exercise and recovery period compared to low CHO trial. There were no differences for plasma insulin, NEFA and glycerol between the two high CHO trials throughout the experiment. Plasma NEFA and glycerol concentrations were lower starting from 60 min point of T1 to the end of the 2 hour recovery in the high CHO with HGI and LGI trials compared with those in the low CHO trial. There were also no differences in the time to complete the 10 km run between the two high CHO trials. The performance in the high CHO with LGI trial was improved when compared to the low CHO trial. However, in this investigation a test breakfast meal was consumed 2 hours prior to the exercise trial, and therefore may have influenced the study findings. This study concludes that the amount rather than the nature of the CHO consumed during the 3-day isoenergetic CHO loading may be the most overriding factor on subsequent metabolism and endurance run performance (Chen et al., 2008). Therefore further studies are required to determine

whether adaptations to the GI of a high CHO diet consumed for a longer period differ from those seen when meals are ingested pre or during short recovery after exercise. In addition, since in a study of Chen et al. (2008) exercise was conducted after breakfast, it is important to investigate of how high CHO diets with HGI and LGI consumed for a few days influence energy metabolism when participants are asked to exercise in the fasted state, in which the rate of fat oxidation is higher than during exercise in the fed state (Van Proeyen et al., 2010) and thus impact of the GI of high CHO diets consumed for a few days may be expected to be more profound.

1.4.4 Impact of GI on exercise performance and capacity

Although a high CHO with HGI ingestion favours glycogen synthesis pre- and post-exercise, a high CHO diet with LGI increases the availability of plasma NEFA thus sparing muscle glycogen utilisation which may enhance performance (Stevenson et al., 2009; Wee et al., 2005). A recent study investigated the effect of ingesting CHO meals (2.0 gCHO/kg BW) with either HGI or LGI 3 hours prior to exercise on endurance running capacity (Wu & Williams, 2006). This study found a greater endurance capacity when the participants ran to exhaustion during the LGI trial than during the isocaloric HGI trial. A higher rate of fat oxidation and lower glycogenolysis observed during exercise may explain a longer time to exhaustion after a LGI diet compared to a HGI diet. Another study demonstrated that there was a relative shift in substrate utilisation from CHO to fat when a LGI CHO meal providing 2.0 g CHO/kg BW was ingested 3 hours before exercise compared with that for a HGI CHO meal. However, there was no difference in endurance running capacity (Wee et al., 1999). When high CHO diets (73% of energy) with HGI and LGI were consumed for 3 days, there were no differences in the rate of fat oxidation

and plasma NEFA concentrations between the HGI and LGI diets during running and there was no difference in exercise performance measured as time to complete a 10 km run (Chen et al., 2008). In terms of exercise performance or capacity, some results indicate that GI is not as important as consuming CHO itself, therefore more research in this area is clearly needed.

1.5 Dietary GI and plasma lipids

1.5.1 Atherogenic dyslipidemia

The abnormalities of circulating lipids and lipoprotein concentration and composition are one of the major causes of the greater risk of cardiovascular heart disease (CHD) (Austin et al., 1988, 1990). When the circulating lipids and lipoproteins are clinically altered, this condition is called dyslipidemia**.** Atherogenic dyslipidemia refer to conditions of high levels of plasma TG, elevated low density lipoprotein (LDL)-cholesterol, and low levels of high density lipoprotein (HDL)-cholesterol (Austin et al., 1990). Relationship between the risk of CHD and high concentration of LDL-cholesterol is evident from several studies (Despres et al., 2000; Jones et al., 2004; Maron et al., 2000). On the other hand, the inverse association between HDL-cholesterol levels and incidence of CHD has been confirmed by many studies (Muntner et al, 2010). Thus, low HDL levels are considered a strong risk for CHD. Elevated TG concentration has also been reported as an independent risk factor for CHD (Austin et al. 1998, Assmann et al. 1998). In addition, it has been shown that distribution of LDL subclasses may be more strongly correlated with CHD than LDL-cholesterol concentrations, especially small dense LDL_3 (Gardner et al., 1996; Wu, 1999). Therefore, atherogenic dyslipidemia phenotype describes a condition

which consists of high plasma TG concentration, high LDL_3 cholesterol concentration and low HDL-cholesterol concentration**.**

The mechanisms by which increased concentration of TG induce atherosclerosis involved the structural abnormalities induced LDL and HDL particles and the accumulation of TRL remnants i.e. chylomicron and VLDL (Karpe et al., 1999). Increase plasma TG concentration has led to an accelerated transfer of esterified cholesterol from HDL and LDL to TRLs which is mediated by cholesterol ester transport protein (CETP) to form TG-enriched, cholesteryl ester-depleted HDL and LDL particles (Brewer, 1999; Davidson, 2010; Griffin, 1997). The TG content of these particles is then hydrolysed by hepatic lipase to form HDL_3 particles which are less anti-atherogenic and LDL_3 particles which are highly atherogenic. In addition, remnants which are formed from chylomicrons and VLDLs after partial removal of their TG by the action of LPL are considered to be atherogenic as well because they are enriched with cholesterol and share many properties of LDL. Moreover, the progressive removal of TG from the core of the VLDL particle, leads to the formation of a smaller and more cholesterol rich particle and by the further action of LPL may be converted to LDL-cholesterol. The atherogenic consequences of raised plasma TG therefore include direct atherogenic effects of chylomicron and VLDL remnant particles, reduced level of HDL and raised level of LDL_3.

High concentration of HDL-cholesterol is protective against CHD since HDL particles mediate efflux of cholesterol from cells of the arterial wall and its subsequent delivery to the liver and steriodogenic organs (van der Velde, 2010). High HDL-cholesterol level signify a high rate at which reverse cholesterol transport

operates and interferes directly with cholesterol deposition in the arterial wall. HDL_2 particle is found to be necessary for the provision of anti-atherogenic function (Johansson et al., 1991, Freedman et al 1998, Asztalos et al 2004). On the other hand, reduced circulating level of HDL will have severely compromised the ability to maintain cellular and circulating cholesterol homeostasis. In particular, there will be reduced ability to remove excess cholesterol from cells. This will lead to accumulation of cholesterol in peripheral cells, leading to down regulation of LDL receptors on the cell membranes, thereby reducing the rate of uptake of LDL from circulation. Furthermore, low HDL-cholesterol concentration when combined with a preponderance of small HDL_3 particle, indicated that the rate of clearance of TRL from the circulation by adipose tissue and skeletal muscle is slow and the rate of production of antherogenic remnants of TRL is high. HDL_3 particles are rapidly catabolised by the liver, leading to reductions in circulating HDL concentrations.

1.5.2 Impact of high carbohydrate diet on plasma lipids

Among dietary approaches to reduce risk of CHD is reduction of saturated fat intake (AHA, 2000). Thus, clinical studies investigated how replacement of saturated or trans unsaturated fats with CHO, monounsaturated or n-6 polyunsaturated fats from vegetable oils impact on plasma lipids (Sacks & Katan, 2002). This randomised clinical trial reports that when CHO are used to replace saturated fats, in a low-fat diet (30% of energy from fat), total cholesterol (TC) and LDL-cholesterol decreased by 5% and 6%, respectively. However, these modest improvements contrast with a decrease in HDL by 9% and an increased in TG by 9%. When total fat is further reduced to 20% of energy (65% of energy from CHO), TC and LDL-cholesterol was decreased by 9% and 12% respectively, whereas HDL-cholesterol decreased by 20%

and TG level increased by 20%. Thus, this provides evidence that increase in CHO intake leads to the adverse changes in HDL-cholesterol and TG.

The notion that high CHO diets are detrimental for CHD risk was confirmed by several experimental studies which have shown that high CHO low fat diet increased fasting and postprandial plasma concentrations of TG and reduced concentration of HDL-cholesterol in healthy sedentary individuals (Culling et al., 2009; Koutsari et al., 2000; Roberts et al., 2008). For example, in Koutsari et al. (2000) study, a high CHO (68% of energy) diet consumed for 3 days by 9 normolipidemic men increased fasting plasma TG and reduced HDL-cholesterol concentrations in comparison to an isoenergetic low fat (66% of energy) diet. In a study of Roberts et al. (2008), 8 healthy individuals consumed isocaloric diets containing a high percentage of energy from CHO or a higher percentage of energy from fat for 3 days in a randomised crossover dietary intervention study. Applying stable isotope tracer techniques, results showed that plasma concentrations of TG were significantly higher in both fasting and postprandial states after a high CHO diet than after a high fat diet. In a more recent study, Culling et al. (2009) examined whether the nature of the CHO diets affects the increased in plasma TG concentrations. Eight non diabetic participants consumed high CHO (68% of energy) with either high starch or high sugar and high fat (50% of energy) diets for 3 days in randomised cross over design. This study found that fasting TG concentrations were greatest following a high sugar diet and lowest following a high fat diet, however there was no difference between high starch and high fat diets. Postprandial TG concentrations were similarly affected by prior of each diet. Therefore, it was suggested that the short term TG raised effect of high CHO dependent on the nature of the CHO, with a greater effect of a sugar-

rich than a complex-CHO-rich diet. Thus, this study provides evidence that different type of CHO produces a difference in lipid profiles and therefore could be important in preventing the risk of CHD.

A study investigating effect of advice to increase CHO and reduce fat intake on dietary profile and plasma lipid concentrations in healthy postmenopausal women reported that increasing CHO intake only up to 50% of energy regardless of body weight reduction also resulted in changes in the lipid profile that were more likely to favour an increased risk of CHD, as TG concentrations were increased and HDL-cholesterol concentrations were reduced (Arefhosseini et al., 2009).

There is some evidence to suggest that replacement of saturated fat with a higher CHO intake, particularly refined CHO, can exacerbate the atherogenic dyslipidemia associated not only with increased TG and reduced HDL-cholesterol but also increased in most atherogenic small LDL particles (Siri-Tarino et al., 2010). It seems that following the hypocaloric CHO restricted diet leads to even more favourable changes. The study of Volek et al. (2009) in which hypocaloric CHO restricted diets were consumed for 12 weeks found consistently reduced glucose (-12%) and insulin (-50%) concentrations, insulin sensitivity (-55%), weight loss (-10%), decreased adiposity (-14%), and more favourable TG (-51%), HDL-cholesterol (13%) and total cholesterol/HDL-cholesterol ratio (-14%) responses. In addition, LDL particle distribution also showed more favourable responses.

Some studies investigated the impact of high CHO intake on plasma lipids in endurance athletes. Brown & Cox (1998) examined the effects of a high CHO (HC:

69% of energy from CHO, 15% of energy from fat,) versus a high fat (HF: 50% of energy from fat, 37% of energy from CHO) diets on plasma lipids and lipoproteins in 32 endurance trained cyclists over a 3-month period and found that from baseline to week 12, there was a significant increase in TC and TG in high CHO group. In an earlier study, Thompson et al. (1984), observed an increase in TG, TC and LDL-cholesterol and a decrease in HDL-cholesterol in distance runners consuming a diet providing high CHO (69% of energy) for 2 weeks compared to a high fat (50% of energy) diet. Despite these dietary effects, HDL-cholesterol concentrations in the athletes remained above values typical of sedentary men. Therefore, it is likely that even in those who are involved in exercise, high CHO diet may induce a detrimental impact on plasma lipid profiles. It remains unclear to which extent high CHO diet impact plasma lipids in individuals who are moderately trained and exercise for health rather than performance.

One of the mechanisms by which increased intake of CHO increased TG is stimulation of hepatic synthesis and secretion of VLDL-TG (Sidossis & Mittendorfer, 1999) and expansion of the fasting TG pool (Jeppesen et al., 1997). This mechanism is supported by a study investigating the effect of diet composition on VLDL-TG metabolism applying *in vivo* isotopically labelled VLDL-TG tracers in healthy participants (Mittendorfer & Sidossis, 2001). Plasma concentration of VLDL-TG was higher after 2 weeks on high CHO (75% energy) diet than an isoenergetic high fat (55% of energy) diet. Hepatic fatty acid oxidation was decreased after the high CHO diet, which presumably increased hepatic fatty acid availability for TG synthesis thus explains the increased in VLDL-TG production in high CHO diet compared to high fat diet. A reduction of the activity of LPL as a

result of high CHO consumption may also explain the increase in plasma TG (Thompson et al., 1984). This is reflected by reduced clearance of VLDL-TG in the fasted state after the high CHO diet (Mittendorfer & Sidossis, 2001; Parks et al., 1999). The high TG concentration enhances the opportunity for the reciprocal transfer of cholesterol and TG between HDL and TRL by the action of CETP which might explain the lower concentration of HDL-cholesterol observed in high CHO diet (Koutsari et al., 2000; Roberts et al., 2008). In addition, lower fat intake is known to reduce the requirement for HDL-mediated cholesterol removal (Velez-Carrasco et al., 1999) which may have also contributed to the lower HDL-cholesterol concentration.

Differently from findings of the above studies in which high CHO diets consumed were isocaloric either to high fat diet (Culling et al., 2009; Koutsari et al., 2000; Roberts et al., 2008) or high fat diet based on monounsaturated fat or n-6 polyunsaturated fat from vegetable oils (Sacks & Katan, 2002), there is evidence to suggest that the consumption of high CHO diet *ad libitum* may benefit plasma lipid profiles. For example, a reduction in plasma concentrations of TC and LDL-cholesterol was found after consuming high CHO *ad libitum* for 7 weeks in men (Archer et al., 2003). The improvement in plasma lipids induced by the *ad libitum* consumption of a high CHO diet was partly mediated by changes in body weight. The notion that high CHO diet improved plasma lipid-related risk of CHD when consumed *ad libitum* was confirmed in recent study conducted on overweight men and women (Kratz et al., 2010). This study showed that switching from moderate fat diet to isocaloric high CHO diet reduced plasma concentrations of HDL-cholesterol and cholesterol content in the larger LDL (LDL_1) fractions, tended to increase

plasma levels of TG, VLDL, intermediate-density lipoprotein and smaller, denser LDL (LDL_3) fractions. These changes were largely reversed when subjects lost weight by consuming this high CHO diet *ad libitum.* Thus, the leading mechanisms by which high CHO diet improve plasma lipids are body weight and body fat reduction.

1.5.3 Impact of GI of high CHO diets on plasma lipids

1.5.3.1 Mechanisms by which GI may modify plasma lipids

It is known that CHO foods with HGI are rapidly digested and absorbed causing a rapid increase in plasma glucose and insulin concentration followed by hypoglycaemia undershoot (Galgani et al., 2006). The hypoglycaemic undershoot seen after HGI food triggers released of counter regulatory hormones (Du et al., 2006; Jenkins et al., 1990) such as glucagon, adrenaline and growth hormones which enables restoration of circulating glucose levels and increases late postprandial circulating NEFA levels (Wolever et al., 1995). On the other hand, CHO foods with LGI take longer to absorb, producing a slower, more controlled and prolonged rise in plasma glucose and insulin concentration and thus attenuates late postprandial NEFA (Wolever et al., 1995). Therefore, these differing responses of plasma glucose, insulin and NEFA to CHO foods with HGI and LGI provide a mechanism by which GI of CHO consumed can be expected to modify concentration of plasma lipids (De Rougemont et al., 2007; Frost et al.; 1999; Kelly et al., 2004; Rizkalla et al., 2004; Sloth et al., 2004) and insulin sensitivity (Clapp & Beth, 2007; Frost et al., 1998; Kiens & Ritcher, 1996; Wolever et al., 1995).

Increased postprandial hyperglycaemia and hyperinsulinemia are also among factors which may increase concentration of TRL (Riccardi et al., 2000) and reciprocal reduction in HDL-cholesterol. This mechanism is supported by findings from second meal studies showing that LGI preload meals compared with HGI preload meals may improve glycaemic responses to standard subsequent meals (Jenkins et al., 1982; Liljeberg & Bjorck, 2000). The glucose response curve to a standard lunch has been shown to be related to plasma NEFA concentration immediately before the lunch (Wolever et al., 1995), and preload meals that improve glucose and insulin responses to a standard lunch are those that prevent a hypoglycaemic undershoot until the second meal (Liljeberg et al., 1999). High circulating NEFA levels after the ingestion of HGI meals result in lipid accumulation in skeletal muscle and liver, causing insulin resistance in these normally insulin-responsive tissues (Frayn, 2001; Petersen & Shulman, 2002), which reduces insulin stimulated glycogen synthesis in skeletal muscle and decreases the ability of insulin to suppress hepatic glucose production and output. High circulating NEFA levels also inhibit insulin-stimulated suppression of NEFA release from adipose tissue, so further increase circulating levels, and trigger a vicious cycle of insulin resistance and increased NEFA levels (Frape et al., 2000). Furthermore, the large insulin demand created by HGI meals leads to overstimulation of β-cells (Ludwig, 2002). High glucose levels have a glucotoxic effect on β-cells, probably as a result of free radical oxidative damage (Augustin et al., 2002). Hyperinsulinemia may reduce β-cell function by causing excess amyloid deposition (Wolever, 2000). High NEFA levels lead to TG accumulation in β-cells, which reduces insulin secretion (Goldstein, 2002). Accordingly, by reducing hyperglycaemia, hyperinsulinemia and NEFA levels LGI foods may decrease the factors contributing to β-cell failure. Thus, this mechanism

may support the improvement in insulin sensitivity observed after the consumption LGI for longer duration (Frost et al., 1996, 1998; Jarvi et al., 1999; Jebb et al., 2010; Rizkalla et al., 2004). Since insulin sensitivity plays an important role in lipid metabolism (Gill & Malkova, 2006) improvement in insulin sensitivity might be the mechanisms by which consumption of LGI diets may be expected to improve plasma lipids.

1.5.3.2 GI and plasma lipids in individuals with metabolic disturbance

Several medium term intervention studies which lasted for 3-4 weeks conducted on patients with increased or advanced CHD (Frost et al., 1996; Frost et al., 1998), type-2 diabetic patients (Jarvi et al., 1999; Luscombe et al., 1999; Rizkalla et al., 2004) and individuals with impaired glucose tolerance (Wolever & Mehling, 2002) suggest that a LGI diet may improve plasma lipid profiles and insulin sensitivity. Findings of these studies have shown that a LGI diet reduced fasting concentrations of TC (Kelly et al., 2004), LDL-cholesterol (Jarvi et al., 1999; Rizkalla et al., 2004) and TG (Rizkalla et al., 2004) and increased HDL-cholesterol (Luscombe et al., 1999), as well as improved insulin sensitivity (Frost et al., 1996, 1998; Jarvi et al., 1999; Rizkalla et al., 2004) and β-cell function (Wolever & Mehling, 2002) when compared with a HGI diet. For example, Rizkalla et al. (2004) examined whether moderate intake of CHO with a LGI diet compared with a HGI diet has beneficial effects on plasma glucose control, lipid metabolism, total fat mass and insulin resistance in type-2 diabetic patients. In this study, with a crossover design, 12 type-2 diabetic men were randomly allocated to two periods of 4 weeks of CHO with either HGI or LGI diet. Results showed that fasting plasma glucose level fell significantly after the LGI diet compared with its basal value but significant change in fasting

plasma insulin. The IAUC for both plasma glucose and insulin were lower after the LGI than HGI diet. Moreover whole-body peripheral insulin sensitivity measured by the euglycaemic-hyperinsulinemic clamp technique was significantly higher after the 4-week LGI diet than after 4-week HGI diet. A LGI diet also induces a decrease in fasting plasma TC, LDL-cholesterol and NEFA. Therefore, the use of a LGI diet for long term basis may be important in the treatment and prevention of diabetes and related disorders. In the most recent intervention study but longer duration, Jebb et al. (2010) investigated the effects the replacement of saturated fatty acid (SFA) with monounsaturated fatty acids or CHO diets with HGI or LGI in participants at risk of developing metabolic syndrome conducted in randomised controlled trial with parallel design for 24 weeks. This study reported that the reduction in SFA decreased TC and LDL-cholesterol and improved insulin sensitivity after a high CHO with LGI diet compared to a HGI diet.

Cross-sectional associations between total CHO and dietary GI intakes and several CHD factors including plasma lipids and insulin sensitivity in a sample of 2,941 Framingham Offspring Participants (McKeown et al., 2009) and 18137 healthy women without diagnosed diabetes (Levitan et al., 2008) were investigated. Dietary intake was assessed by a food frequency questionnaire and categorized by quintiles of dietary intake. It was found that dietary GI was positively associated with fasting TG in the highest quintile of intake fasting insulin and inversely associated with HDL-cholesterol. These cross-sectional findings support the hypothesis that a HGI diet unfavourably affects CHD risk factors and therefore, substitution of high with low GI dietary CHO may have reduced the risk of CHD.

However, some other studies conducted on patients with CHD or type-2 diabetes reported no effect of CHO diets with contrasting GI on lipids profile and insulin sensitivity (Kelly et al., 2004; 2005; Liese et al., 2005). A Cochrane meta-analysis investigated the effect of LGI diets, over a minimum of 4 weeks in randomised controlled trials (RCT), on CHD and on risk factors for CHD (Kelly et al., 2004). Participants were adults with at least one major risk factor for CHD such as abnormal lipids, diabetes or being overweight. The findings from fifteen RCT showed no evidence that LGI diets have an effect on LDL-cholesterol or HDL-cholesterol, TG, fasting glucose or fasting insulin levels and evidence that LGI diets reduced CHD and CHD risk factors was weak. Data on 979 adults with normal (67%) and impaired (33%) glucose tolerance from the Insulin Resistance Atherosclerosis Study (1992-1994) showed no association was observed between GI and fasting insulin sensitivity after adjustment for demographic characteristics or family history of diabetes, energy expenditure, and smoking (Liese et al., 2005).

1.5.3.3 GI and plasma lipids in healthy individuals

In healthy individuals evidence on the impact of the GI on metabolic risk factors including plasma lipids and insulin sensitivity is lacking. Previous medium term intervention studies reported that consuming LGI foods was associated with improvement of plasma lipids and insulin sensitivity in healthy sedentary individuals (Clapp & Beth, 2007; De Rougemont et al., 2007; Fajcsak et al., 2008; Jeppesen et al., 1997; Kiens & Ritcher, 1996). In a study of Clapp & Beth (2007), 7 healthy women consumed high CHO (60% of energy) diets with either HGI or LGI for 20 days in counterbalanced order. Glucose and insulin responses to the diet, insulin sensitivity and lipids profile were measured over the last 7 days. On the LGI diet,

average glucose and insulin levels were 40% lower, insulin sensitivity was >20% higher and LDL-cholesterol was lower than in the HGI diet. In addition, in the fasting stage rate of fat oxidation supplied approximately 45% of energy expenditure in LGI diet compared to 38% in HGI diet. De Rougemont et al. (2007) reported an improvement in lipid profiles in an overweight non-diabetic group after 5 weeks on LGI diet. The LGI diet significantly decreased TC by 9.6%, LDL-cholesterol by 8.6% and both LDLcholeterol:HDL-cholesterol ratio (10.1%) and total cholesterol:HDL-cholesterol ratio (8.5%) compared to HGI diet. Meanwhile, Kiens & Ritcher (1996) showed that a LGI diet improved insulin sensitivity compared to an isoenergetic HGI diet while consuming a diet with an energy composition common in Western countries (47% of energy from CHO) for 30 days in healthy young men. Other intervention studies conducted on healthy overweight/obese pre-pubertal children (Fajsack et al., 2008) and healthy postmenopausal women (Jeppesen et al., 1997) observed an improvement in CHD risk factors when changing from HGI diet to LGI diets. Thus, the improvement in lipid profiles and insulin sensitivity after LGI diet as evidence from the above studies may prevent those healthy sedentary individuals from the risk of CHD.

However, other intervention and cross sectional studies conducted in healthy individuals do not necessarily support the notion that CHO intake with LGI diet is more beneficial in relation to plasma lipids and insulin sensitivity than HGI diet (Lau et al., 2005; Shikany et al., 2009; Sloth et al., 2004). Sloth et al. (2004) investigated the long term effect of low fat high CHO with either HGI or LGI diets on the risk factors for type-2 diabetes and ischemic heart disease in overweight healthy participants. This study was a 10 weeks parallel, randomised intervention trial with

two matched groups and both HGI and LGI diets were matched in total energy, energy density, dietary fibre and macronutrient composition. No significant differences were observed between groups in fasting serum insulin, homeostasis model assessment for relative insulin resistance ($HOMA_{IR}$), TG, NEFA or HDL-cholesterol. Only LDL-cholesterol was decreased and TC tended to decrease after a LGI diet compared with a HGI diet. In contrast, TC and LDL-cholesterol concentrations were increased after a LGI diet compared with HGI diet consumed by healthy overweight/obese men for 4 weeks in randomised crossover study (Shikany et al., 2009). Thus, HGI and LGI diets diets of 4 weeks duration had no consistent effects on CHD risk factors in this group of overweight/obese men. Furthermore, the Inter99 cross sectional study observed that habitual intake of diets with a HGI and high GL or diets with a high content of total CHO including simple sugars was not associated with the probability of having insulin resistance in 5,675 non diabetic men and women at 30 - 60 years (Lau et al., 2005).

Thus, findings on how GI of high CHO diets modify insulin sensitivity and plasma lipids in healthy individuals require further confirmation. To our best knowledge there are no studies investigating impact of the GI of high CHO diets in healthy physically active individuals. As mentioned above in endurance trained individuals high CHO intake consumed for the purposes of muscle glycogen increase may be detrimental to plasma lipids (Brown & Cox, 1998; Thompson et al., 1984). It remains unclear whether consideration of LGI of high CHO may prevent from unfavourable changes in plasma lipids when CHO diets are consumed for the increase in muscle glycogen content. Thus, impact of the GI of high CHO diets on plasma lipids in healthy physical active individuals will be investigated in this thesis.

1.5.4 Impact of GI of CHO diets on endothelial dysfunction

Endothelial function describes the ability of endothelium of the blood vessels to interact with vascular smooth muscle to influence blood flow (Cines et al., 1998). The endothelium is considered an active organ with many physical and chemical properties and main responsibility to keep vascular-tissue homeostasis by modulating vasodilation and vasoconstriction, controlling production of prothrombotic and antithrombotic components, and fibrynolytics and antifibrynolitics and intervening in immunological processes (Landmesser et al., 2004; Libby et al., 2002; Moncada et al., 2001).

There is accumulating evidence that endothelial dysfunction represents one of the earliest events in cardiovascular disease and is currently considered an independent atherosclerotic disease risk factor (Moyna & Thompson, 2004). Endothelial dysfunction is characterized partly by increased levels of chronic systemic low-grade inflammation (Pearson et al., 2003), impairment of atheroprotective substances such as nitric oxide and soluble isoforms of cellular adhesion molecules (CAMs) such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Hope & Meradith, 2003; Meydani, 2003). The initial stages of atherosclerosis are characterized by adhesion of circulating leukocytes to the endothelial cells and subsequent transendothelial migration which is mediated in part by ICAM-1 and VCAM-1, expressed on the endothelial membrane, in response to several inflammatory cytokines (Springer, 1994). CAMs are later on detached from the membrane and are found in the circulation in their soluble form which can be detected in the serum. Soluble ICAM-1 and VCAM-1 are increased in conditions with an inflammatory component (Gearing & Newman, 1993) and in atherosclerosis

(Poredos, 2001) resulting in coronary artery disease (Hwang et al., 1997; Ridker et al., 1998). Furthermore increased levels of ICAM-1 and VCAM-1 are associated with diabetes mellitus (Meigs et al., 2004, hypertension (Boulbou et al., 2005) and dyslipidemia (Hackman et al., 1996; Kressel et al., 2009).

Dyslipidemia, the most important risk factor of CHD have been shown to be associated with increased expression of ICAM-1 and VCAM-1 (Abe et al., 1998; Hackman et al., 1996). Abe et al. (1998) examined the association between CAMs and risk factors occurring with hypertriglyceridemia in patients with hypertriglyceridemia and low HDL-cholesterol compared with healthy individuals. This study found that ICAM-1 and VCAM-1 were higher in the patients than the healthy individuals and that the higher CAMs level in the patients occurred independently of diabetes mellitus and other risk factors. Hackman et al. (1996) observed higher levels of VCAM-1 in patients with hypertriglyceridemia in comparison to patients with hypercholesterolemia and healthy individuals. Levels of ICAM-1 were significantly increased in both hypertriglyceridemia and hypercholesterolemia patients compared with the healthy individuals. Thus, increased levels of ICAM-1 and VCAM-1 in patients with hyperlipidemia may be considered as another risk factor of atherosclerosis.

It has been also suggested that oxidative stress i.e. the imbalance between free radical production and in vivo antioxidant defences may be an important mechanism linking acute hyperglycaemia to increased cardiovascular risk (Ceriello, 2000). Hyperglycaemia induces oxidative stress and reduces antioxidant defences in both healthy individuals and those with type-2 diabetes (Ceriello et al., 1998). The chronic

consumption of a HGI diet may lead to chronically high oxidative stress and a LGI diet appears to be beneficial in reducing oxidative stress. The high CHO with LGI diet was reported to decrease endothelial dysfunction and risk factors of type-2 diabetes and CHD in comparison to high CHO with HGI diet (Hare-Bruun 2008; Lavi et al., 2009; McCarty, 2004).

1.6 Aims of the studies

- To investigate whether high CHO with HGI and LGI meals defined by using the GI values of foods present within a meal produce significant differences in plasma glucose response in moderately active individuals and thus can be used for the main experimental studies (Chapter 3).

- To investigate the impact of consuming a high CHO diet with either HGI or LGI for 5 days on energy substrate utilisation during running at 65% $\dot{V}O_2$ max in the fasted state and running capacity measured as time to exhaustion in physically active men (Chapter 4) and women (Chapter 5).

- To investigate the impact of the consumption of high CHO diets with HGI and LGI for 5 days on fasting concentration of plasma lipids such as total, LDL- and HDL-cholesterol and TG, and circulating biomarkers of endothelial activation such as ICAM-1and VCAM-1in physically active healthy individuals. Since plasma insulin concentrations are related to insulin sensitivity, its measurement will be performed (Chapter 6).

Chapter 2 General Methods

2.1 Participants

All studies were conducted with the approval of University of Glasgow's Medical Faculty Ethics Committee for non-clinical research involving human participants, material or data. Participants were recruited via an advertisement in the University's news letter, news desk, e-mails and posters (Appendix I). Posters were located at several sports and recreational clubs in Glasgow and in the Glasgow University campus. When a volunteer contacted us expressing an interest to participate in the study, the purpose and procedures of the study were explained including possible risks and discomforts involved via e-mail, telephone or in person. They were also given the study information sheet (Appendix II) to take away and make their decision. If the individuals decided to take part in the study, they were asked to sign a consent form (Appendix III), to complete a health screen form (Appendix IV) and a simple physical activity questionnaire (Appendix V) so as to ensure that the participants were physically active and involved in moderate to strenuous exercise at least three times a week. The participants' information was stored on a University of Glasgow password protected computer server. Each participant was entered as a code number rather than by name for identification protection. This is in accordance with the Data Protection Act.

Participants of the studies were young healthy moderately active men and women. To be eligible for inclusion in all studies, participants had to be aged between 18-35 years, body mass index (BMI) of between 18.5 to 25 kg/m^2 and recreationally active, exercising at least three times per week. All participants were non-vegetarian, non-

smokers and not on weight reducing diets. Women participants had a regular menstrual cycle and were not pregnant. None of the participants had any diagnosed cardiovascular or metabolic disease and none were consuming medication or drugs known to influence lipid or carbohydrate metabolism.

2.2 Anthropometry

2.2.1 Height and weight

Height was measured using a portable stadiometer (Seca, Invicta Plastics LTD, Leicester, UK). Participants were measured barefoot with their back positioned against a fixed backboard and their arms relaxed in the lateral position. The head was also positioned against the backboard, with the line of eyesight perpendicular to the backboard. Measurement was performed when the participant was positioned and relaxed, and a moveable headboard was lowered on to the top of the head with light pressure allowing hair compression. The measurement was made to the nearest 0.01m.

Body weight was measured using digital scales (TBF-300, TANITA, Cranlea, UK). Participants were weighed wearing lightweight clothing without wearing shoes. Body weight was measured with both feet flat on the balance and with arms positioned in the lateral position. The measurement was made to the nearest 0.05 kg.

The derived values for height and weight measurements were used to calculate BMI according to the formula, weight (kg) divided by the square of height (m) (kg/m^2) (Marfell-Jones, 2006).

2.2.2 Body composition

Components of body fat and fat free mass were measured through leg-to-leg bioelectrical impedance analysis (TBF-300, TANITA, Cranlea, UK) (Jebb et al., 2000). TANITA scales determine the electrical impedance or resistance to the flow of an electric current on body tissues, which estimates total body water. The speed of travel is dependent on how much lean body tissue and fat tissue exists in the body. Lean tissue, such as skeletal muscle, contains a large amount of water and electrolytes, which are excellent conductors of electricity and increase the speed of the current. Fat or adipose tissue contains very little water and slows the transmission of the current (ACSM, 1995). In the system, two footpad electrodes are incorporated into the platform of an electronic scale. The measurements were taken while participants were in a standing position with the electrodes in contact with bare feet.

2.3 Resting Metabolic Rate

Resting metabolic rate (RMR) was measured by the indirect calorimetry ventilated hood system (Deltatrac, Datex Instrumentation Corporation, Helsinki, Finland) which estimates rates of fat and CHO oxidation by continuously monitoring oxygen (O_2) consumption and carbon dioxide (CO_2) production. The test was carried out in the metabolic investigation room with a thermoneutral environment (21-24^{o}C) in the morning after participants' overnight fast and 24 hours abstention from exercise. Participants lay comfortably on the examination bed in the supine position with their arms at their sides and their legs straight and uncrossed. An eye mask was offered on their demand to protect them from the annoying lights of the ceiling. After the participants had rested for 10 minutes, a ventilated hood was placed over each

participant's head to allow analysis of expired gas. The participant was monitored throughout the measurement to ensure that sleeping, talking and excess movement did not occur. Oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory quotient and RMR values were read and recorded every 60 seconds for the duration of 30 minutes. For each measurement, the first 10 minutes were excluded to ensure steady state values were used and the averaged RMR was calculated.

2.4 Preliminary exercise tests

All participants underwent a resting electrocardiogram (ECG) which was conducted and interpreted by a physician to exclude any participant with hypertrophic cardiomyopathy or prolonged QT syndromes before commencing any exercise testing. The preliminary exercise tests were conducted to determine the speed that elicits 65% of $\dot{V}O_2$ max for each participant. The participants performed two preliminary exercise tests conducted on a motorised treadmill (Woodway PPS55medII, Woodway GmBH, Germany) which were;

1) an incremental submaximal running test to determine the relationship between submaximal running speed and $\dot{V}O_2$ and,
2) an uphill incremental treadmill running test at constant speed to exhaustion to determine $\dot{V}O_2$ max.

Using the results from submaximal and maximal exercise tests, running speed equivalent to 65% $\dot{V}O_2$ max were determined and selected to be the running speed during the main exercise trials.

2.4.1 Submaximal exercise testing

A submaximal test was employed to determine the relationship between submaximal running speed and oxygen uptake which consisted of four to five stages. Participants performed a continuous running of submaximal test on a treadmill by means of an incremental protocol with each stage lasting four minutes. The initial speed of the treadmill was set between 7.0 and 9.0 km/hr for men and between 6.0 and 8.0 km/hr for women depending on the participant's training status. Throughout the test, the treadmill's gradient remained unchanged whilst the speed was increased by 1.0 to 1.5 km/h after each four-minute stage. An expired air collection was taken during the last minute of each stage using the Douglas bag technique (Consolazio et al., 1963), heart rate was monitored using short-range telemetry (Polar S610i, Polar Electro, Finland) and ratings of perceived exertion (RPE) were recorded using the Borg scale (Borg, 1982). The first three minutes of every stage constituted a period of adjustment of steady state to be achieved. This submaximal test end point was when the participant reached around 85% of estimated maximal heart rate [85% x (220-age)]. A regression equation describing the linear relationship between $\dot{V}O_2$ and running speed from the results of this test were determined.

2.4.2 Determination of maximal oxygen consumption ($\dot{V}O_2$ max)

The $\dot{V}O_2$ max of each participant was determined using a modified version of the protocol devised by Taylor et al. (1955). This involved a continuous incremental running test to exhaustion on a treadmill. The running test was divided into three-minute stages. All the stages were conducted at the participant's self-selected running speed. The treadmill speed was kept constant throughout the test while the inclination of the treadmill was increased by 3-5 % every three minutes from an initial grade of 4-8 %. The starting gradient and the increase in gradient with each stage were determined individually, based on participant ability, to elicit a test duration between seven and twelve minutes. Expired air samples were collected using the Douglas bag technique, heart rate was monitored and RPE were recorded using the Borg scale during the last minute of each stage. Strong verbal encouragement was given to the participants during the test. The test was open-ended and concluded with a final expired air collection after participants signal that they could only manage 1 more minute. The $\dot{V}O_2$ value obtained during the last expired air collection was taken as the $\dot{V}O_2$ max value. The criteria to establish the determination of $\dot{V}O_2$ max are a plateau in $\dot{V}O_2$ with increasing exercise intensity, an RER greater than 1.1, and a maximal heart rate within ± 10 beats/min from predicted (220 – age) (Howley et al., 1995).

2.5 Heart rate measurements

Heart rate was recorded at rest and monitored during the preliminary tests and exercise sessions using short range telemetry (Polar S610i, Polar Electro, Finland) positioned on the participants' chest.

2.6 Ratings of perceived exertion measurements

The participants' perception of fatigue was measured using the Borg scale during the preliminary tests and exercise trials. This scale consists of numbers ranging from 6 to 20, in which a rating 6 indicates no exertion at all and 20 would be maximal exertion. The Borg scale was shown to the participants during the collection of expired air and participants expressed their own perceived rate of exertion by pointing at the corresponding rate.

2.7 Measurement of oxygen uptake and carbon dioxide production.

$\dot{V}O_2$ and $\dot{V}CO_2$ were determined at rest in fasted state and during all exercise test sessions. Samples of expired air were collected using 100 litre Douglas bag (Hans Rudolph 2100 three-way stop cock, Hans Rudolph Inc, Kansas City, USA) for resting samples and during submaximal test and main exercise trials in which the volumes of expired air were low. For large volume samples, a 150 litre Douglas bag was used for collecting expired air samples during maximal test. Participant, whilst wearing a nose clip, breathed through a mouthpiece fitted to a lightweight one way respiratory valve (2700 Series Large 2-way NRBV, Hans Rudolph Inc, Kansas City, USA), which in turn was connected to a 120 cm long lightweight tube with a diameter of 38 mm. The tubing terminated at a two-way valve which opened and closed the Douglas bag.

A known volume (range between 200-350 ml/min) of expired air was extracted through the sampling port of the Douglas bag at a constant flow rate, controlled by a flow meter. This air passed into a gas analyser (Servomex 1440 Gas Analyser, Servomax Group Limited, East Sussex, England) to determine the percentage of O_2

and of CO_2. The remaining volume of expired air in each Douglas bag was measured by evacuation through a dry gas meter (Harvard Apparatus Inc, Holliston, USA). The temperature of the air in Douglas bag was measured during evacuation and read from a thermometer connected.

The analyser was calibrated before each sample analysis with nitrogen, a calibration gas (BOC Gases, BOC limited, Surrey, UK) and room air, and the barometric pressure was also recorded. The measured expired gas volumes ($\dot{V}_E$) were corrected to standard temperature and pressure for a dry gas ($\dot{V}_{E\,STPD}$) using the universal gas equation (Appendix VI). Inspired gas volume ($\dot{V}_I$) was derived using the Haldane transformation (Appendix VII) and used to calculate $\dot{V}O_2$ and $\dot{V}CO_2$ in l/min (Appendix VIII).

2.8 Substrate oxidation and energy expenditure

The method that was used to measure substrate oxidation was indirect calorimetry, a technique based on the measurement of $\dot{V}O_2$ and $\dot{V}CO_2$. This method relies on the assumption that $\dot{V}O_2$ and $\dot{V}CO_2$ accurately reflects tissue O_2 consumption and CO_2 production (Frayn, 1983). These measurements were also used to quantify the contributions of fat and CHO to energy expenditure (EE). The rate of fat and CHO oxidation were calculated using the equations described by Frayn (1983) for the resting state (Chapter 6) and by Ferrannini (1988) during exercise (Chapter 4 and Chapter 5), without measurement of urinary nitrogen excretion (for the estimation of protein oxidation). EE provided by fat oxidation was calculated by multiplying rate of fat oxidation by 9.3 (for both resting and exercise measurements) and energy

provided by CHO was calculated by multiplying the rate of CHO oxidation by 3.7 and 4.1 for resting and exercise measurement, respectively.

2.8.1 Calculating rates of fat and carbohydrate oxidation and energy expenditure for resting conditions.

Different CHO may have differences in their stoichiometry although it is generally assumed that these differences are only small and have minimal impact on the calculation of substrate oxidation. Under resting condition, plasma glucose is the main CHO source and was used as the representative of CHO (Frayn, 1983).

The stoichiometric equation for glucose oxidation is as followed:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 38\ ATP$$

Oxidizing 1 g of CHO 0.746 L O_2, produces 0.746 L CO_2 and provides 3.7 kcal energy.

Oxidation of fat (palmitic acid) occurs according to the following equation:

$$C_{16}H_{32}O_{12} + 23O_2 \longrightarrow 16CO_2 + 16H_2O + 130\ ATP$$

Oxidizing 1 g of fat uses 1.989 L O_2, produces 1.419 L CO_2 and provides 9.3 kcal energy.

Rates of fat and CHO oxidation can be calculated using simultaneous equations.

$\dot{V}O_2$ = [0.746 x CHO oxidation] + [1.989 x fat oxidation]

$\dot{V}CO_2$ = [0.746 x CHO oxidation] + [1.419 x fat oxidation]

Therefore:

Rate of fat oxidation (g/min) = $(\dot{V}O_2 - \dot{V}CO_2)/0.57$

Rate of CHO oxidation (g/min) = $(1.40 \times \dot{V}CO_2 - \dot{V}O_2)/0.30$

EE was calculated as;

EE (kcal) = [rate of CHO oxidation x 3.7 kcal] + [rate of fat oxidation x 9.3 kcal]

2.8.2 Calculating rates of fat and carbohydrate oxidation and energy expenditure during exercise

Glycogen is often the predominant fuel used during exercise of moderate intensity (Romijn et al., 2000; van Loon et al., 2001). Therefore, the estimation of substrate oxidation during exercise was based on the stoichiometry equation derived by Ferrannini (1988).

Oxidation of glycogen occurs according to following equation:

$$(C_6H_{12}O_6)_n + (6O_2)_n \longrightarrow (6CO_2)_n + (6H_2O)_n$$

Oxidizing 1 g of glycogen uses 0.828 O_2, produces 0.828 L CO_2 and provides 4.1 kcal energy.

Oxidation of fat (palmitic acid) occurs according to the following equation:

$$C_{16}H_{32}O_{12} + 23O_2 \longrightarrow 16CO_2 + 16H_2O$$

Oxidising 1 g of fat uses 1.989 L O_2, produces 1.419 L CO_2 and provides 9.3 kcal energy.

Rate of fat and CHO oxidation can be calculated using simultaneous equations.

$\dot{V}O_2$ = [0.828 x CHO oxidation] + [1.989 x fat oxidation]

$\dot{V}CO_2$ = [0.828 x GI oxidation] + [1.419 x fat oxidation]

Therefore:

Rate of fat oxidation (g/min) = $(\dot{V}O_2 - \dot{V}CO_2)/0.57$

Rate of CHO oxidation (g/min) = $[\dot{V}O_2 - 1.989(\text{fat oxidation})]/0.828$

EE (kcal) = [rate of CHO oxidation x 4.1 kcal] + [rate of fat oxidation x 9.3 kcal]

2.9 Estimation of energy requirement

The energy intake of the prescribed diets was based on the participant's habitual energy requirements, which were calculated by adding EE of planned and structured exercise estimated from 5 day physical activity diaries and data in physical activity tables (Ainsworth et al., 2000) to the multiplier of RMR measured (section 2.4) and sedentary physical activity level (PAL).

The participants completed the exercise diaries for 5 days (Appendix IX) and the researcher discussed with participants the duration and intensity of all planned and structured exercise sessions. Thus, for the calculation of EE of individual exercise session, rate of EE (kcal/h/kg body weight) of the corresponding activity was obtained from the physical activity tables (Ainsworth et al., 2000) and multiplied by time spent in this exercise session (in hours) and body mass (in kg). Values of EE of each exercise session were summed to obtain exercise EE for each day.

To calculate the participants' daily energy requirement, RMR for the duration of the sedentary activities minus the time spent in exercise was multiplied by a sedentary PAL of 1.5 in case of men and 1.4 in case of women (Bonomi et al., 2010) and the value obtained was added to the EE of the exercise sessions of the corresponding day. The value of this energy requirement was used for the development of high CHO diets with HGI and LGI.

2.10 Development of high carbohydrate diets with high and low glycaemic index

The high CHO diets with HGI and LGI were designed to be isoenergetic and the food items were prescribed on an individual basis, relative to the participants previously determined energy requirement (section 2.9), in order to maintain participant's body weight. The GI diets comprised of normal foods, excluding alcohol. The sources of CHO with HGI were cereal, rice, wholemeal breads, jam, biscuits and Lucozade™ while LGI were All Bran™, porridge, pasta, rye bread, oatcakes and apple juice. The researcher discussed with participants the foods they were able to consume before designing the experimental diets.

The GI of each CHO food in the high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) diets was taken from the GI tables available in the literature (Aston et al., 2008; Foster-Powell et al., 2002; Henry et al., 2005), where glucose was chosen as the reference food. In an attempt to achieve maximal compliance, an effort was made to choose foods that were palatable and easy to prepare. Daily calorie intake, available CHO, fat, protein, sugar and fibre content were kept as similar as possible between HC-HGI and HC-LGI diets. The

menu of the high CHO diets was shown to the subjects prior to their dietary interventions to confirm that they would agree to consume all the foods. The GI of the overall diet was calculated from the weighted means of the GIs of each food with the weighting based on the proportion of total meal carbohydrate contributed by the foods (Wolever & Jenkins, 1986). A dietary analysis programme (Diet 5^{TM}, Robert Gordon University, Aberdeen) was used for the prescription of the experimental diets.

Participants were provided with all the food and menus, which informed them of the exact amount of each food they were required to consume and when, during the HC-HGI and HC-LGI diets. Digital food scales and written instructions explaining how to cook and prepare the prescribed foods were included. To eliminate any influence of cooking methods on glycaemic response and to increase compliance, the menus were based on processed and easy-to prepare foods. Any deviations from these instructions were recorded by the participants. The researcher kept in close contact with the participants, who were encouraged to contact the researcher if they had any questions concerning the experimental diets. For the Control diet, participants performed a 5 day weighed food diary (Appendix X) using the digital scales provided and were asked to follow their usual or habitual diet during this period.

2.11 Dietary analysis, calculation of dietary glycaemic index and glycaemic load

Dietary intake of energy and macronutrients from the participant's habitual dietary records, as well as from the consumed experimental diets were calculated using the Diet 5^{TM} programme. Dietary GI and GL were also estimated from participants'

habitual dietary records and from consumed experimental diets based on the following steps. First, the GI of each CHO food was obtained from the most up-to-date published sources at the time (Foster-Powell et al., 2002; Henry et al., 2005 and Aston et al., 2008). Second, the GL of each CHO-containing food was estimated by multiplying the CHO content of the food consumed by the GI of that food divided by 100 (Salmeron et al., 1997). The GL, calculated in this way, for all CHO-containing foods were summed to give the daily GL. Finally, to estimate the daily GI, the daily GL was divided by total daily CHO intake and then that value multiplied by 100 (Wolever & Jenkins, 1986).

The GI calculation of the test meal is summarized below (Wolever et al., 2006);

$$\text{GL of each CHO food} = (\text{GI} \times \text{gAvCHO})/100$$

$$\text{GI of each test meal} = \frac{\text{GI} \times \text{gAvCHO}}{\text{gGAvCHO}} \times 100$$

Where gAvCHO was grams of available CHO of the food, gGAvCHO was grams of available CHO in the entire meal.

2.12 Blood collection and plasma preparation

A cannula (Venflon 18G, Becton Dickinson Ltd., Helsingborg, Sweden) was inserted in an antecubital vein of the participant. Blood samples at rest (Chapter 6) and during exercise (Chapter 4 and Chapter 5) were collected into a pre-cooled 7.5 ml ethylenediamine tetra-acetic acid (EDTA) Vacutainer^{TM} tube (BD Vacutainer Systems, Plymouth, UK) and were centrifuged at centrifugal force of 1509 x g for 15 minutes at 4^{o}C. After centrifugation, aliquots of plasma were transferred using a

disposable plastic Pasteur pipette into labelled 1.5-ml Eppendorf tubes (Eppendorf AG, Hamburg, Germany). The aliquoted plasma was then stored at -80^{o}C until analysis.

2.13 Plasma analysis

2.13.1 Plasma insulin measurements

Insulin concentrations were determined using the Mercodia Ultrasensitive Insulin ELISA (Mercodia AB, Sylveniusgatan 8A, Uppsala, Sweden) for exercise measurements (Chapter 4 and Chapter 5) whereas Mercodia Insulin ELISA (Mercodia AB, Sylveniusgatan 8A, Uppsala, Sweden) was used for resting measurements (Chapter 6). In principle, both Mercodia Insulin and Ultrasensitive Insulin ELISA and Mercodia Insulin ELISA are solid phase two-site enzyme immunoassay and based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation, insulin in the samples reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to a microtitration well. A washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding a stop solution (0.5 MH_2SO_4) to give a colorimetric endpoint that was read spectrophotometrically.

Calibrators at concentrations 0, 0.15, 1.0, 3.0, 10 and 20 mU/l (recombinant human insulin), quality control and plasma samples 25µl were pipetted into the appropriate wells followed by 100µl of Enzyme Conjugate (peroxidase conjugated mouse

monoclonal anti-insulin) and incubated on a plate shaker for 1 hour at room temperature (18-25^{o}C). The plate was then washed 6 times with 350µl Wash Buffer that had been diluted with 800ml of redistilled water. After each wash, the plate was inverted and tapped against absorbent paper. Substrate TMB 200 µl was then added into each well and incubated for 30 minutes at room temperature. The reaction was stopped by adding 50µl of Stop Solution into each well and the plate was placed on a shaker to ensure mixing for approximately 5 seconds. Finally, optical density was read at 450 nm using the Mercodia HS Insulin computer program (Multiskan Spectrum spectrophotometer, Thermo Fisher Scientific, Surrey, UK). The coefficient of variation for the insulin measurement was <4%.

2.13.2 Plasma total cholesterol, HDL-cholesterol, triglyceride, non-esterified fatty acids, glycerol and glucose measurements

Plasma concentrations of TC, HDL-cholesterol and TG (Roche Diagnostics Gmbh, Mannheim, Germany), NEFA (Wako Chemicals, Neuss, Germany), glycerol (Randox, Northern Ireland, UK) and glucose (Randox Laboratories Ltd. Co. Antrim, Ireland) were determined by homogeneous enzymatic colorimetric (HDL-cholesterol) and enzymatic colorimetric methods using available kits in the biochemistry lab of the Department of Clinical Biochemistry, Royal Infirmary, Glasgow. Low density lipoprotein (LDL) was calculated by the Friedwald equation (Friedwald et al., 1972):

LDL-cholesterol (mg/dl) = (Total cholesterol) – (HDL cholesterol + TAG/5)

All samples for each participant were analysed in the automatic analyser (ILab 600). Coefficient of variation was <3.1%.

2.13.3 Soluble vascular cell adhesion molecule-1 measurements

Soluble vascular cell adhesion molecule-1 (VCAM-1) and inter cellular adhesion molecule-1 (ICAM-1) were measured using a commercially available immunoassay kit (R&D System Inc, Minneapolis, USA). All samples for each participant were analysed in a single run analyser. The accuracy and precision of the assays were monitored using quality sera (Mercodia AB, Uppsala, Sweden; R&D Systems Inc, Minneapolis, USA). Assays were performed in the biochemistry laboratory of the Department of Human Nutrition, University of Glasgow. Coefficient of variation was <4%.

2.13.4 Homeostasis model assessment of insulin resistance ($HOMA_{IR}$)

$HOMA_{IR}$ technique was used to validate surrogate measure of insulin resistance (Matthews et al. 1985) and calculated according to which:

Insulin resistance = Fasting Glucose* Fasting Insulin/22.5 (Matthews et al., 1985)

Chapter 3 The effect of high and low glycaemic index of high carbohydrate meals on postprandial plasma glucose responses in healthy women.

3.1 Introduction

The GI is a method of functionally ranking CHO foods based on their actual postprandial plasma glucose response (Jenkins et al., 1981). GI is defined as the plasma glucose response measured as area under the curve in response to a 50 g CHO portion of a test food expressed as a percentage of the area under the curve following consumption of 50 g CHO of a reference food consumed by the same individual under standard conditions (FAO, 1998). The extent and duration of plasma glucose rise after a meal are influenced by the rate of hydrolysis of CHO foods in the gastrointestinal tract and the rate of gastric emptying, which determines the absorption rate from small intestine into the bloodstream (Bornet et al., 1987; Jenkins et al., 2002; Welch et al., 1987) and therefore determines the GI value (Jenkins et al., 1982). A CHO with a HGI results in rapid absorption of glucose and a large and fast rise in blood insulin which is followed by hypoglycaemia. A CHO with a LGI results in a slower and smaller rise in plasma glucose and insulin levels.

The development of HGI and LGI diets used in research studies is normally based on the published GI tables (Aston et al., 2008; Foster-Powell et al., 2002; Henry et al., 2005). Some researchers have found that the GI of CHO foods predict the relative glycaemic effect of mixed meals (Brand-Miller et al., 2003; Collier et al., 1986; Le Floch et al., 1991; Wolever et al., 2006), with protein and fat having negligible

effects (Wolever et al., 2006; Wolever & Bolognesi, 1996a). However, other studies have found that there was no association between the GI of the meal calculated from the GI tables and the measured GI of mixed breakfast meals (Flint et al., 2004; Alfenas & Mattes, 2005). It has been suggested that the GI, based on tests of single foods, may not apply in the setting of mixed meals containing representative amounts of fat and protein (Coulston, 1984; Franz et al., 2002; Pi-Sunyer, 2002) and cannot predict the glycaemic response to that meal (Coulston et al., 1987). In addition, a recent study by Galgani et al. (2006) suggested that foods with contrasting glycaemic indexes do not always induce a proportionally comparable difference in plasma glucose when consumed in large amounts.

It has been reported that both the amount and source of CHO influence the postprandial glucose and insulin responses of mixed meals in non-diabetic subjects (Wolever & Bolognesi, 1996b). The GL is defined as the measure which incorporates both quantity and quality of the dietary CHO consumed and has been proposed as an indicator of the glucose responses and insulin demand induced by individual foods across a wide range of portion sizes or a serving of foods (Brand-Miller et al., 2003). Although GI is usually tested on individual foods, there are methods described whereby the GI of the meals and habitual diets can be estimated (Wolever & Jenkins, 1996b, Salmeron et al., 1997).

Taking into consideration the above evidence, it is obvious that plasma glucose and insulin responses to high or low GI diets prescribed using the published GI tables (Aston et al., 2008; Foster-Powell et al., 2002; Henry et al., 2005) may be different from expected. Therefore in research studies investigating impact of HGI and LGI

foods, first of all it is important to make sure that the prescribed of HGI and LGI diets produce different responses in plasma glucose. Without proper evaluation of glucose responses to the diets prescribed using GI tables, interpretation of results might be misleading.

Since the main experimental chapters of this thesis are aiming to investigate the impact of high CHO diets with HGI and LGI consumed for 5 days on exercise energy metabolism in men and women, and on fasting plasma lipids and insulin sensitivity in moderate physically active individuals, the aim of this pilot study was to investigate whether high CHO with HGI and LGI meals defined by using the GI values of foods present within a meal produce significant differences in plasma glucose response in moderately active individuals.

3.2 Participants and Methods

3.2.1 Participants

Eight healthy, young women aged 23 ± 3 years and with body mass index 21.8 ± 1.0 kg/m^2 participated in this study. They were exercising regularly, at least two-three structured endurance or resistance exercise sessions per week. The present study was conducted with the approval of University of Glasgow's Medical Faculty Ethics Committee and participants provided written consent.

3.2.2. Study design

Each participant, in randomised counterbalanced order, participated in two dietary intervention trials lasting for 5 hours: in one trial they consumed a HC-HGI diet and

another HC-LGI diet. The dietary interventions were separated by a washout period of 7 days. For 2 days before the first trial, the participants were asked to refrain from participating in any vigorous physical activity and not to consume alcohol. Prior the first trial participants recorded their dietary intake and physical activity and they were asked to replicate this before the second trial to minimise differences in diet and lifestyle between experiment trials.

3.2.3 Main trials

The participants arrived at the laboratory after a 12-hour overnight fast, at approximately 8.30 a.m. The baseline finger prick capillary blood sampling was taken after the participant's left hand had been immersed for 10 minutes in a bath of water heated to 42^{o}C to ensure increased blood flow and arterialisation of the sample. The finger was swabbed with an alcohol swipe and prick was performed by digital puncture, using Accu-Chek Softclix lancets (Roche Diagnostic, Welwyn Garden City, UK). The initial blood droplet was wiped away and then another blood droplet was absorbed by the strip previously inserted into the blood glucose meter (HemoCue β-glucose system, HemoCue AB, Angelholm, Sweden), and the reading of the plasma glucose concentration was recorded. Then the participants consumed a breakfast meal within 10 minutes. Finger-prick blood samples were obtained at 30, 60, 90, 120, 150, 180, 210, 240 and 300 minutes after the baseline blood sample using the same blood sampling procedure. Morning snacks and lunch were provided to the participants at 120 and 240 minutes after baseline. The participants were asked to stay within the testing area, executing only sedate behaviour like lying, sitting, reading and studying. The test meals of the trial days were isoenergetic in both HC-

HGI and HC-LGI diets. All meals were prepared and weighed with an accuracy of 1g.

3.2.4 Development of experimental diets

Daily energy requirements of the participants were calculated by multiplying the measured RMR (see General Methods, section 2.3) with a PAL of 1.4 to get the sedentary energy expenditure. This energy requirement was divided into two representing the energy requirement for half day, to get the energy intake of the study meals. The proportions of energy from CHO, fat, and protein (70%, 15% and 15%, respectively) were similar in the prescribed HC-HGI and HC-LGI diets. The menu of prescribed HC-HGI and HC-LGI meals are presented in Table 3.1. The development of the experimental diets is described in detail in section 2.10, General Methods.

3.2.5 Dietary analysis, calculation of dietary glycaemic index and glycaemic load

The energy and nutrient intakes of the HC-HGI and HC-LGI diets were calculated using the Diet 5^{TM} programme. Dietary GI and GL were also estimated, as described in section 2.11, General Methods.

3.2.6 Blood glucose measurements

Both fasting and postprandial plasma glucose concentrations were analysed immediately using a HemoCue β-glucose system (HemoCue AB, Angelholm, Sweden). Duplicate measurements were taken at each time point to control for any variation. The mean of these two values from each time point were used as the final value.

3.2.7 Area under the curve and Incremental area under the curve calculations

The GI of the meals consumed over 300 minutes was calculated from the area under the plasma glucose response curve above the fasting plasma glucose concentration, ignoring any area beneath fasting values (Wolever 2004; Wolever & Jenkins 1986). The integrated area under the postprandial glucose curve was calculated using the trapezoidal method (Wolever & Jenkins, 1986) and was used to assess the plasma glucose responses for the whole period of observation. In the response for glucose plasma concentration versus time, the graph area was split into vertical segments. The total AUC for plasma glucose responses was calculated by adding all the segments together. The incremental area under the curve (IAUC) was given by subtracting the rectangular area (obtained by multiplying time of observation with the baseline concentration of glucose) from total AUC. To obtain a time-averaged IAUC, the IAUC was divided by the duration of the observation period.

3.2.8 Statistical analysis

Results are presented as means ± standard errors of the mean (SEM) unless otherwise stated. Data were tested for normality using the Anderson-Darling test before statistical analysis. Differences between energy and nutrient intakes, and total IAUC were compared using paired T-test. Data were analysed by using SPSS statistical software (version 18.0 for Windows, SPSS, Chicago, Ill., USA). Glucose responses during the 300 minutes experimental trials were compared by two-factor (trial x time) repeated-measures analysis of variance (ANOVA) with Tukey post hoc tests being used to locate the differences. Data were analysed using the Statistica software program (Statistica for Windows, version 6). All statistical significance was set at $P<0.05$.

Table 3.1 The menu of the high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) meals

Meal	HC-HGI	HC-LGI
Breakfast	Coco Pops (50g) + semi-skimmed milk (100g), Wholemeal bread (35g) + jam (15g) + low-fat spread (4g) Tea (260g) + semi-skimmed milk (40g)	All bran (50g) + semi-skimmed milk (100g), Burgen bread (35g) + jam (15g)+ low fat spread (4g) Tea (260g) + semi-skimmed milk (40g)
Morning Snacks	Wholemeal bread (70g) + lean ham (25g) + low-fat spread (6g) + cucumber (20g) + tomatoes (20g) Lucozade original (170g)	White Pita bread (60g) + lean ham (25g) + low fat spread (6g) + cucumber (20g) + tomatoes (20g) Lentil soup (200g) Unsweetened apple juice (170g)
Lunch	Instant Potato (100g) + Peas (40g) + Chunky Chicken in Gravy (150g), Lucozade original (165g) Bananas (50g)	Spaghetti (cooked weight 150g) + Pasta sauce (100g) + mince meat in tomato sauce (100g) Unsweetened apple juice (165g) Low fat yogurt (100g) Orange (50g)

Note: This menu was prescribed to the participants with energy requirements of approximately 1500 kcal/day

3.3 Results

3.3.1 Dietary intakes

The energy and nutrient intakes, GI and GL of HC-HGI and HC-LGI diets are presented in Table 3.2. Both the HC-HGI and HC-LGI diets were isocaloric and there were no significant differences in available CHO, sugars, non-milk extrinsic sugars (NMES), starch, fibre, fat and protein between the two experimental diets. The GI and GL in the HC-HGI diet were significantly higher (P<0.001) than in the HC-LGI diet.

3.3.2 Incremental area under the glucose responses curve

The time-averaged incremental area under the glucose versus time curve for glucose responses over 300 minutes was significantly higher (Figure 3.1; P<0.05) in the HC-HGI diet (3.29 ± 0.38 mmol/L) than the HC-LGI diet (1.50 ± 0.42 mmol/L). The concentration of glucose following the HC-HGI diet was significantly higher (Figure 3.1; P<0.05) at 30, 90, 150, 180, 270 and 300 minutes in comparison to the HC-LGI diet.

Table 3.2 Energy and nutrient intakes, percentage (%) of energy from macronutrients, glycaemic index (GI) and glycaemic load (GL) of high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) diets consumed over the duration of 300 minutes. Values are mean ± SEM, *n*=8.

	HC-HGI	HC-LGI
Nutrient Intake		
Available CHO (g)	205 ± 6	205 ± 7
Sugar (g)	35 ± 3	31 ± 3
NMES (g)	62 ± 3	60 ± 2
Starch (g)	108 ± 3	110 ± 3
Fibre (g)	13 ± 1	14 ± 1
Fat (g)	20 ± 1	20 ± 2
Protein (g)	43 ± 3	44 ± 3
CHO (g/kgBM/day)	3.4 ± 0.2	3.4 ± 0.2
Energy Intake (MJ)	4.9 ± 0.2	5.0 ± 0.2
Energy from CHO (%)	70 ± 1	70 ± 0
Energy from Fat (%)	15 ± 0	15 ± 0
Energy from Protein (%)	15 ± 0	15 ± 0
GI	71 ± 2	35 ± 0 [a]
GL	148 ± 11	80 ± 6 [a]

NMES – Non-milk extrinsic sugars. [a] HC-LGI trial was significantly different ($P< 0.01$) from HC-HGI trial.

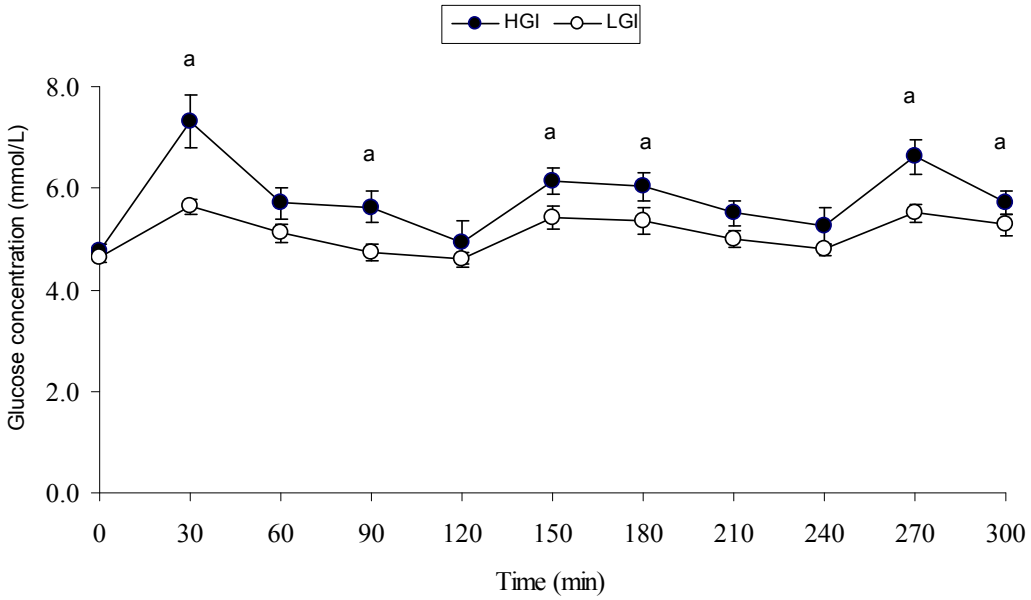


Figure 3.1 The time-averaged incremental area under glucose versus time curve over 300 minutes after consumption high carbohydrate-high glycaemic index (HC-HGI) diet and high carbohydrate-low glycaemic index (HC-LGI) diet, *n*=8.

[a] HC-HGI diet significantly different ($P<0.05$) than HC-LGI diet. Meals were consumed at 0, 120, and 240 minutes.

3.4 Discussion

The main finding of this study is that prescribing high CHO diets with HGI and LGI based on the GI values of CHO foods present within a meal produced a significant difference in postprandial plasma glucose responses in normoglycaemic individuals. We found that plasma glucose responses over the 300 minutes dietary observation in HC-HGI diet were higher ($P<0.05$) than HC-LGI diet.

In the present study, the finding that HC-HGI diet elicited a greater plasma glucose response than HC-LGI diet is consistent with the findings from Wolever et al. (2006) and Nansel et al. (2008) indicating that GI CHO containing foods present within a mixed meal is a significant determinant of the glycaemic responses of mixed meals. GI largely depends on the rate at which the CHO is digested and absorbed from the small gut into the bloodstream (Jenkins et al., 2002). The quickly digested and absorbed CHO foods will produce a higher increase in postprandial glycaemia. Therefore, the difference in plasma glucose responses between HC-HGI and HC-LGI diets observed in the present study was due to the differences in the rates of digestion and absorption between both high CHO diets. This is supported by evidence that HGI meals produce a faster rate of digestion and absorption than LGI meals (Englyst et al., 2003).

Both HC-HGI and HC-LGI meals were prepared based on the participants' energy requirements and were consumed by the participants in the laboratory. The energy and nutrient intakes were not different between the experimental meals. The fat and protein content were similar between the two experimental meals to minimise variability. It has been shown that fat and protein modify the glycaemic response to

CHO meals by slowing gastric emptying (Welch et al. 1987) and increasing insulin secretion (Nuttall et al., 1984), respectively. Dietary analysis revealed consistently and significantly higher GI and GL in the HC-HGI diet compared to HC-LGI diet. The extent of the postprandial plasma glucose response results mainly from the effect of the GI and the amount of available CHO contained in the meals (Wolever & Bolognesi, 1996b), therefore both GI and GL are important determinants of postprandial glycaemic responses. It cannot be advised to prescribe diets based on GI tables alone, as the GL of the meals has such a profound impact on the postprandial metabolic responses to CHO foods (Brand-Miller et al., 2003; Galgani et al., 2006). In addition, as the amount of CHO in a meal increases, a proportionally, almost linear rise in plasma glucose and incremental area under the curve is seen (Chew et al, 1988).

The participants who took part in this study were all moderately active women. It has been shown that training status does not influence the GI determination in women (Mettler et al., 2008). In contrast, the GI of CHO containing foods was dependent on training status in men (Mettler et al., 2006). Furthermore, studies on the impact of GI on glucose responses have been carried out on people with metabolic disorders, sedentary and obese individuals which found lower glycaemic responses after LGI diet compared to HGI diet (Brand-Miller, 2003). Thus, the present study gives valuable evidence that the GI diets created from existing GI tables, also produces significant differences to glycaemic response in healthy physically active individuals. However, the HGI and LGI diets in the present study were prescribed for active individuals and may not be suitable for patients with metabolic disorder due to high energy from CHO.

The GI of mixed meals has also been shown to correlate positively with insulin responses (Brand-Miller et al., 2003; Wolever & Bolognesi, 1996a). In the present study, the postprandial insulinemic responses accompanying the glycaemic responses of the high CHO diets were not measured. However, there appears to be a close correlation between glycaemic and insulinemic responses for many CHO-rich foods and for pure CHO (Englyst et al., 2003; Holt et al., 1997). As the purpose of the present study was to compare the plasma glucose responses of high CHO meals between HGI and LGI, glucose measurements should be enough. However correction for changes in plasma volume was not conducted. In addition, to consume three meals within the space of 300 min may have resulted in an accumulated effect on plasma glucose responses, as all meals contained a high percentage of CHO.

Since results from Suh et al. (2002) showed that glucose flux during both rest and postprandial state was not affected by menstrual cycle phase, the wash out period between HGI and LGI trials was only 7 days. This reduced the length of this pilot study and also prevented changes in life style between the two trials, as dietary intake and physical activity were controlled in the days before the dietary experiments in the present study to minimise variability. We believe that this control is important regardless of the evidence suggesting that postprandial glycaemic responses to food are not affected by controlling participants' prior diet and activity (Campbell et al., 2003).

In conclusion, in healthy physically active individuals high CHO diets with HGI and LGI prescribed using GI values available for CHO containing foods produce significant differences in plasma glucose responses. Therefore these meals can be

used in the main experimental trials to investigate the impact of GI of high CHO diets on exercise energy metabolism and capacity, fasting plasma lipids and insulin resistance.

Chapter 4 The effect of glycaemic index of high carbohydrate diets consumed for five days on exercise energy metabolism and running capacity in men

4.1 Introduction

It is well established that increased dietary CHO intake for several days before endurance events increases muscle glycogen concentration (Rauch et al., 1995; Tarnopolsky et al., 2001) and enhances performance (Brewer et al. 1988; Rauch et al. 1995). It is also known that optimizing glycogen content with high CHO diets occurs at the cost of diminished availability and contribution of fat towards exercise energy expenditure (Brewer et al., 1988; Coyle et al., 2001). This compromised fat oxidation, in addition to enhanced pre-exercise muscle glycogen availability, could be expected to enhance the rates of glycogenolysis and glycogen depletion (Arkinstall et al., 2004; Hargreaves et al., 1995; Wojtaszewski et al., 2003), a condition known to be accompanied by an impairment in oxidative energy provision (Gibala et al., 2002), disturbances in excitation-contraction coupling (Hargreaves, 2004), and thus attenuate the enhancement of exercise performance expected from a high CHO intake.

Several recent studies have suggested that lipid availability and fat oxidation during endurance exercise conducted under conditions of a high CHO intake might be influenced by the GI. First, there is evidence to suggest that consuming CHO with a LGI diet 2-3 hours before endurance exercise results in increased availability of plasma NEFA and thus a higher rate of total fat oxidation (Febbraio et al., 2000;

Kirwan et al., 2001; Sparks et al., 1998; Wee et al., 1999; Wu et al., 2003; Wu & Williams, 2006). Second, three recent studies have suggested that a LGI diet consumed during 24 hour recovery between bouts of prolonged strenuous exercise may also result in greater availability of plasma NEFA (Stevenson et al., 2005b, 2009; Trenell et al., 2008) and sometimes (Stevenson et al., 2005b), but not always (Stevenson et al., 1009; Trenell et al., 2008), a higher rate of total fat oxidation during subsequent exercise conducted in the fasted state.

There is some evidence to suggest that availability of fasting NEFA could also be influenced by the GI of moderate CHO diets consumed for an extended period (Kiens & Richter, 1996). In contrast, 24 hour post-exercise recovery studies (Stevenson et al., 2005b, 2009; Trenell et al., 2008), Chen et al. (2008) reported no impact of the GI of the CHO diets consumed for 3 days on exercise energy substrate utilisation and exercise performance. Therefore, further studies are required to determine whether adaptations to the GI of a high CHO diet consumed for longer periods differ from those seen in shorter intervention studies.

The aim of the present study was to investigate the impact of consuming a high CHO diet with either HGI or LGI for 5 days on energy substrate utilisation during running conducted in the fasted state in physically active men. As in previous research (Chen et al., 2008; Febbraio et al., 2000; Sparks et al., 1998; Stevenson et al., 2005b; Wee et al., 1999; Wu & Williams, 2006), our study also aimed to evaluate whether the GI of high CHO diets impacts on exercise capacity.

4.2 Participants and Methods

4.2.1 Participants

Nine physically active men participated in the present study. Participant characteristics are shown in Table 4.1. Inclusion and exclusion criteria, recruitment and ethical approval process are described in detail in section 2.1 of General Methods. All participants had been involved in regular endurance training for at least 4 years and some had been involved in endurance type events and competitive sports. The present study was conducted with the approval of University of Glasgow's Medical Faculty Ethics Committee and participants provided written consent.

4.2.2 Study design

Each participant, in randomised counterbalanced order, performed three treadmill runs to exhaustion at 65% $\dot{V}O_2$ max: one following their habitual diet (Control trial), another after 5 days on a HC-HGI diet, and a third after 5 days on a HC-LGI diet. The exercise trials were separated by a washout period of a minimum of 11 days. During the 5 days leading up to the first treadmill run, the participants recorded all planned and structured exercise conducted and were asked to replicate this before their second and third runs. For 2 days before the running trials, the participants were asked to limit themselves to activities of daily living and slow walking or cycling for personal transport over short distances and were asked not to consume alcohol. The participants recorded all fluids they consumed during 24 hours leading up to the first running trial and were asked to replicate this before the second and third trials. To ensure euhydration, the participants were instructed to consume about a litre of water the night before and ~500 ml in the morning before each running trial. All running

trials were performed under similar experimental and environmental conditions. Two weeks before the main running trial, the participants undertook a familiarisation run to ensure that their predicted running speed corresponded to 65% $\dot{V}O_2$ max and to allow the participants to become familiar with the experiment protocol.

4.2.3 Preliminary exercise test

Before the familiarization run and treadmill runs to exhaustion, two preliminary exercise tests were conducted to find the speed that elicited 65% $\dot{V}O_2$ max for each participant. In the first, the steady-state relationship between submaximal $\dot{V}O_2$ and treadmill speed was established (see General Methods, section 2.4.1). In the second, $\dot{V}O_2$ max was determined during uphill running at constant speed which range from 9 to 11.5 km/h, as described in section 2.4.2 of General Methods.

4.2.4 Main exercise trials

The participants arrived at the laboratory after a 12-hour overnight fast, at approximately 8.30 a.m. They were weighed and a cannula (Venflon 18G, Becton Dickinson Ltd, Helsingborg, Sweden) was inserted in an antecubital vein. After a brief warm up consisting of 5 minutes of continuous running at 60% $\dot{V}O_2$ max followed by 5 minutes of stretching, the participants ran to exhaustion on the treadmill at a speed equivalent to 65% of $\dot{V}O_2$ max. During the last minute of every 15 minute stage throughout the run, expired air samples were collected using the Douglas bag technique, heart rate was monitored using short- range telemetry (Polar S610i, Polar Electro, Finland) and RPE were recorded using the Borg scale. These measurements were followed by blood sample collection. After each blood sample

the cannula was flushed with saline solution (0.9% w/v Sodium Chloride Intravenous Infusion BP, B.Braun Melsungen AG, Melsungen Germany) to keep it patent throughout the experiment. Figure 4.1 presents the schematic of the experimental design. During trials, participants were allowed to drink water and an electric fan was used to cool them. Strong verbal encouragement was also given to the participants throughout the run and especially when near to exhaustion. Exhaustion was defined as the point at which the participants were no longer able to maintain the prescribed running speed. When the participants signalled that they could only manage 2 minutes, expired air was immediately collected, heart rate was recorded and a blood sample was taken.

4.2.5 Development of high CHO diets with high and low glycaemic index

The energy intake of the prescribed diets was based on the participants' habitual energy requirement (see General Methods, section 2.9). The proportions of energy from CHO, fat, and protein (70%, 15% and 15%, respectively) were similar in the prescribed HC-HGI and HC-LGI diets. An example of prescribed HC-HGI and HC-LGI meals are presented in Table 4.2. The development of the experimental diets is described in detail in section 2.10, General Methods.

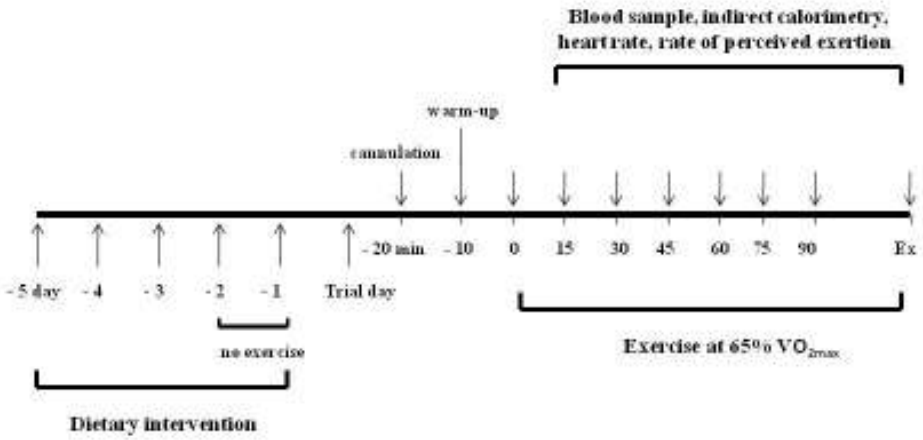


Figure 4.1 Schematic representation of the study design

4.2.6 Dietary analysis, calculation of dietary glycaemic index and glycaemic load

Dietary intake of energy and macronutrients from participants' habitual dietary records (Control trial) as well as from the consumed experimental diets were calculated using a Diet 5^{TM} programme. Dietary GI and GL were also estimated from the participants' habitual diet records and from the consumed experimental diets, as described in section 2.11, General Methods.

4.2.7 Measurement of substrate oxidation and energy expenditure

Expired air was collected into a Douglas bag over 60 seconds and immediately analysed for O_2 and CO_2 concentrations (Servomex 1440, Crowborough, UK). The

expired gas volume was determined using a dry gas meter (Harvard, Kent, UK), and $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated using the Haldane transformation (see General Methods, section 2.7). EE and substrate oxidation during running were estimated from $\dot{V}O_2$ and $\dot{V}CO_2$ using indirect calorimetry, neglecting protein oxidation (Ferrannini, 1988), as described in section 2.8.2, General Methods.

4.2.8 Anthropometry and body composition

Measurements of body weight and body fat were taken using bioelectrical impedance scales (TBF-300, TANITA, Cranlea, UK) and height was determined as described in section 2.2 of General Methods.

4.2.9 Plasma preparation and analysis

Blood samples were collected into a pre-cooled 7.5 ml EDTA Vacutainer™ tube (BD Vacutainer Systems, Plymouth, UK) and centrifuged at centrifugal force of 1509 x g for 15 minutes at 4°C. After centrifugation, aliquots of plasma were transferred using a disposable plastic Pasteur pipette into labelled 1.5-ml Eppendorf tubes (Eppendorf AG, Hamburg, Germany). The aliquoted plasma was then stored at -80°C for later analysis of insulin (see section 2.13.1, General Methods), glucose, NEFA and glycerol (see section 2.13.2, General Methods).

4.2.10 Power calculation

The *post hoc* power calculation was carried out using Minitab statistical software (version 15 for Windows). Using a standard deviation (SD) for difference in fat oxidation of 0.06 and SD for difference in CHO oxidation of 0.15, 9 participants

would be sufficient to detect difference of 0.07 g/min in case of fat oxidation and 0.085 g/min in case of CHO oxidation with a power of 85%.

4.2.11 Statistical analysis

Results are presented as means ± SEM unless otherwise stated. Data were tested for normality using the Anderson-Darling test before statistical analysis. Reponses during the exercise period were compared by two-factor (trial x time) ANOVA, with Tukey *post hoc* test being used to locate the differences. Differences between dietary profiles and those at the point of exhaustion were compared by one factor (trial) ANOVA. Statistical significance was set at $P<0.05$. Data were analysed using the Statistica software program (Statistica for Windows, version 6).

Table 4.1 Participant characteristics. Values are means ± standard deviation (SD), *n*=9

Characteristics	Males
Age (years)	24 ± 4
Weight (kg)	72 ± 8
Height (cm)	179 ± 7
BMI (kg/m^2)	22 ± 2
Fat (%)	10 ± 4
Fat free mass (kg)	60 ± 9
Maximum oxygen consumption (ml/kg/min)	60 ± 4

Table 4.2 Example of prescribed high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) meals consumed for the duration of 5 days.

Meal	HC-HGI	HC-LGI
Breakfast	Coco Pops (100g) + semi-skimmed milk (200g) Wholemeal bread (70g) + jam (30g) + low-fat spread (8g) Tea (260g) + semi-skimmed milk (40g)	All bran (100g) + semi-skimmed milk (200g), Burgen bread (70g) + jam (30g) + low fat spread (8g) Tea (200g) + semi-skimmed milk (40g)
Snack	Apples (100g) Bananas (100g)	Pear (100g) Orange (100g)
Lunch	Wholemeal bread (140g) + lean ham (50g) + low-fat spread (12g) + cucumber (40g) + tomatoes (40g) Lucozade original (340g)	White Pita bread (120g) + lean ham (50g) + low fat spread (12g) + cucumber (40g) + tomatoes (40g) Lentil soup (400g) Unsweetened apple juice (340g)
Snack	Rice Cakes (30g) Lucozade original (330g)	Cheese flavoured oatcakes (30g) Unsweetened apple juice (330g)
Evening meal	Instant Potato (200g) + Peas (80g) + Chunky Chicken in Gravy (300g) Lucozade original (330g) Bananas (100g)	Spaghetti (cooked weight 300g) + Pasta sauce (200g) + mince meat in tomato sauce (200g) Unsweetened apple juice (330g), Low fat yogurt (200g) Orange (100g)
Evening snack	Wholemeal bread (70g) + low-fat spread (5g) + jam (15g) Tea (260g) + semi-skimmed milk (40g)	Burgen bread (70 g) + low fat spread (5g) + jam (15g) Tea (260g) + semi-skimmed milk (40g)

Note: This menu was prescribed to subject with energy requirements of approximately 2900 kcal/day

4.3 Results

4.3.1 Dietary intake

Daily dietary intakes for the 5 days preceding the run to exhaustion are presented in Table 4.3. Both the HC-HGI and HC-LGI diets were isocaloric to the habitual diet and provided energy that was very similar to the predicted energy requirements (12.40 ± 0.34 MJ). Daily intake of available CHO and the percentage of energy provided by CHO was significantly higher (P<0.001) in the two experimental trials than the Control trial. There were no significant differences in available CHO, NMES, starch, fibre intakes and the percentage of energy provided by CHO between HC-HGI and HC-LGI diets. The sugar content was not different between the two experiment diets and the control trial. The GI and GL in the HC-HGI diet were significantly higher (P<0.001) than in the Control trial. The GI of the HC-LGI diet was significantly lower (P<0.001) compared to the Control trial. GI and GL of the HC-HGI diet were significantly higher (P<0.001) than those of HC-LGI.

Body weight (Control trial, 74.1 kg ± 6.4; HC-HGI trial, 74.1kg ± 6.9; HC-LGI trial, 74.3kg ± 6.8) and body fat percentage (Control trial, 9.8 % ± 3.4; HC-HGI trial, 10.1 % ± 6.9; HC-LGI trial, 10.1 % ± 3.9) before the run to exhaustion were not significantly different between Control, HC-HGI and HC-LGI trials. Values are means ± standard deviation (SD), n=9.

4.3.2 Plasma glucose, insulin, non-esterified fatty acids and glycerol

Concentrations of plasma glucose, insulin, NEFA and glycerol measured during 90 minutes of running and at the point of exhaustion (Figure 4.2 a-d) were not

significantly different between the HC-HGI and HC-LGI trials. During the 90 minutes of running and at the point of exhaustion, plasma glycerol concentration in the two experimental trials was significantly lower (Figure 4.2c; $P<0.05$) than in the Control trial. At exhaustion, plasma glucose concentrations in the HC-LGI trial tended ($P=0.06$) to be higher than in the Control trial, but were similar between the HC-HGI and HC-LGI trials. The concentrations of plasma insulin and NEFA during running and at the point of exhaustion exercise were not significantly different between trials.

4.3.3 Fat and CHO oxidation

The rates of fat and CHO oxidation during 90 minutes of running and at the point of exhaustion (Figure 4.3) were not significantly different between the HC-HGI and HC-LGI trials. During 90 min of running, the rate of fat oxidation in both experimental trials was significantly lower (Figure 4.3; $P<0.05$), and the rate of CHO oxidation higher (Figure 4.3: $P<0.05$) than in Control trial. At the point of exhaustion, compared with the Control trial the rate of fat oxidation was lower (Figure 4.3; $P<0.05$) and the rate of CHO oxidation higher (Figure 4.3; $P<0.05$) only in the HC-LGI trial. The proportion of energy from fat and CHO during 90 minutes of running is presented in Figure 4.4.

4.3.4 Heart rate, ratings of perceived exertion and oxygen consumption

There were no differences in HR, RPE and $\dot{V}O_2$ during 90 minutes of running and at the point of exhaustion (Table 4.4) between trials.

Table 4.3 Daily macronutrient and energy intakes, percentage (%) of energy from macronutrients, glycaemic index (GI) and glycaemic load (GL) of habitual (Control), high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) diets consumed over the duration of 5 days prior run to exhaustion at 65% $\dot{V}O_2$ max. Values are mean ± SEM, *n*=9.

	Control	HC-HGI	HC-LGI
Nutrient Intake			
Available CHO (g)	349 ± 27	511 ± 11 [a]	508 ± 12 [a]
Sugar (g)	80 ± 9	65 ± 16	49 ± 14
NMES (g)	96 ± 15	194 ± 15 [a]	195 ± 12 [a]
Starch (g)	186 ± 13	255 ± 12 [a]	266 ± 9 [a]
Fibre (g)	29 ± 4	39 ± 1	39 ± 3
Fat (g)	74 ± 4	47 ± 2 [a]	47 ± 2 [a]
Protein (g)	115 ± 7	102 ± 3	107 ± 2
CHO (g/kgBM/day)	4.6 ± 0.5	7.4 ± 0.2 [a]	7.3 ± 0.2 [a]
Energy Intake (MJ)	11.0 ± 0.5	12.5 ± 0.4	12.3 ± 0.3
Energy from CHO (%)	53 ± 2	71 ± 0 [a]	70 ± 1 [a]
Energy from Fat (%)	26 ± 2	15 ± 0 [a]	15 ± 0 [a]
Energy from Protein (%)	18 ± 1	14 ± 0 [a]	15 ± 0 [a]
GI	56 ± 1	71 ± 1 [a]	36 ± 0 [a b]
GL	171 ± 17	391 ± 9 [a]	216 ± 6 [b]

NMES – Non-milk extrinsic sugars. [a] HC-HGI and HC-LGI trials significantly different ($P< 0.01$) from Control trial, [b] HC-LGI trial significantly different ($P< 0.01$) from HC-HGI trial.

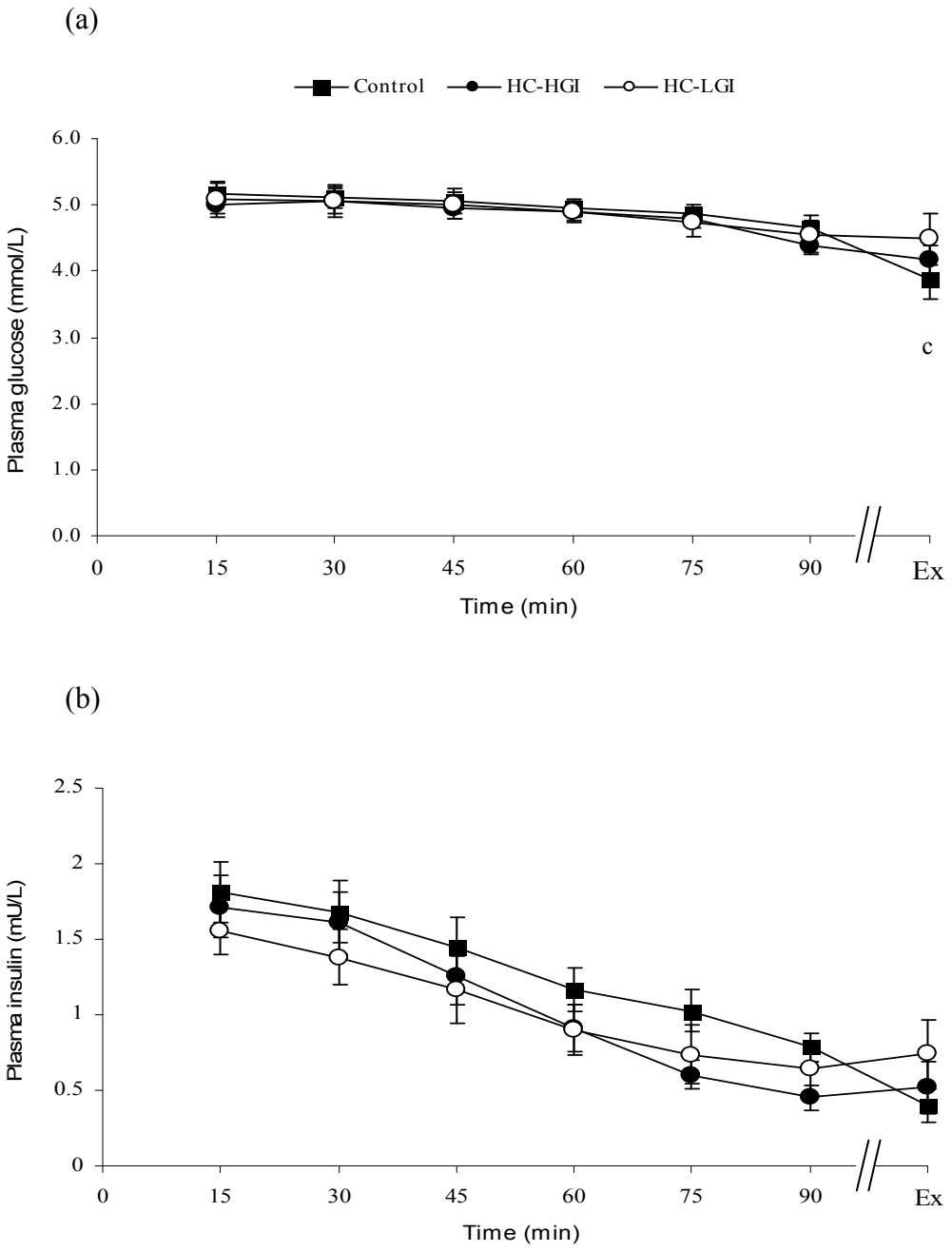
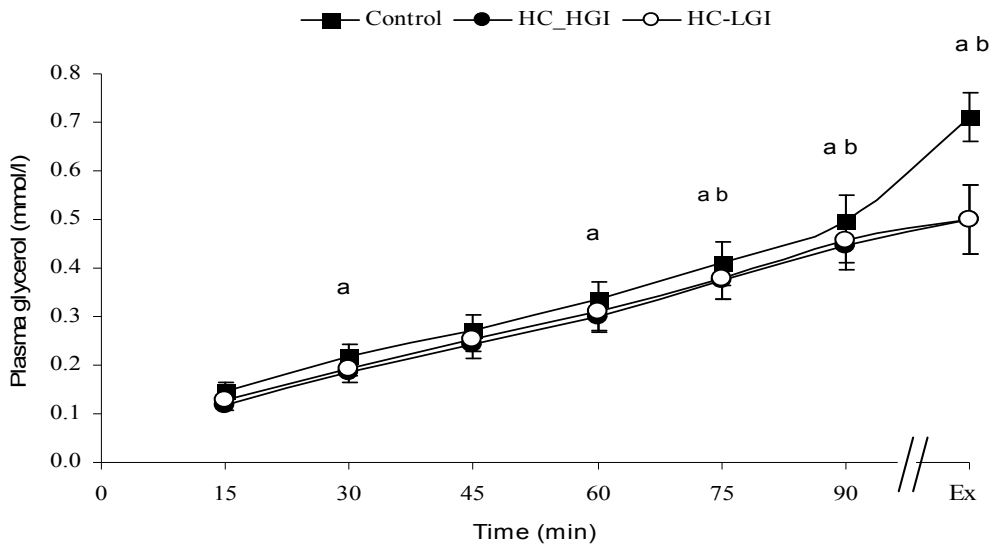


Figure 4.2 Plasma concentrations of (a) glucose (mmol/L) and (b) insulin (mU/L) for the duration of 90 min running at 65% $\dot{V}O_2$ max and at the point of exhaustion in Control trial (■), high carbohydrate-high glycaemic index (HC-HGI) trial (●) and high carbohydrate-low glycaemic index (HC-LGI) trial (○). Values are means ± SEM; *n*=9 for 15-90 min, and *n*=8 at exhaustion (Ex). [c] tendency for difference (p=0.06) between Control and HC-LGI trials.

(c)



(d)

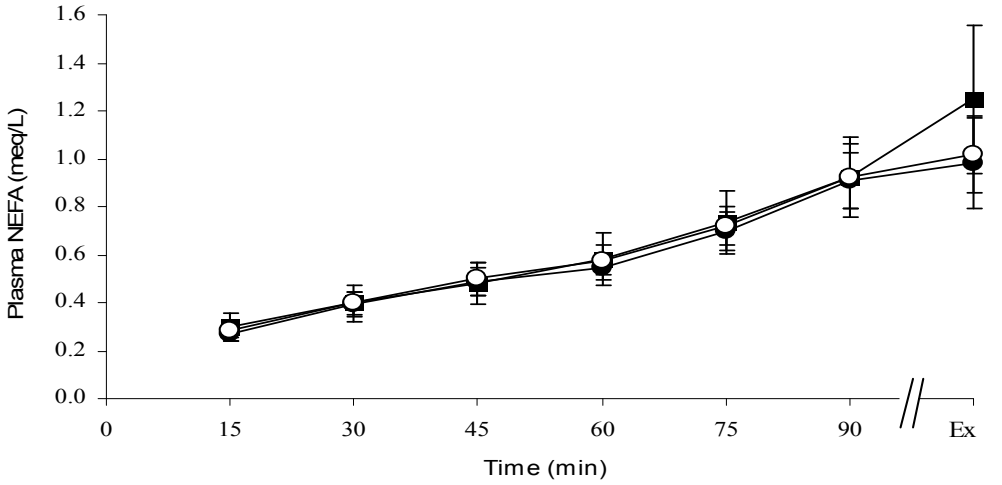


Figure 4.2 (continue) Plasma concentrations of (c) glycerol (mmol/L) and (d) NEFA (meq/L) for the duration of 90 min running at 65% $\dot{V}O_2$ max and at the point of exhaustion in Control trial (■), high carbohydrate-high glycaemic index (HC-HGI) trial (●) and high carbohydrate-low glycaemic index (HC-LGI) trial (○). Values are means ± SEM; *n*=9 for 15-90 min, and *n*=8 at exhaustion (Ex). [a] Control trial significantly different ($P< 0.05$) from HC-HGI trial; [b] Control trial significantly different ($P< 0.001$) from HC-LGI trial.

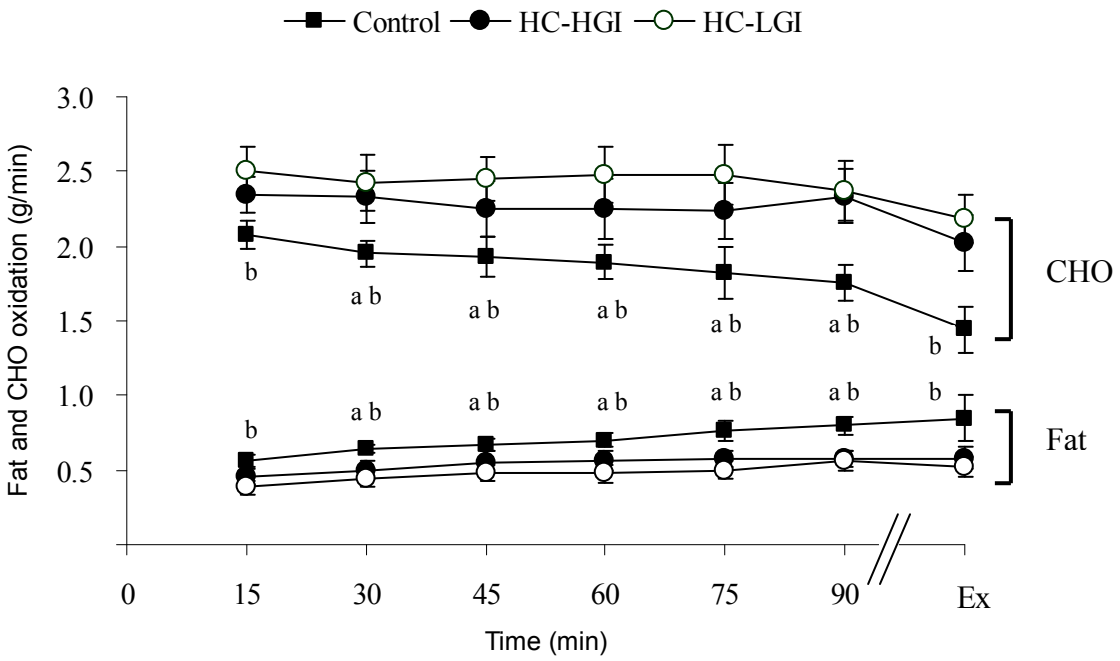


Figure 4.3 Rate of fat and CHO oxidation (g/min) for the duration of 90 min running at 65% $\dot{V}O_2$ max and at the point of exhaustion in Control trial (■), high carbohydrate-high glycaemic index (HC-HGI) trial (●) and high carbohydrate-low glycaemic index (HC-LGI) trial (○). Values are means ± SEM; *n*=9 for 15-90 min, and *n*=8 at exhaustion (Ex). [a] Control trial significantly different ($P< 0.05$) from HC-HGI trial, [b] Control trial significantly different ($P< 0.001$) from HC-LGI trial.

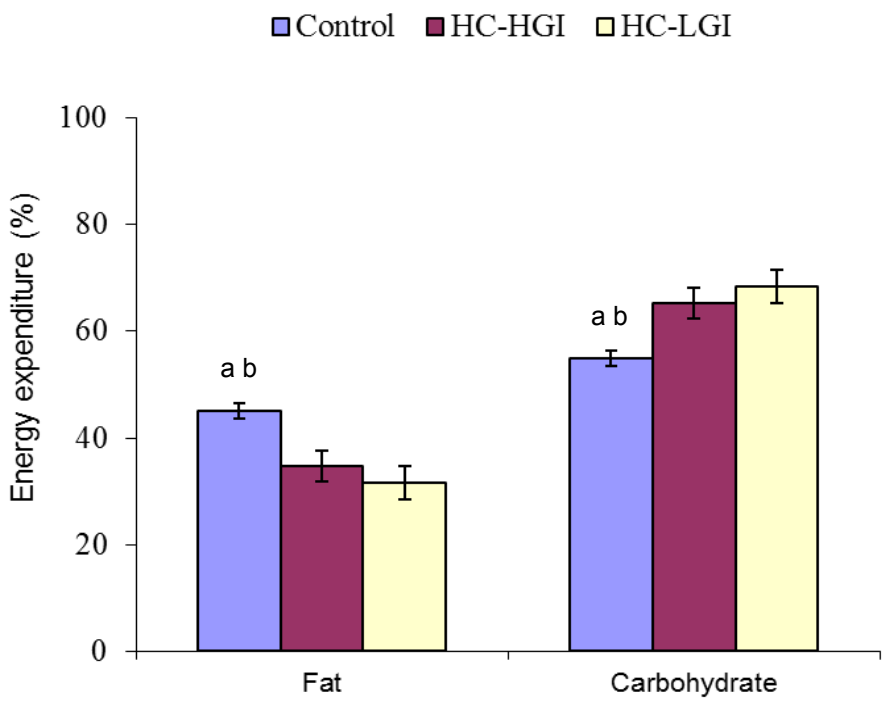


Figure 4.4 Percentage of energy expenditure (%) from fat and CHO during 90 min of running in the Control trial, high carbohydrate-high glycaemic index (HC-HGI) trial and high carbohydrate-low glycaemic index (HC-LGI) trial.

Values are means ± SEM, *n*=*9*. [a] Control trial significantly different ($P< 0.05$) from HC-HGI trial, [b] Control trial significantly different ($P< 0.001$) from HC-LGI trial.

Table 4.4 Oxygen consumption ($\dot{V}O_2$, ml/kg/min), rate of perceived exertion (RPE) and heart rate (HR, beats/min) during running on the treadmill at 65% $\dot{V}O_2$ max to exhaustion (Ex) in the Control, high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) trials. Values are means ± SEM; *n*=9 for 15-90 min, and *n*=8 at exhaustion (Ex).

	15 min	30 min	45 min	60 min	75 min	90 min	Ex
$\dot{V}O_2$							
Control	38.2 ± 0.7	38.9 ± 0.8	39.6 ± 0.9	40.0 ± 0.9	40.8 ± 0.9	41.0 ± 1.2	40.6 ± 1.4
HC-HGI	38.4 ± 0.9	39.5 ± 1.1	40.3 ± 1.1	40.2 ± 1.0	40.4 ± 1.2	41.6 ± 1.3	42.5± 2.5
HC-LGI	38.3 ± 1.2	39.0 ± 1.2	40.0 ± 1.1	40.0 ± 1.2	40.5 ± 1.2	41.0 ± 1.2	41.4 ± 1.4
RPE							
Control	11 ± 0	12 ± 0	13 ± 0	13 ± 0	14 ± 1	15 ± 1	17 ± 1
HC-HGI	11 ± 0	12 ± 0	13 ± 0	13 ± 1	15 ± 1	15 ± 1	18 ± 1
HC-LGI	10 ± 1	12 ± 0	12 ± 0	13 ± 0	14 ± 1	16 ± 1	18 ± 1
HR							
Control	148 ± 4	154 ± 4	157 ± 4	159 ± 5	162 ± 5	164 ± 6	167 ± 6
HC-HGI	148 ± 2	155 ± 3	159 ± 4	162 ± 5	164 ± 5	166 ± 5	171 ± 6
HC-LGI	148 ± 4	155 ± 4	160 ± 4	161 ± 4	164 ± 5	167 ± 5	172 ± 6

4.3.5 Time to exhaustion and running distance

Of the nine participants, one participant stopped exercising before exhaustion in the one of the trials because of a hip problem. Therefore, results for time to exhaustion and running distance are presented for only eight participants. Time to exhaustion (Control trial, 114 min ± 15; HC-HGI, 107 min ± 18; HC-LGI trial, 110 min ± 18) and distance covered (Control trial, 19 km ± 3; HC-HGI, 18 km ± 5; HC-LGI trial, 19 km ± 5) were not significantly different between trials. These data are presented as mean ± standard deviations (SD).

4.4 Discussion

The main finding of the present study is that the extent to which high CHO diets consumed for 5 days reduce the rate of fat oxidation during running in the fasted state is not influenced by the GI of the diet. We also found that GI of the high CHO diets consumed for 5 days has no impact on running capacity. From a practical perspective, our findings suggest that when HGI diets are consumed for 3-5 days leading up to an endurance event, it is not necessary to consider the GI of the high CHO diets.

As reported previously (Arkinstall et al., 2004; Brewer et al., 1988; Chen et al., 2008; Coyle et al., 2001; Wojtaszewski et al., 2003), the rate of total fat oxidation and thus the contribution of fat to running energy expenditure was lower following both high CHO trials than in the Control trial. Also, the plasma concentration of NEFA during the 90 minutes of running after both high CHO diets was strikingly similar to the concentration measured during the Control trial. Thus, the reduced oxidation of fat observed during running after the high CHO diets may be a reflection of the reduced

availability and utilisation of IMTG, the main contributor of non-plasma derived fatty acids (Roepstorff et al., 2005; van Loon et al., 2003). This suggestion receives support from the evidence that IMTG content is modified by dietary macronutrient composition (Roepstorff et al., 2005).

Previous evidence suggests that high CHO LGI diets consumed during 24 hour recovery periods between bouts of prolonged strenuous exercise may result in greater availability of plasma NEFA (Stevenson et al., 2005b; Trenell et al., 2008) and sometimes (Stevenson et al., 2005b) but not always (Trenell et al., 2008), in a higher rate of fat oxidation during subsequent exercise conducted in the fasted state. However, in our study, the GI of high CHO diets consumed for 5 days had no impact on plasma NEFA measured during running to exhaustion. In addition, the rate of total fat and CHO oxidation was not different between the two high CHO trials, which are consistent with the finding of Chen et al. (2008) that the amount rather than GI of the GL consumed during a 3-day isoenergetic CHO loading is the most overriding factor for subsequent exercise energy metabolism. Therefore, when high CHO diets are consumed for a few days before an endurance event, the expected reduction in fat oxidation (Arkinstall et al., 2004; Brewer et al., 1988; Coyle et al., 2001) and thus compensatory increase in rate of muscle glycogen utilisation (Arkinstall et al., 2004; Wojtaszewski et al., 2003) cannot be prevented by lowering the GI of the foods consumed.

We found that the GI of high CHO diets consumed for 5 days had no impact on exercise capacity measured as time to exhaustion during endurance run in the fasted state. This finding is in line with recent evidence suggesting that GI of high CHO

diets consumed for 3 days has no impact on time taken to complete 10-km run (Chen et al., 2008). Brewer et al. (1988) observed no impact of quality of CHO consumed on exercise performance in their study, in which time to complete treadmill run to exhaustion was measured in two groups of experienced runners who were required to obtain 70% of energy from CHO for 3 days, either by supplementing their normal diets with complex (synonymous with LGI) or simple (synonymous with high HGI) CHO.

In the present study, exercise running capacity measured after the high CHO trials consumed for 5 consecutive days was not different from that in the Control trial. This finding is quite unexpected, since increased dietary CHO intake elevates muscle and liver glycogen concentrations (Arkinstall et al., 2004; Burke & Hawley, 2006; Tarnopolsky et al., 2001; Wojtaszewski et al., 2003), which prolongs time to exhaustion in trials lasting over 90 minutes (Brewer et al., 1988; Hawley et al., 1997). During both the HC-HGI and HC-LGI trials in the present study, CHO intake was on average 2.8 g/kg BW/day higher than in the Control trial and amounted of 7.3-7.4 g/kg BW/day, which is only slightly lower that the commonly recommended value of 8-10 g/kg BW/day for optimum muscle glycogen storage (Burke & Hawley, 2006; Bussau et al., 2002). It should be noted however, that diet of the Control trial was not strictly controlled and coincided with participants' habitual diet. Thus, although intake of available CHO in both high CHO trials was significantly higher than in the Control trial, the difference in CHO intake between habitual and high CHO diets in some participants may have been insufficient to promote reasonable increase in muscle glycogen and thus increase exercise capacity. This may have contributed to the fact that there was no significant difference in exercise capacity

between the Control and high CHO trials. In addition, since the rate of muscle glycogen utilisation and thus the development of fatigue are very sensitive to exercise intensity it is possible that the validity of the assessment of exercise capacity has been diminished by asking participant to run at 65% rather than at 70% of $\dot{V}O_2$ max.

Although the rates of fat and CHO oxidation during 90 minutes of running were very similar in all three trials, the rate of fat oxidation at the point of exhaustion in the HC-LGI trial was lower, and the rate of CHO oxidation was higher, than in the Control trial. This coincided with a tendency for plasma glucose at the point of exhaustion to be higher in the HC-LGI trial than in the Control trial. This implies that high CHO diets based on LGI foods may be better at delaying the point at which availability of CHO stores is limited during consecutive bouts of exercise, and at improving exercise capacity or performance, than high CHO diets based on HGI foods. Indeed, Chen et al. (2008) found that 3 days of a high CHO diet with low but not high GI foods reduced the time taken to complete a 10-km run performed after 1 hour of steady state running. It should be noted, however, that Chen et al. (2008) observed significantly higher plasma glucose concentrations during the final stages of exercise in both high-CHO trials than in their low-CHO trial.

It is widely accepted that substrate selection during exercise is dictated not only by the pre-exercise muscle glycogen concentration (Harvey et al., 2007) but also by the availability of IMTG (van Loon et al., 2003). Thus, the fact that we found no difference in rate of fat and CHO oxidation between HC-HGI and HC-LGI trials does not necessarily imply that the expected increase in muscle glycogen (Arkinstall

et al., 2004; Burke & Hawley, 2006; Tarnopolsky et al., 2001; Wojtaszewski et al., 2003) and reduction in IMTG (Coyle et al., 2001) were not influenced by the GI of the two diets. Indeed, it has been reported that after glycogen-depleting exercise, a high CHO diet with HGI consumed over a recovery period of 24 hours resulted in greater muscle glycogen resynthesis than a LGI diet (Burke et al., 1993), and that during 3 hours of the postprandial period muscle glycogen concentration increased by 15% after a HGI meal but remained unchanged after a LGI meal (Wee et al., 2005). In addition, there is some evidence to suggest that consumption of a high CHO diet with LGI may lead to less accumulation (Kiens & Richter, 1996; Trenell et al., 2008) and reduced utilisation (Stevenson et al., 2009; Trenell et al., 2008) of IMTG during subsequent exercise compared to a HGI diet. Regardless of the above evidence, the role of the GI of high CHO diets consumed for several days in modifying the content of intramuscular energy substrates remains unclear and requires further investigation.

Due to the concern that foods with contrasting GI do not always induce a proportionally comparable difference in plasma glucose (Galgani et al., 2006), we evaluated retrospectively whether diets used in the present study could be expected to modify glycaemia differently in the pilot study (Chapter 3). Using a crossover design, the plasma glucose response (HemoCue AB, Angelholm, Sweden) to high CHO diets with a low and high GI was measured in 8 physically active healthy participants. We found that the time-averaged incremental area under the glucose versus time curve for glucose responses was significantly higher ($P<0.05$) in the HC-HGI meals (3.29 ± 0.38 mmol/L) than the HC-LGI meals (1.50 ± 0.42 mmol/L).

Thus, the diets prescribed in the present study may be expected to result in a different metabolic response.

We conclude that the extent by which high CHO diets consumed for the duration of 5 days reduces rate of fat and increases rate of CHO oxidation during subsequent running exercise in the fasted state is not influenced by GI and that GI of high CHO diets commonly consumed during days leading up to an athletic endurance event has no impact on exercise capacity.

Chapter 5 The effect of glycaemic index of high carbohydrate diets consumed for five days on exercise energy metabolism and running capacity in women.

5.1 Introduction

High dietary CHO intake for several days before competition is known to increase muscle glycogen stores, with subsequent ergogenic performance benefits often seen in events longer than 90 min in duration (Sedlock, 2008). It is known that optimizing glycogen content occurs at the cost of diminished availability and oxidation of fat (Brewer et al., 1988; Coyle et al., 2001). This compromised fat oxidation, in addition to enhanced pre-exercise muscle glycogen availability, could be expected to enhance the rates of glycogenolysis and glycogen depletion (Arkinstall et al., 2004; Hargreaves et al., 1995; Wojtaszewski et al., 2003), and thus reduce the benefits of high CHO diets. Thus, in the search for strategies to improve athletic performance, research on nutritional procedures which theoretically may protect from the reduction in fat oxidation and attenuate the rate of muscle glycogen depletion is required.

Studies in which 1-day CHO loading protocols were used suggest that in relation to the attenuation of the decrease in fat oxidation, GI may be an important consideration. Indeed, in men, LGI diet consumed during 24 hour recovery between bouts of prolonged strenuous exercise resulted in greater availability of plasma NEFA (Stevenson et al., 2005b, 2009; Trenell et al., 2008) and a higher rate of total fat oxidation during subsequent exercise conducted in the fasted state (Stevenson et

al., 2005b). However, recent study conducted on men investigating the impact of 3-day high CHO diets with HGI and LGI on energy metabolism during run after consumption of test breakfast (Chen et al., 2008) and our study on men investigating impact of 5-day on high CHO diets with HGI and LGI during run in the fasted state (Chapter 4) found that during running, the significant reduction in fat oxidation induced by high CHO consumption was independent of the GI of the high CHO. We found that the plasma concentrations of NEFA during the 90 minutes run after the 5-day high CHO diets and their habitual diet were similar while the concentration of glycerol in both high CHO trials were significantly lower than in the Control trial. This suggests that in men high CHO diets consumed for few days reduce the availability and utilisation of IMTG rather than the availability and oxidation of NEFA.

Women oxidise more lipid and less CHO and protein than men during endurance exercise (Tarnopolsky et al., 2008). This increase in fat oxidation is associated with higher IMTG content (Devries et al., 2007; Hoeg et al., 2009; Roepstorff et al. 2006; Tarnopolsky et al., 2007) and use (Roepstroff et al., 2006; Steffensen et al., 2002) as well as greater adipocyte lipolysis (Tarnopolsky et al., 2008). This may contribute to the phenomenon of high fat oxidation which occurs regardless of high CHO intake. For example, in moderately trained women, after 4 days of a high CHO diet (68% of energy), fat still contributed 60% of EE in the last 60 min of 2 hours exercise at 67% $\dot{V}O_2$ max in a fasted state (Wallis et al., 2006). To the best of our knowledge there are no studies investigating how the GI of high CHO diets consumed for several days impacts on energy substrate oxidation in moderately trained women.

The aim of this study therefore was to investigate the impact of the GI of high CHO diets over 5 days on energy substrate utilisation during running in the fasted state and to determine whether the GI of high CHO diets has an impact on exercise capacity in physically active women.

5.2 Participants and Methods

5.2.1 Participants

Nine physically active women participated in this study. Participant characteristics are shown in Table 5.1. Inclusion and exclusion criteria, recruitment and ethical approval process are described in detail in section 2.1 of General Methods. All participants had been involved in regular endurance training for at least 4 years and some had been involved in endurance type events and competitive sports. They were eumenorrheic and completed all three trials in the same phase of their menstrual cycle. This study was conducted with the approval of University of Glasgow's Medical Faculty Ethics Committee and participants provided written consent.

5.2.2 Study design

Each participant, in randomised counterbalanced order, performed three treadmill runs to exhaustion at 65% $\dot{V}O_2$ max: one after following their habitual diet (Control trial), another after 5 days on a HC-HGI diet and a third after 5 days on a HC-LGI diet. The exercise trials were separated by a washout period of at least 11 days. During 5 days leading up to the first treadmill run, participants recorded all planned and structured exercise conducted and were asked to replicate this prior the second and third runs. For two days prior to the running trials, the participants were asked to

limit themselves to activities of daily living and slow walking or cycling for personal transport over short distances and were asked not to consume alcohol. The participants recorded all fluid they consumed during 24 hours leading up to the first running trial and were asked to replicate this before the second and third runs. To ensure euhydration, the participants were instructed to consume about a litre of water the night before and ~500 ml in the morning before each running trial. All running trials were performed under similar experimental and environmental conditions. Two weeks before the main running trial, the participants undertook a familiarisation run to ensure that their predicted running speed corresponded to 65% $\dot{V}O_2$ max and to allow the participants to become familiar with the experiment protocol.

5.2.3 Preliminary exercise test

Before the familiarization run and treadmill runs to exhaustion, two preliminary exercise tests were conducted to find the speed that elicited 65% $\dot{V}O_2$ max for each participant. In the first, the steady-state relationship between submaximal $\dot{V}O_2$ and treadmill speed was established (see General Methods, section 2.4.1). In the second, $\dot{V}O_2$ max was determined during uphill running at constant speed which ranged from 7.5 to 10 km/h as described in section 2.4.2 of General Methods.

5.2.4 Main exercise trials

The participants arrived at the laboratory after a 12-hour overnight fast, at approximately 8:30 a.m. They were weighed and a cannula (Venflon 18G, Becton Dickinson Ltd, Helsingborg, Sweden) was inserted in an antecubital vein.

After a brief warm up consisting of 5 minutes continuous running at 60% $\dot{V}O_2$ max followed by 5 minutes of stretching, the participants ran to exhaustion on the treadmill at a speed equivalent to 65% of $\dot{V}O_2$ max. During the last minute of every 15 minute stage throughout the run, expired air samples were collected using the Douglas bag technique, heart rate was monitored using short-range telemetry (Polar S610i, Polar Electro, Finland) and RPE were recorded using the Borg scale and a blood sample taken with an indwelling cannula flushed with saline solution (0.9% w/v Sodium Chloride Intravenous Infusion BP, B.Braun Melsungen AG, Melsungen Germany) to keep it patent throughout the experiment. During trials, participants were allowed to drink water and cooled by an electric fan. Strong verbal encouragement was also given to the participants throughout the run and especially when near to exhaustion. Exhaustion was defined as the point at which the participants were no longer able to maintain the prescribed running speed. When a participant signalled that they could only manage a further 2 minutes, expired air was immediately collected, heart rate was recorded and a blood sample was taken.

5.2.5 Development of high CHO diets with high and low glycaemic index

The energy intake of the prescribed diets was based on the participants' habitual energy requirements (see General Methods, section 2.9). The proportions of energy from CHO, fat and protein (70%, 15% and 15% respectively) were similar in all prescribed diets. The example of prescribed HC-HGI and HC-LGI meals are presented in Table 4.2 in Chapter 4. The development of the experimental diets is described in detail in section 2.11, General Methods.

5.2.6 Dietary analysis, calculation of dietary glycaemic index and glycaemic load

Dietary intake of energy and macronutrients from participants' habitual dietary records (Control trial) as well as from the consumed experimental diets were calculated using a Diet 5^{TM} programme. Dietary GI and GL, which quantifies the overall glycaemic effect of a portion of food (Wolever & Jenkins, 1986; Salmeron et al., 1997) were also estimated from participants' habitual diet records and from consumed experimental diets, as described in section 2.11, General Methods.

5.2.7 Measurement of substrate oxidation and energy expenditure

Expired air was collected into a Douglas bag over 60 seconds and immediately analysed for O_2 and CO_2 concentrations (Servomex 1440, Crowborough, UK). The expired gas volume was determined using a dry gas meter (Harvard, Kent, UK), and $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated using the Haldane transformation (see General Methods, section 2.7). EE and substrate oxidation during running were estimated from $\dot{V}O_2$ and $\dot{V}CO_2$ using indirect calorimetry, neglecting protein oxidation (Ferrannini, 1988), as described in section 2.8.2, General Methods.

5.2.8 Anthropometry and body composition

Measurements of body mass and body fat were taken using bioelectrical impedance scales (TBF-300, TANITA, Cranlea, UK) and height was determined as described in section 2.2 of General Methods.

5.2.9 Plasma preparation and analysis

Blood samples were collected into a pre-cooled 7.5 ml EDTA Vacutainer^{TM} tube (BD Vacutainer Systems, Plymouth, UK) and centrifuged at centrifugal force of 1509 x g for 15 minutes at 4^{o}C. After centrifugation, aliquots of plasma were transferred using a disposable plastic Pasteur pipette into labelled 1.5-ml Eppendorf tubes (Eppendorf AG, Hamburg, Germany). The aliquoted plasma was then stored at -80^{o}C for later analysis of insulin (see section 2.13.1, General Methods), glucose, NEFA and glycerol (see section 2.13.2, General Methods).

5.2.10 Power calculation

The *post hoc* power calculation was carried out using Minitab statistical software (version 15 for Windows). Using a SD for difference in fat oxidation of 0.07 and SD for difference in CHO oxidation of 0.20, 9 participants would be sufficient to detect difference of 0.08 g/min in case of fat oxidation and 0.23 g/min in case of CHO oxidation with a power of 85%.

5.2.11 Statistical analysis

Results are presented as means ± SEM unless otherwise stated. Data were tested for normality using the Anderson-Darling test before statistical analysis. Reponses during the exercise period were compared by two-factor (trial x time) ANOVA, with Tukey *post hoc* test being used to locate the differences. Differences between energy and nutrient intake and in the parameters at the point of exhaustion were compared by one-factor (trial) ANOVA. Statistical significance was set at $P<0.05$. Data were analysed using the Statistica software program (Statistica for Windows, version 6).

Table 5.1 Participant characteristics. Values are mean ± standard deviation (SD), *n*=9

Characteristics	Women
Age (years)	25 ± 4
Weight (kg)	54 ± 4
Height (cm)	165 ± 5
BMI (kg/m^2)	20 ± 1
Fat (%)	17 ± 3
Fat free mass (kg)	44 ± 3
Maximum oxygen consumption (ml/kg/min)	53 ± 5

5.3 Results

5.3.1 Dietary intake

Daily dietary intakes for the 5 days preceding the run to exhaustion are presented in Table 5.2. Both the HC-HGI and HC-LGI diets were isocaloric with the habitual diet and provided energy which was very similar to the predicted energy requirements (9.32 ± 1.14 MJ). Daily intake of available CHO and the percentage of energy provided by the CHO were significantly higher (P<0.001) in the two experimental trials than the Control trial. There were no significant differences in available CHO, NMES, starch and fibre intakes and the percentage of energy provided by CHO between the HC-HGI and HCLGI diets. The GI and GL of the HC-HGI diet were significantly higher (P<0.001) than in the Control trial. The GI of the HC-LGI diet was significantly lower (P<0.001), and the GL of the HC-LGI was significantly higher than in the Control. The GI and GL of the HC-HGI diet were significantly higher (P<0.001) than those of HC-LGI diet.

Body weight (Control trial, 53.5 kg ± 4.2; HC-HGI trial, 53.8 kg ± 4.0; HC-LGI trial, 53.7 kg ± 4.7) and body fat percentage (Control trial, 17.2% ± 4.2; HC-HGI trial, 16.9% ± 3.4; HC-LGI trial, 17.1% ± 4.3) did not differ between trials. Values are means ± SD, n=9.

5.3.2 Plasma glucose, insulin, non-esterified fatty acids and glycerol

Concentrations of plasma glucose, insulin, NEFA and glycerol measured during 90 minutes of run (Figure 5.1 a - d) were not significantly different between the HC-HGI trial and HC-LGI trial. During 90 minutes of running, plasma glycerol

concentration in the two experimental trials was significantly lower (Figure 5.1c; $P<0.05$) than in the Control trial. Plasma glucose, insulin and NEFA concentrations during 90 minutes run (Figure 5.1 a, b and d respectively) and at exhaustion were not significantly different between trials (Table 5.3). At exhaustion glycerol tended to be lower ($P= 0.06$) in the HC-LGI trial in comparison to Control trial

5.3.3 Fat and CHO oxidation

The rates of fat and CHO oxidation during 90 minutes of running (Figure 5.2 a & b) and at the point of exhaustion (Table 5.3) were not significantly different between HC-HGI and HC-LGI trials. During 90 minutes of running, the rate of fat oxidation was significantly lower (Figure 5.2 a; $P<0.05$) and the rate of CHO oxidation was significantly higher (Figure 5.2 b; $P<0.05$) in the HC-LGI compared with the Control trial. At the point of exhaustion, there was no difference in the rate of fat oxidation and CHO oxidation between the two experimental trials and there were no differences in substrate oxidation between both high CHO trials and the Control trial. The proportion of energy from fat and CHO is presented in Figure 5.3.

5.3.4 Heart rate, ratings of perceived exertion and oxygen consumption

There were no differences in HR, RPE or $\dot{V}O_2$ during 90 minutes of running and at the point of exhaustion (Table 5.4) between all the trials.

Table 5.2 Daily macronutrient and energy intakes, percentage (%) of energy from macronutrients, glycaemic index (GI) and glycaemic load (GL) of habitual (Control), high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) diets consumed over 5days before run to exhaustion at 65% $\dot{V}O_2$ max. Values are means ± SEM, *n*=9.

	Control	HC-HGI	HC-LGI
Nutrient Intake			
Available CHO (g)	263 ± 26	400 ± 29[a]	393 ± 29[a]
Sugar (g)	98 ± 14	67 ± 8	65 ± 13
NMES (g)	48 ± 6	120 ± 22[a]	128 ± 14[a]
Starch (g)	100 ± 13	208 ± 17[a]	184 ± 18[a]
Fibre (g)	27 ± 2	32 ± 2	32 ± 2
Fat (g)	120 ± 20	93 ± 12	112 ± 18
Protein (g)	112 ± 18	78 ± 7	83 ± 4
CHO (g/kgBM/day)	4.8 ± 0.9	7.9 ± 1.4[a]	7.7 ± 1.3[a]
CHO (g/FFM/day)	5.7 ± 1.0	9.6 ± 1.9[a]	9.4 ± 2.0[a]
Energy Intake (MJ)	8.0 ± 0.5	9.3 ± 0.4	9.0 ± 0.3
Energy from CHO (%)	53 ± 3	69 ± 1[a]	69 ± 1[a]
Energy from Fat (%)	28 ± 3	15 ± 1[a]	15 ± 1[a]
Energy from Protein (%)	18 ± 2	15 ± 1[a]	15 ± 0[a]
GI	47 ± 1	69 ± 2[a]	39 ± 0[a b]
GL	116 ± 12	278 ± 26[a]	152 ± 14[a b]

NMES – Non-milk extrinsic sugars. [a] HC-HGI and HC-LGI trials significantly different ($P< 0.001$) from Control trial, [b] HC-LGI trial significantly different ($P< 0.001$) from HC-HGI trial.

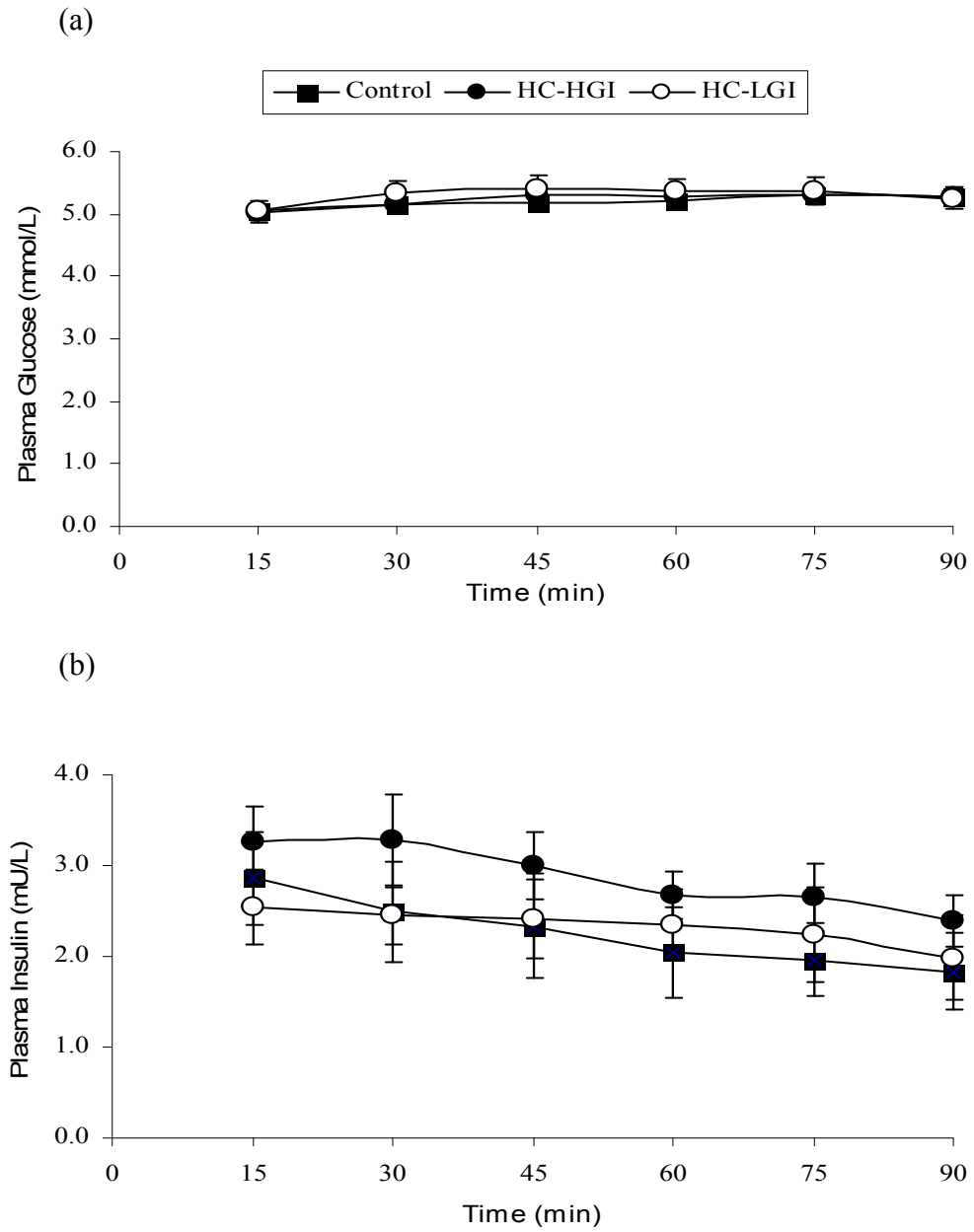


Figure 5.1 Plasma concentrations of (a) glucose (mmol/L) and (b) insulin (mU/L) during 90 min of running at 65% $\dot{V}O_2$ max in the Control trial (■), high carbohydrate-high glycaemic index (HC-HGI) trial (●) and high carbohydrate-low glycaemic index (HC-LGI) (○) trial. Values are mean ± SEM, *n*=9

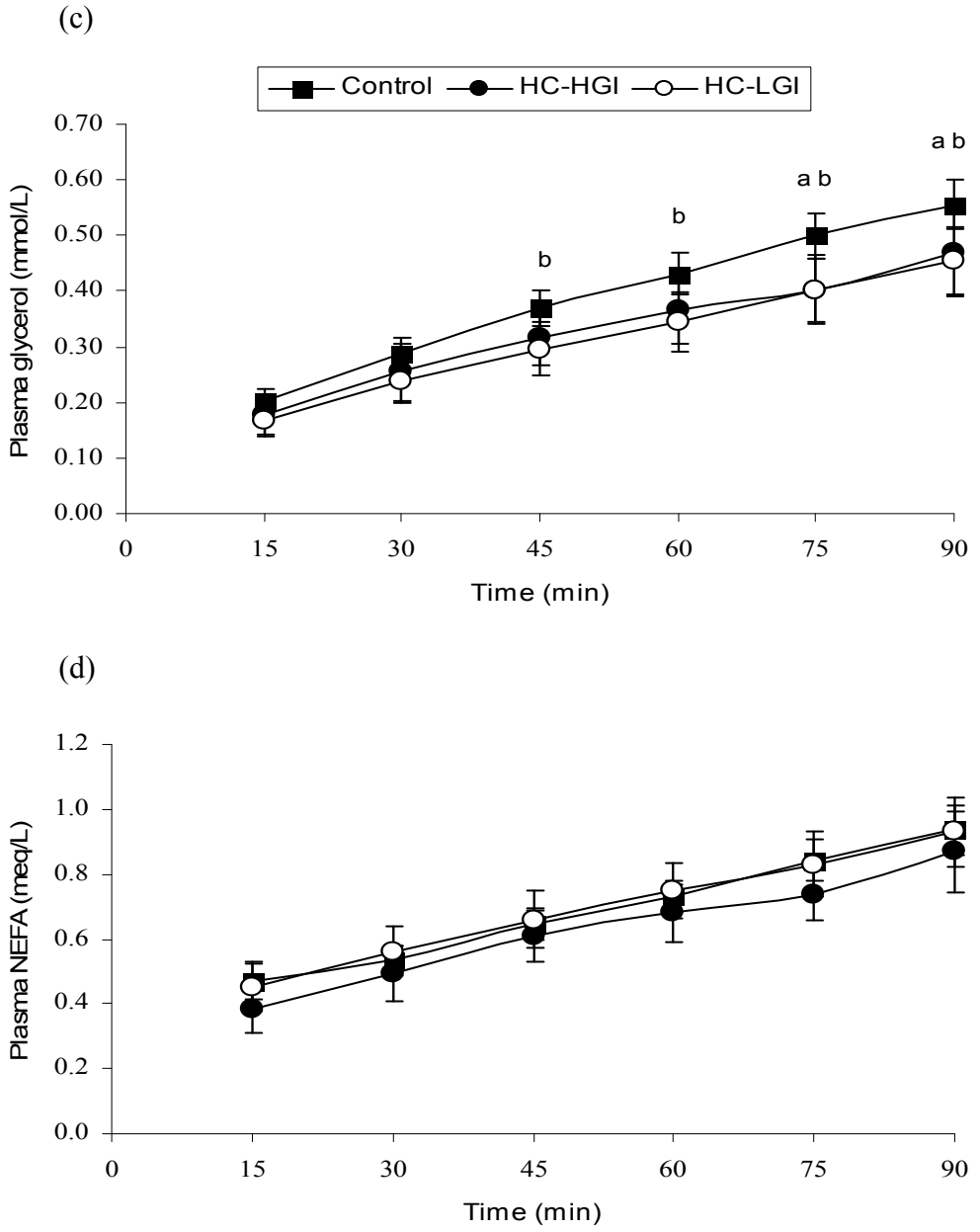


Figure 5.1 (continue) Plasma concentrations of (c) glycerol (mmol/L) and (d) and non-esterified fatty acids (meq/L) during 90 min of running at 65% $\dot{V}O_2$ max in the Control trial (■), high carbohydrate-high glycaemic index (HC-HGI) trial (●) and high carbohydrate-low glycaemic index (HC-LGI) trial (○). Values are mean ± SEM, n=9. [a] Control trial significantly different ($p < 0.05$) from HC-HGI trial, [b] Control trial significantly different ($P < 0.001$) from HC-LGI trial.

Table 5.3 Metabolic responses, rate of fat and CHO oxidation, oxygen consumption ($\dot{V}O_2$), rate of perceived exertion (RPE) and heart rate (HR) at the point of exhaustion in the Control, high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) trials. Values are mean ± SEM, *n*=9.

	Control	HC-HGI	HC-LGI
Glucose (mmol/L)	4.97 ± 0.17	4.97 ± 0.24	4.95 ± 0.23
Insulin (mU/L)	1.76 ± 0.56	1.98 ± 0.23	1.79 ± 0.19
NEFA (meq/L)	1.51 ± 0.17	1.36 ± 0.17	1.38 ± 0.15
Glycerol (mmol/L)	0.75 ± 0.06	0.65 ± 0.08	0.58 ± 0.15
Rate of fat oxidation (g/min)	0.57 ± 0.05	0.49 ± 0.06	0.51 ± 0.04
Rate of CHO oxidation (g/min)	1.00 ± 0.11	1.09 ± 0.15	1.04 ± 0.11
Oxygen consumption ($\dot{V}O_2$, ml/kg/min)	35.7 ± 1.1	33.9 ± 0.9	33.7 ± 1.2
Rate of perceived exertion (RPE)	17 ± 1	17 ± 1	17 ± 1
Heart rate (beats/min)	167 ± 4	163 ± 4	162 ± 5

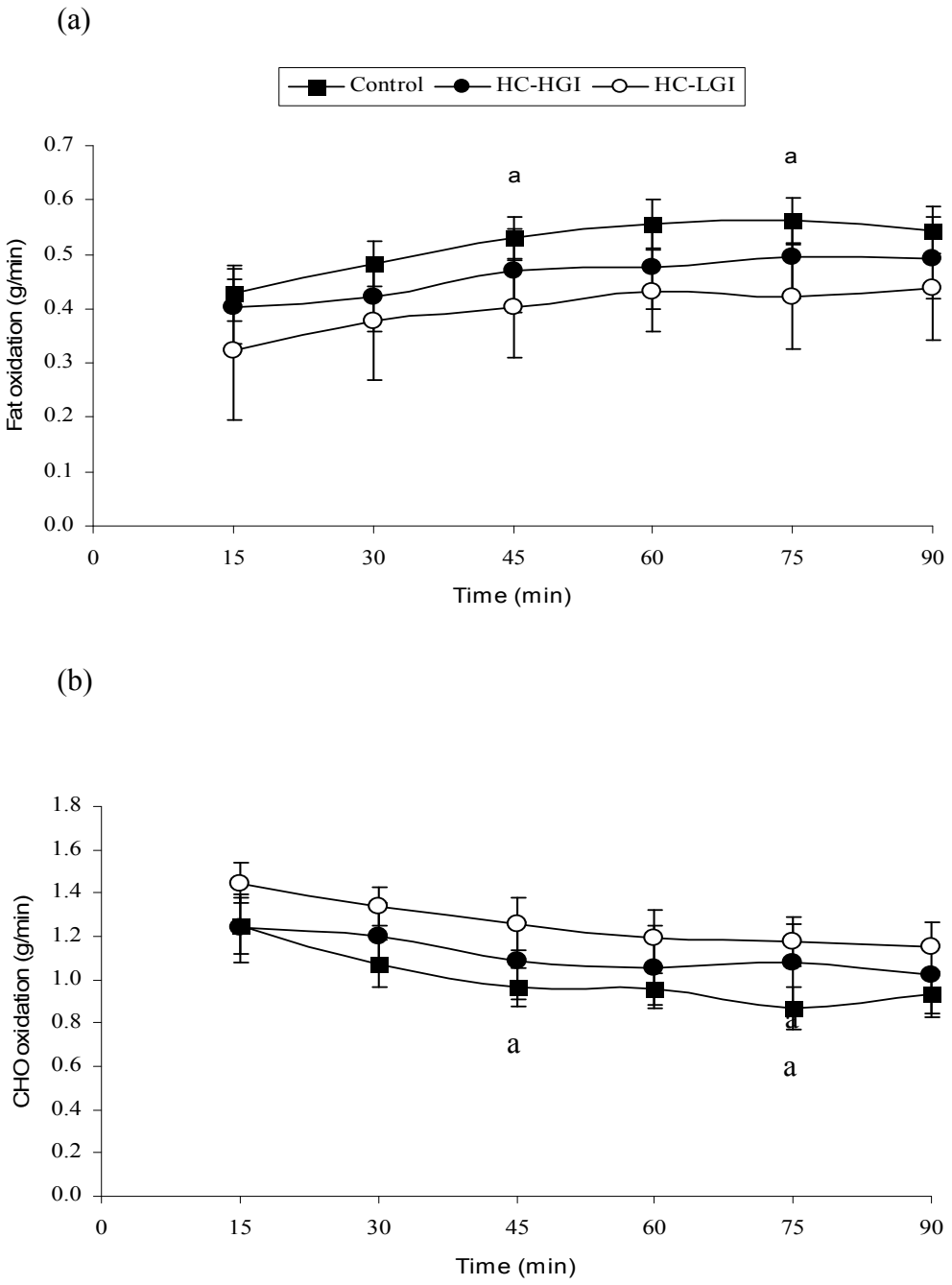


Figure 5.2 Rates of (a) fat oxidation and (b) carbohydrate oxidation (g/min) throughout the run to exhaustion at 65% $\dot{V}O_2$ max in the Control trial (■), high carbohydrate-high glycaemic index (HC-HGI) trial (●) and high carbohydrate-low glycaemic index (HC-LGI) trial (○). Values are mean ± SEM, n=9. [a] Control trial significantly different (P<0.05) from HC-LGI trial.

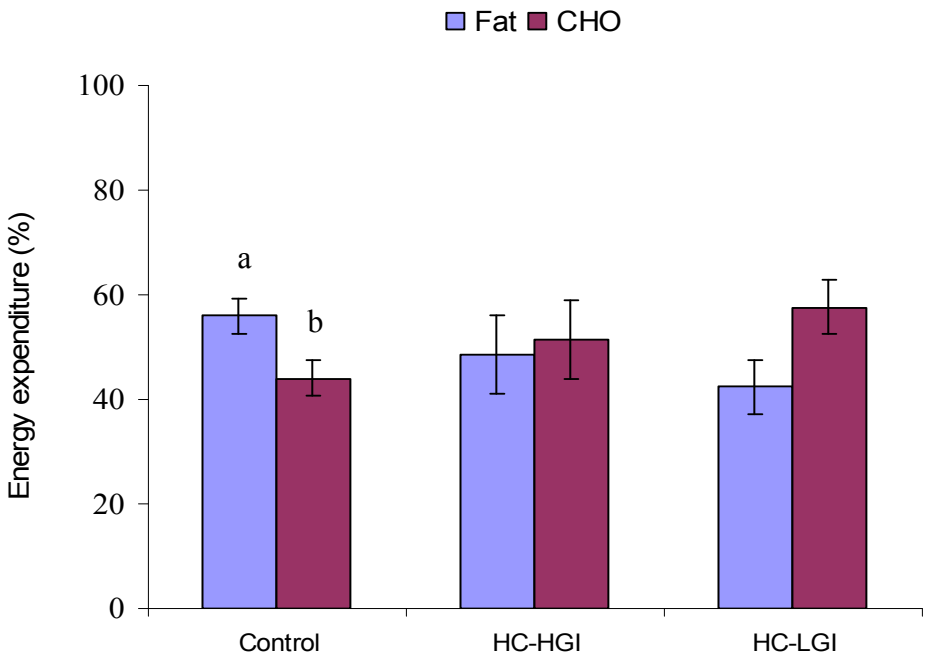


Figure 5.3 Percentage (%) of energy expenditure from fat and CHO during 90 min of running in the Control trial, high carbohydrate-high glycaemic index (HC-HGI) trial and high carbohydrate-low glycaemic index (HC-LGI) trial. Values are mean ± SEM, n=9. [a] Control trial significantly different (P<0.05) from fat oxidation in HC-LGI trial, [b] Control trial significantly different (P<0.05) from CHO oxidation in HC-LGI trial.

Table 5.4 Oxygen consumption ($\dot{V}O_2$, ml/kg/min), rate of perceived exertion (RPE) and heart rate (HR, beats/min) during running on the treadmill at 65% $\dot{V}O_2$ max to exhaustion (Ex) in the Control, high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) trials. Values are mean ± SEM, *n*=9.

	15 min	30 min	45 min	60 min	75 min	90 min
$\dot{V}O_2$						
Control	34.9 ± 1.0	34.0 ± 0.8	34.2 ± 1.0	34.9 ± 1.0	34.6 ± 0.9	34.7 ± 0.9
HC-HGI	33.8 ± 0.9	33.6 ± 0.9	33.5 ± 1.1	33.2 ± 0.8	34.0 ± 1.4	33.4 ± 1.2
HC-LGI	33.5 ± 1.2	33.1 ± 1.2	33.3 ± 1.1	33.0 ± 1.2	32.8 ± 1.1	33.5 ± 1.1
RPE						
Control	10 ± 1	11 ± 0	12 ± 0	12 ± 1	13 ± 1	14 ± 1
HC-HGI	10 ± 1	11 ± 1	10 ± 1	12 ± 0	13 ± 1	14 ± 1
HC-LGI	9 ± 1	11 ± 0	12 ± 0	13 ± 0	14 ± 0	14 ± 1
HR						
Control	158 ± 4	160 ± 4	159 ± 4	161 ± 4	163 ± 3	162 ± 4
HC-HGI	156 ± 4	157 ± 4	158 ± 4	159 ± 4	159 ± 4	159 ± 4
HC-LGI	154 ± 4	157 ± 5	159 ± 5	158 ± 5	159 ± 5	161 ± 5

5.3.5 Time to exhaustion and running distance

Time to exhaustion (Control trial, 149 min ± 30; HC-HGI trial, 147 min ± 21; HC-LGI trial, 142 min ± 34) and distance covered (Control trial, 23 km ± 4; HC-HGI trial, 23 km ± 4; HC-LGI trial, 22 km ± 6) were not significantly different between trials. Values are mean ± SD.

5.4 Discussion

The main finding of the present study is that the rate of fat oxidation during running in the fasted state in women was not influenced by the GI of high CHO diets consumed for 5 days. We also found that the GI of high CHO diets consumed for 5 days had no impact on running capacity.

The findings that in healthy women, GI of high CHO consumed for 5 days have no impact on the rate of fat and CHO oxidation during subsequent running exercise in the fasted state is in consistent with findings from the study with similar design conducted on men (Chapter 4) and the study investigating the influence of three days on high CHO with different GI on exercise energy substrate utilisation (Chen et al., 2008). The finding of the present study that there were no differences in fat and CHO oxidation between HC-HGI and HC-LGI trials cannot be related to the small number of study participants. Power calculations indicated that with nine participants at power of 0.85%, difference of 0.08 g/min in case of fat oxidation could be detected. In addition, the lack of a difference between the two high CHO trials cannot be explained by no difference in substrate utilisation during 5 days of the dietary interventions since our pilot study demonstrated that the prescribed high CHO diets based on foods with contrasting GI induced a proportionally comparable

difference in plasma glucose (Chapter 3). Thus, in both moderately trained men and women the consideration of the GI has no impact on exercise energy substrate utilisation when high CHO diets are consumed for several days before endurance exercise.

We found that compared to the habitual diet, the HC-LGI diet reduced while high HC-HGI diet had no impact on fat oxidation. In our previous study in men (Chapter 4), high CHO diets with both HGI and LGI consumed for 5 days significantly reduced the rate of fat oxidation and increased the rate of CHO oxidation during 90 minutes of running at 65% $\dot{V}O_2$ max. This difference between men and women may be explained by the higher reduced in the availability and thus oxidation of IMTG during exercise after HC-LGI trial in women. Indeed, content of IMTG, the main contributor of non-plasma derived fatty acids during exercise (Roepstorff et al., 2005; Tarnopolsky et al., 2007) can be influenced by the GI of the diet. For example, it has been shown that the consumption of a high CHO with LGI diet for 24 hr reduces IMTG content to a greater extent when compared to a high CHO HGI diet (Trenell et al., 2008). In addition, it has been reported that a moderate intake of CHO with LGI diet for 30 days (Kiens & Ritcher, 1986) results in lower IMTG accumulation compared a HGI diet. The responses of plasma glycerol concentration during 90 minutes of exercise in our study also support this. We found that although the glycerol concentration was lower in both high CHO trials, significant difference from the Control trial was found at more time points in the HC-LGI trial. More research is needed to confirm these findings before the advice on the GI of high CHO diets in relation to fat utilisation during exercise can be provided for women.

In our study, 56%, 49% and 42% of energy was obtained from fat in the Control, HC-HGI and HC-LGI trials during running at 65% $\dot{V}O_2$ max, respectively while in our previous study with the same design conducted in men, fat contributed 45%, 35% and 32% towards energy, respectively. The contribution of fat as energy during run in the Control, HC-HGI and HC-LGI trials between men and women was compared using unpaired t-test (SPSS, version 18.0 for Windows, SPSS, Chicago, Ill., USA) and results showed that energy from fat were significantly higher in women than in men in the Control (P=0.01), HC-HGI (P=0.05) and HC-LGI (P=0.04) trials. Thus, these two studies confirm women oxidise more fat during endurance type of exercise than men (Knechtle et al., 2004; Venables et al., 2005; Wallis et al., 2006). These sex differences can be explained by women having a higher IMTG content (Devries et al., 2007; Hoeg et al., 2009; Roepstorff et al., 2006; Tarnopolsky et al., 2007) and use (Roepstroff et al., 2006; Steffensen et al., 2002). This is related to the higher protein content of major enzymes involved in long and medium chain fatty acid oxidation found in women than in men (Maher et al., 2010). In addition, enhanced oxidation of fat during exercise in women may reflect a reduced rate of muscle glycogen (Tarnopolsky & Ruby, 2001), and plasma glucose utilisation (Devries et al., 2006; Horton et al., 2006; Tarnopolsky et al., 2007).

Despite of the different rates of fat and CHO oxidation between the HC-LGI trial and the Control trial, and plasma glycerol concentration between both high CHO trials and the Control trial during 90 min of running, there was no difference between all measured variables at the point of exhaustion. We also found that the GI of high CHO diets consumed for 5 days had no impact on exercise capacity measured as time to exhaustion. This is in line with our previous study in men (Chapter 4).

In the present study in women, 5 days high CHO diets did not affect running capacity which is contrary to the promotion of muscle glycogen storage by increased dietary CHO which should prolong time to exhaustion in trials lasting over 90 minutes (Sedlock, 2008). As reported previously (James et al., 2001; Sedlock, 2008, Tarnopolsky et al., 2001, Walker et al., 2000), women have the capacity to accumulate supranormal muscle glycogen levels in response to high CHO intake. Although muscle glycogen concentration was not measured in this study, it should be expected that sufficient CHO was consumed by our participants to promote increased muscle glycogen. It is known that women who attempt to CHO load should be particularly attentive to both total energy intake and relative CHO intake (> 8 g/kg BW/day) (James et al., 2001; Sedlock, 2008; Walker et al., 2000). In our study, participants consumed CHO 7.9 and 7.7 g/kg BW/day in HC-HGI and HC-LGI diet, respectively, only slightly below the amount required to optimally increase muscle glycogen stores.

This study was aimed to conduct all trials on the same menstrual cycle phase. However, it was not always achieved and this is unlikely to compromise the quality of our data. The different phases of the menstrual cycle seem to have no influence on whole body substrate oxidation in moderately active women, either at rest or during 90 minutes of moderate-intensity exercise (Horton et al., 2006). On the other hand, other studies found that plasma glucose kinetics and CHO oxidation during moderate-intensity exercise are lower during the luteal compared with the follicular of menstrual phase (Zderic et al., 2001), and that endurance performance may only be improved in the mid-luteal phase compared with the early follicular phase (Oosthuyse & Bosch, 2010). James et al. (2001) showed that three days of a CHO

loading regimen resulted in a substantial increase in glycogen levels in the vastus lateralis muscle, irrespective of menstrual cycle phase. However, Oosthuyse & Bosch (2010) reported that following a CHO loading diet will super-compensate muscle glycogen stores in the early follicular phase to values attained in the luteal phase.

In conclusion, in women rate of fat oxidation during running in the fasted state running capacity is not influenced by the GI of high CHO diets consumed for 5 days. We also found that in women, the rate of fat oxidation during running in the fasted state is reduced only after consumption of high CHO with LGI diet.

Chapter 6 Effect of high carbohydrate diets with high and low glycaemic index consumed for five days on insulin sensitivity and fasting plasma lipids in healthy physically active individuals.

6.1 Introduction

The abnormalities of the circulating lipids and lipoprotein concentration and composition are one of the major causes of the greater risk of CHD (Austin et al., 1988, 1990). When the circulating lipids and lipoproteins are clinically altered, this condition is called dyslipidemia**.** The atherogenic dyslipidemia refer to conditions of high levels of plasma TG, elevated LDL-cholesterol, preponderance of small dense LDL_3 particles and low levels of HDL-cholesterol (Austin et al., 1990). High CHO diets are recommended for lowering the risk of CHD because they decrease plasma LDL-cholesterol concentrations (Sacks and Katan, 2002). However, unfavourable effects of high CHO diets are an increase in plasma TG concentration and reduced concentration of HDL-cholesterol (Culling et al., 2009; Koutsari et al., 2000; Roberts et al., 2008). There was evidence to suggest that replacement of saturated fat with a higher CHO intake, particularly refined CHO, can exacerbate the atherogenic dyslipidemia associated not only with increased TG and reduced HDL-cholesterol but also increased in most atherogenic small LDL particles (Siri-Tarino et al., 2010). This unfavourable effect of high CHO intake on plasma lipids was also demonstrated in endurance athletes. In a study by Thompson et al. (1984), an increase in TG, TC and LDL-cholesterol and a decrease in HDL-cholesterol in distance runners consuming a diet providing high CHO (69% of energy) for 2 weeks compared to a high fat (50% of energy) diet was observed. Brown & Cox (1998) examined the

effects of a high CHO (HC: 69% of energy from CHO, 15% fat) versus a high fat (HF: 50% fat, 37% CHO) diet on plasma lipids and lipoproteins in 32 endurance trained cyclists over a 3-month period and found that from baseline to week 12, there was a significant increased in TC and TG in high CHO group. Therefore, it is likely that even in those who are involved in exercise, high CHO diets may induce detrimental impact on plasma lipid profiles.

Several studies reported that healthy sedentary individuals consuming LGI food was associated with improvement of CHD risk factors including plasma lipids and insulin sensitivity (Clapp & Beth, 2007; De Rougemont et al., 2007; Fajcsak et al., 2008; Jeppesen et al., 1997; Kiens & Ritcher, 1996). The differing responses of glucose, insulin and NEFA to CHO foods with HGI and LGI provides a mechanism by which high CHO diet with LGI consumed can be expected to prevent from CHD risk (Frayn, 2001; Petersen & Shulman, 2002). However, studies conducted in healthy individuals not necessarily support the notion that LGI diet is more beneficial in relation to plasma lipids and insulin sensitivity than HGI diet (Lau et al., 2005; Shikany et al., 2009; Sloth et al., 2004). Thus, findings on how GI of high CHO diets effects plasma lipids in healthy individuals require further confirmation. In addition, there are no studies which consider whether GI may prevent from detrimental changes in plasma lipids when high CHO diets are consumed for muscle glycogen accumulation in physically active individuals (Brown & Cox, 1998; Thompson et al., 1984).

There is evidence available from *in vitro* studies that even a short-term increase in glucose concentration leads to the increased expression of adhesion molecules such as ICAM-1 and VCAM-1 (Altannavch et al., 2004). The repeated increase in post-meal glucose responses after a high CHO with HGI diet may be expected to impact on the expression of ICAM-1 and VCAM-1 and thus the plasma concentration of these CAMs to a greater degree than the LGI diet. In addition, dyslipidemia and risk of CHD are known to be associated with increased expression of ICAM-1 and VCAM-1 (Abe et al., 1998; Hackman et al., 1996). Therefore, a LGI diet may be important for the prevention of atherosclerosis by means of modifying the expression of these adhesion molecules. So far, there are no studies which have tested this hypothesis.

The aim of this study was to investigate the impact of the consumption of high CHO diet with HGI and LGI for the duration of 5 days on insulin sensitivity and plasma concentration of fasting lipids and circulating biomarkers of endothelial activation such as VCAM-1 and ICAM-1 in moderately physically active healthy individuals.

6.2 Participants and Methods

6.2.1 Participants

Seventeen participants (10 men and 7 women) participated in this study. Some of these participants were the same participants as in Chapter 4 and Chapter 5. Inclusion and exclusion criteria, recruitment and ethical approval process are described in detail in section 2.1 of General Methods. This study was conducted with the approval of University of Glasgow's Medical Faculty Ethics Committee and all participants

gave their written informed consent. The participant characteristics are presented in Table 6.1.

6.2.2 Study design

Each participant's fasting blood and expired air samples were collected and body composition was measured on three occasions in randomised counterbalanced order: one after following their habitual diet (Control trial), another after 5 days on HC-HGI diet and a third after 5 days on a HC-LGI diet. The trials were separated by a washout period of a minimum 11 days. During 5 days leading up to the first trial, participants recorded all planned and structured exercise conducted and were asked to replicate this before their second and third trials. For 2 days before the trials, participants were asked to limit themselves to activities of daily living and slow walking or cycling for personal transport over short distances and were asked not to consume alcohol. Two weeks before the main trial, each participant's RMR was measured using Deltatrac (Datex Instrumentation Corporation, Helsinki, Finland) (see General Methods, section 2.3) and $\dot{V}O_2$ max was determined during uphill running on the treadmill at a constant speed which range from 8 to 11.5 km/h (see General Methods, section 2.2.2).

6.2.3 Main trials

The participants arrived at the laboratory after a 12 h overnight fast, at approximately 8.30 a.m. They were weighed and then rested in a seated position for 10 minutes before a baseline blood sample (7 ml collected into a EDTA tube) was drawn and expired air was collected using the Douglas bag technique (see General Methods, section 2.7).

6.2.4 Development of high carbohydrate diets with high and low glycaemic index

The energy intake of the prescribed diets was based on the participant's habitual energy requirements as described in detail in section 2.9 of General Methods. The proportions of energy from CHO, fat, and protein (approximately 70%, 15% and 15%, respectively) were similar in the prescribed HC-HGI and HC-LGI diets. The example of prescribed HC-HGI and HC-LGI meals are presented in Table 4.2 in Chapter 4. The development of the experimental diets is described in detail in section 2.10, General Methods.

6.2.5 Dietary analysis, calculation of dietary glycaemic index and glycaemic load

Dietary intake of energy and macronutrients from participant's habitual dietary records (Control trial) as well as from the consumed experimental diets were calculated using a Diet 5^{TM} programme. Dietary GI and GL were also estimated from participants' habitual diet records and from consumed experimental diets, as described in section 2.11, General Methods.

6.2.6 Measurement of substrate oxidation

Expired air was collected into a Douglas bag over 60 seconds and immediately analysed for O_2 and CO_2 concentrations (Servomex 1440, Crowborough, UK). The expired gas volume was determined using a dry gas meter (Harvard, Kent, UK) and $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated using the Haldane transformation (see General Methods, section 2.7). The rates of fasting fat and CHO oxidation (g/min) were

calculated using the stoichiometric equations by Frayn (1983) from the $\dot{V}O_2$ and $\dot{V}CO_2$ values (see General Methods, section 2.8.1).

6.2.7 Plasma preparation and analysis

Blood samples were collected into a pre-cooled 7.5 ml EDTA Vacutainer™ tube (BD Vacutainer Systems, Plymouth, UK) and centrifuged at centrifugal force of 1509 x g for 15 minutes at 4°C. After centrifugation, aliquots of plasma were transferred using a disposable plastic Pasteur pipette into labelled 1.5 ml Eppendorf tubes (Eppendorf AG, Hamburg, Germany). The aliquoted plasma was then stored at -80°C for later analysis of insulin (see section 2.13.1, General Methods), glucose, NEFA, TC, LDL-cholesterol, TG and HDL-cholesterol (see section 2.13.2, General Methods). $HOMA_{IR}$ was calculated as described in section 2.13.4 of General Methods.

6.2.8 Power calculation

The *post hoc* power calculation was carried out using Minitab statistical software (version 15 for Windows). Using a SD for difference in plasma TG concentration of 0.19 and SD for difference for HDL-cholesterol concentration of 0.09, 17 participants would be sufficient to detect difference of 0.15mmol/L in case of TG and 0.07mmol/L in case of HDL-cholesterol with a power of 85%.

6.2.9 Statistical analysis

Results are presented as means ± SEM unless otherwise stated. Data were tested for normality using the Anderson-Darling test before statistical analysis. Differences between energy and nutrient intake and those between metabolic profiles were

compared by one factor (trial) ANOVA. Statistical significance was set at $P < 0.05$. Data were analysed using the Statistica software programme (Statistica for Windows, version 6).

Table 6.1 Participant characteristics. Values are means ± standard deviation (SD), *n*=17

Characteristics	Men (n=10)	Women (n= 7)
Age (years)	24 ± 4	26 ± 5
Weight (kg)	73 ± 9	53 ± 6
Height (cm)	179 ± 7	165 ± 4
BMI (kg/m^2)	23 ± 2	20 ± 1
Fat (%)	10 ± 4	18 ± 4
Fat free mass (kg)	65 ± 7	45 ± 2
Maximum oxygen consumption (ml/kg/min)	61 ± 4	53 ± 2

6.3 Results

6.3.1 Dietary intake

Daily dietary intakes for 5 days preceding trials are presented in Table 6.2. Both the HC-HGI and HC-LGI diets were isocaloric with the habitual diet and provided energy which was very similar to the predicted energy requirements. Daily intake of available CHO and the percentage of energy provided by CHO was significantly higher (P<0.001) in the high CHO trials than the Control trial. There were no significant differences in available CHO, NMES, starch, fibre intakes and the percentage of energy provided by CHO between the HC-HGI and HC-LGI trials. The sugar content was not different between the two experimental diets and the Control trial. The GI and GL of the HC-HGI diet were significantly higher (P<0.001) than in the Control trial. The GI of the HC-LGI diet was significantly lower (P<0.001), and the GL of the HC-LGI was significantly higher than in the Control. The GI and GL of the HC-HGI diet were significantly higher (P<0.001) than those of HC-LGI diet.

6.3.2 Plasma total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride and non-esterified fatty acids

Fasting concentrations of plasma TC, HDL-cholesterol, LDL-cholesterol, TG and NEFA were not significantly different between the HC-HGI and HC-LGI trials (Figure 6.1 a - d). The concentration of plasma TG was significantly higher (Figure 6.1 c; P<0.05) and the concentration of plasma HDL-cholesterol was significantly lower (Figure 6.1d; P<0.05) in both high CHO trials than the Control trial.

Table 6.2 Daily macronutrient (g) and energy intake (MJ), percentage (%) of energy from macronutrients, glycaemic index (GI) and glycaemic load (GL) of habitual (Control), high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) diets. Values are means ± SEM, *n*=17.

	Control	HC-HGI	HC-LGI
Nutrient intake			
Available CHO (kg)	294 ± 24	478 ± 22[a]	475 ± 23[a]
Sugar (g)	94 ± 6	112 ± 15	128 ± 21
NMES (g)	69 ± 10	160 ± 14[a]	170 ± 11[a]
Starch (g)	151 ± 14	232 ± 10[a]	230 ± 12[a]
Fibre (g)	27 ± 2	35 ± 1	33 ± 2
Fat (g)	62 ± 5	43 ± 2[a]	46 ± 3[a]
Protein (g)	108 ± 6	93 ± 3	94 ± 4
CHO (g/kg BW/day)	4.6 ± 0.3	7.3 ± 0.3[a]	7.1 ± 0.3[a]
Energy intake (MJ)	9.6 ± 0.5	11.0 ± 0.5	11.0 ± 0.5
Energy from CHO (%)	53 ± 2	70 ± 1[a]	70 ± 1[a]
Energy from fat (%)	27 ± 1	15 ± 1[a]	15 ± 0[a]
Energy from protein (%)	18 ± 1	15 ± 0[a]	15 ± 0[a]
Glycaemic index	53 ± 2	70 ± 1[a]	39 ± 0[a b]
Glycaemic load	158 ± 12	388 ±17[a]	189 ± 10[a b]

Notes: CHO=carbohydrate, NMES=non-milk extrinsic sugars

[a] HC-HGI and HC-LGI trials significantly different ($p<0.05$) from Control trial, [b] HC-LGI trial significantly different ($p<0.05$) from HC-HGI trial

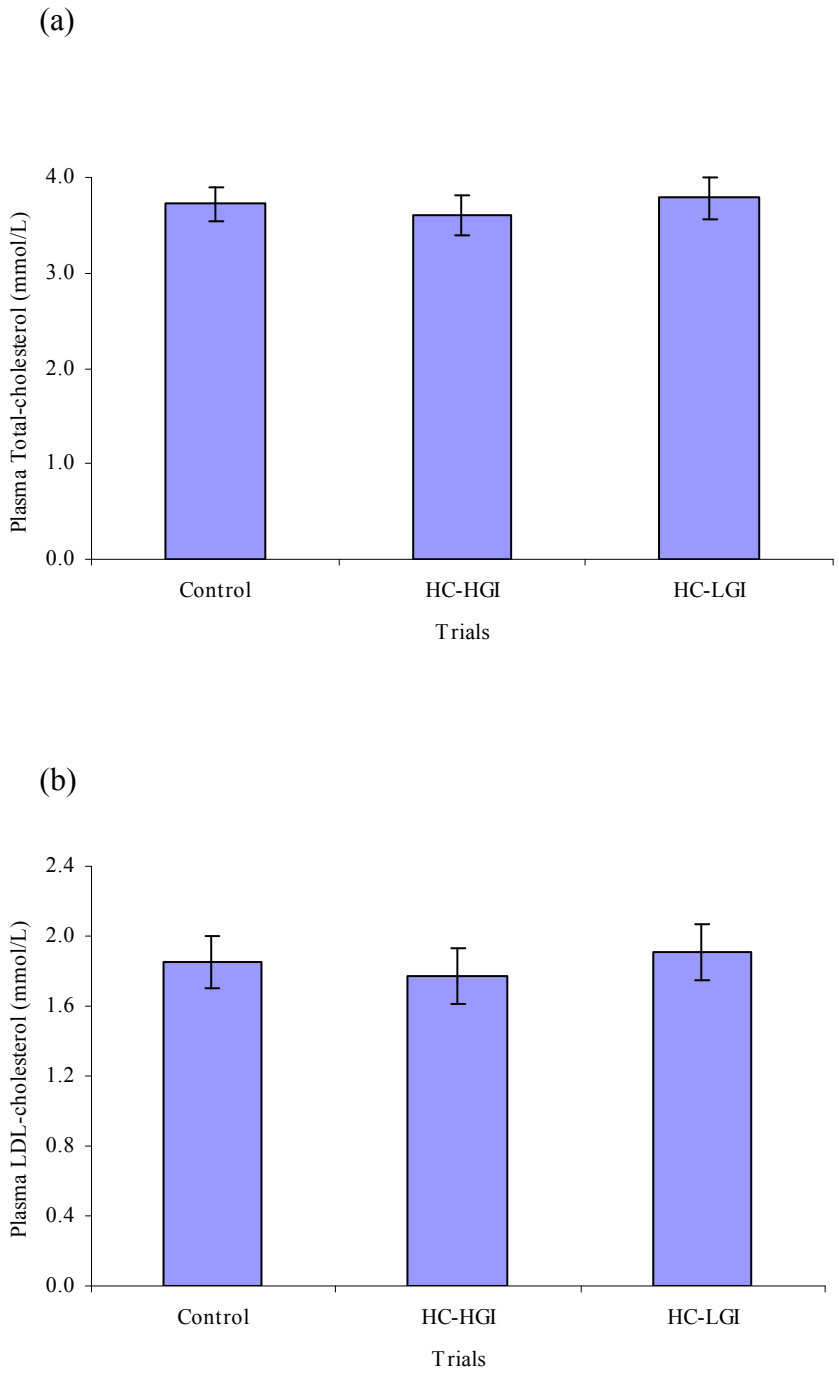


Figure 6.1 Fasting plasma concentrations of (a) total cholesterol (mmol/L) and (b) LDL-cholesterol (mmol/L) in habitual (Control), high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) trials. Values are means ± SEM, n=17.

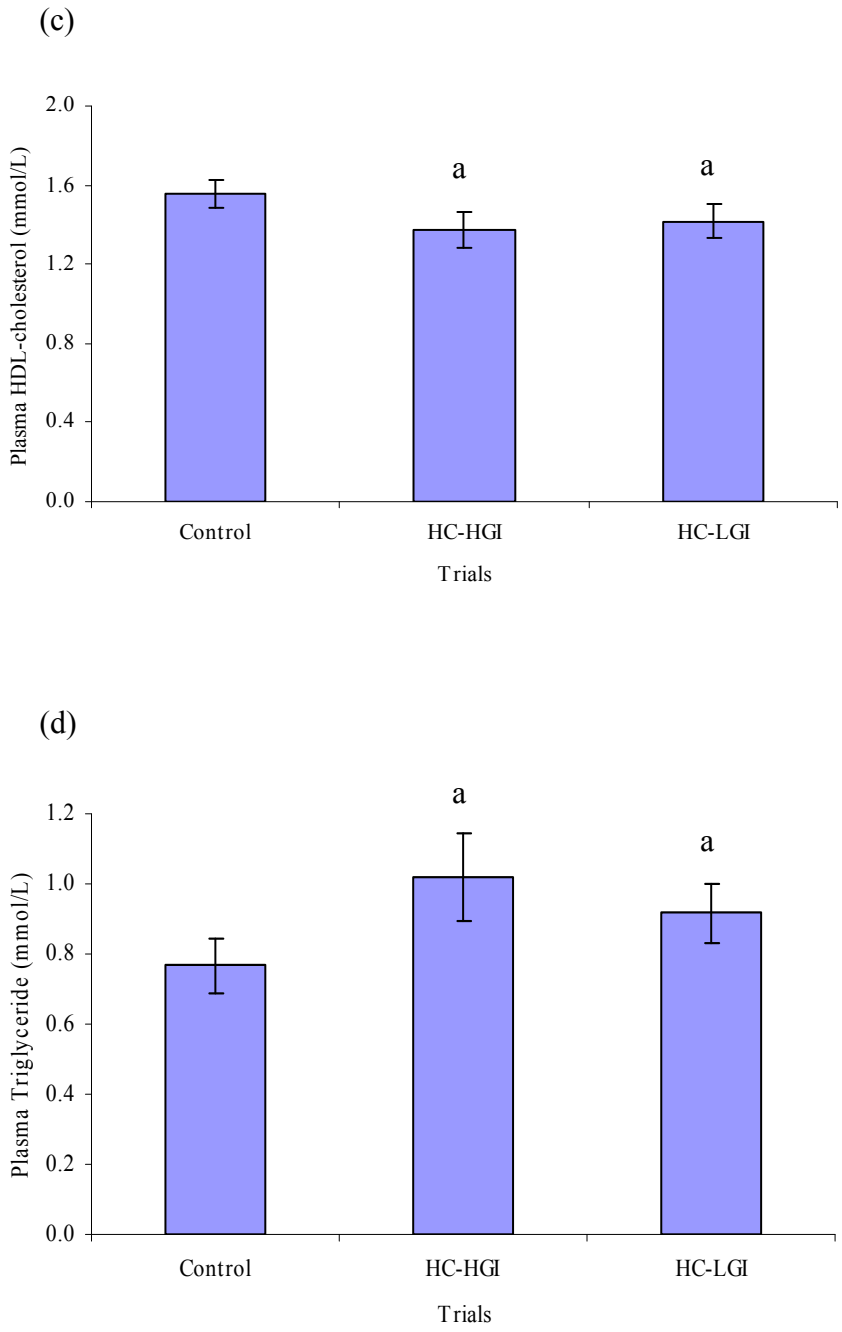


Figure 6.1 (continue) Fasting plasma concentrations of (c) HDL- cholesterol (mmol/L) and (d) triglyceride (mmol/L) in habitual (Control), high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) trials. Values are means ± SEM, n=17. [a] HC-HGI and HC-LGI trials significantly different (P< 0.05) from Control trial.

6.3.3 Plasma glucose, insulin and homeostasis model assessment for relative insulin resistance ($HOMA_{IR}$)

The concentration of fasting plasma glucose, insulin and $HOMA_{IR}$ (Table 6.3) were not significantly different between the HC-HGI and HC-LGI trials. In comparison to the Control trial, plasma concentration of insulin and glucose, and $HOMA_{IR}$ were not significantly different from both the high CHO trials.

6.3.4 Fat and CHO oxidation

The rates of fat and CHO oxidation were not significantly different between HC-HGI and HC-LGI trials (Table 6.3). Compared with the Control trial, the rate of fat oxidation and the rate of CHO oxidation were not different from the high CHO trials.

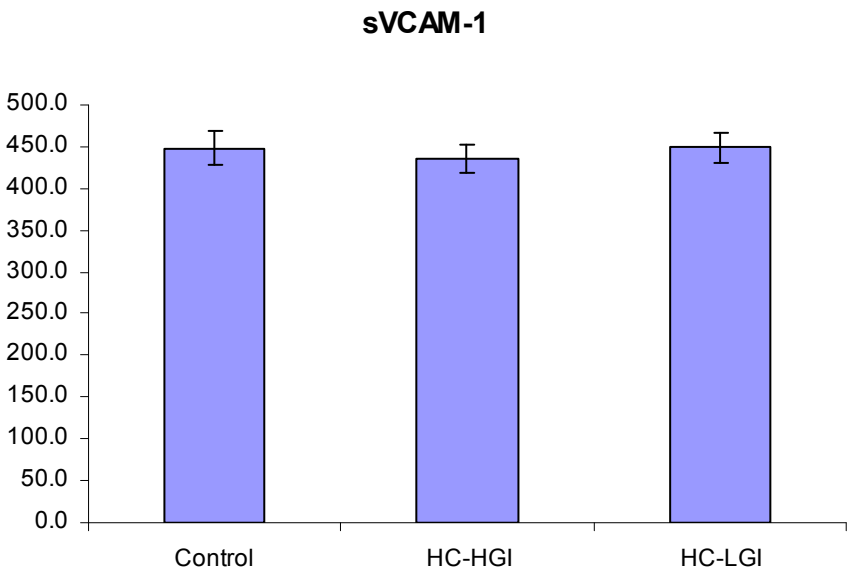
6.3.5 Biomarkers of endothelial activation

No significant differences were found between HC-HGI and HC-LGI trials for sVCAM-1 (Figure 6.2 a) and sICAM-1 (Figure 6.2 b). Values of soluble ICAM-1 and sVCAM-1 after the high CHO trials were not different from values in the Control trial.

Table 6.3 Fasting plasma concentration of non-esterified fatty acids (NEFA), glucose, insulin and $HOMA_{IR}$ in habitual (Control), high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) trials. Values are means ± SEM, n=17.

Parameters	Control	HC-HGI	HC-LGI
NEFA (meq/L)	0.43 ± 0.04	0.49 ± 0.07	0.47 ± 0.05
Glucose (mmol/L)	4.91 ± 0.07	4.78 ± 0.06	4.98 ± 0.09
Insulin (mU/L)	3.68 ± 0.27	3.53 ± 0.27	3.83 ± 0.36
$HOMA_{IR}$	0.80 ± 0.08	0.76 ± 0.06	0.85 ± 0.08
CHO oxidation (g/min)	0.18 ± 0.02	0.18 ± 0.02	0.19 ± 0.02
Fat oxidation (g/min)	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01

(a)



(b)

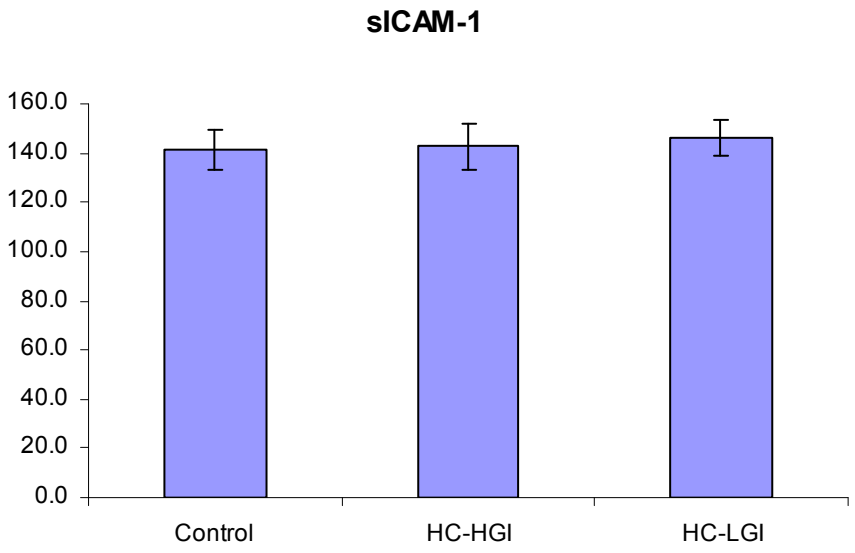


Figure 6.2 Fasting plasma concentrations of (a) sVCAM-1 (ng/ml) and (b) sICAM-1 (ng/ml) in habitual (Control), high carbohydrate-high glycaemic index (HC-HGI) and high carbohydrate-low glycaemic index (HC-LGI) trials. Values are means ± SEM, n=17.

6.4 Discussion

The main finding of the present study is that in moderately physically active individuals, the extent to which high CHO consumed for the duration of 5 days increases plasma concentration of TG and reduces concentration of HDL-cholesterol is not influenced by the GI of the diets. These findings imply that in healthy individuals when high CHO diets are consumed for short duration, consideration of GI is not important for the protection of detrimental changes in plasma lipids. We also found that the GI of high CHO diets consumed for 5 days has no impact on insulin sensitivity and the biomarkers of endothelial activation.

The HC-HGI and HC-LGI diets in this study were prescribed to have a significant difference in GI and GL and the compliance by the participants was well achieved. Available CHO content in both high CHO diets was significantly higher than in the Control trial. However, sugar content between the high CHO trials and Control trial were kept the same because when large amounts of sugars are included in the high CHO diets, alterations of plasma lipids may be observed, usually elevation of plasma TG and depression of HDL-cholesterol concentrations (Frayn & Klingman, 1995).

We found that compared to the habitual diet, the high CHO diets consumed for 5 days increased the concentration of plasma TG. This finding is in agreement with previous short term intervention studies in sedentary individuals (Culling et al., 2009; Koutsari et al., 2000; Roberts et al., 2008) and endurance athletes (Brown & Cox, 1998; Thompson et al., 1984). One of the mechanisms by which increased intake of CHO increased TG is stimulation of hepatic synthesis and secretion of VLDL-TG (Mittendorfer & Sidossis, 2001; Sidossis & Mittendorfer, 1999) and expansion of the

fasting TG pool (Jeppesen et al. 1997). At the same time, VLDL-TG clearance in humans in the fasted state is known to be reduced after high CHO diet (Parks et al., 1999). The high TG concentration enhances the opportunity for the reciprocal transfer of cholesterol and TG between HDL-cholesterol and TRL by the action of CETP (Davidson, 2010) which might explain the lower concentration of HDL-cholesterol observed in the both high CHO trials. In addition, lower fat intake is known to reduce the requirement for HDL-mediated cholesterol removal (Velez-Carrasco et al., 1999), which may have also contributed to the lower HDL-cholesterol concentration. It should be noted that both in the above studies (Brown & Cox, 1998; Culling et al., 2009; Koutsari et al., 2000; Roberts et al., 2008; Thompson et al., 1984) and in the present study, all diets consumed were isocaloric and that in the high CHO trials, CHO contributed approximately 70% of energy intake. Nevertheless, when high CHO diets are consumed *ad libitum* and for longer duration, the effect on plasma lipids due to body weight reduction may be beneficial (Archer et al., 2003; Kratz et al., 2010).

Our findings that there were no differences in fasting plasma concentrations of TG and HDL-cholesterol between HGI and LGI trials are consistent with findings of some intervention studies (Shikany et al., 2009; Sloth et al., 2004). However, our findings differ from other intervention studies which have shown that LGI diets improve plasma lipids when compared to HGI diets in healthy sedentary individuals (Clapp & Beth, 2007; De Rougemont et al., 2007). The differences between findings in the present study and other studies could be explained by the difference in the duration of the intervention trials. Indeed, in our study the dietary intervention lasted for 5 days while in study of Clapp & Beth (2007) and De Rougemont et al. (2007)

studies were conducted for 20 days and 5 weeks, respectively. Therefore, LGI would be more advantageous for those with increased risk or advanced CHD (Frost et al., 1996; Frost et al., 1998; Jebb et al., 2010), type-2 diabetes (Jarvi et al., 1999; Luscombe et al., 1999; Wolever et al. 1992), and individuals with impaired glucose tolerance (Wolever & Mehling, 1992). In addition, it seems that duration of the intervention study should be long enough to achieve favourable impact.

In the present study, plasma concentrations of glucose and insulin and $HOMA_{IR}$ in the HC-HGI trial were not different from the HC-LGI trial. This is supported by evidence that a moderate intake of CHO diets with HGI and LGI do not influence insulin sensitivity in healthy individuals when consumed for 30 days (Kiens & Ritcher, 1996) or 10 weeks (Sloth et al., 2004). On the other hand, consuming a high CHO with LGI diet for a period of 20 days (Clapp & Beth, 2007) or 5 weeks (De Rougemont et al., 2007) resulted in increased insulin sensitivity and thus improved insulin-mediated glucose uptake in healthy individuals. Attenuation of the plasma NEFA rebound may be a mechanism by which LGI diets could improve insulin sensitivity (Wolever et al., 1995). In the present study, plasma concentrations of glucose and insulin and $HOMA_{IR}$ were not different between the high CHO trials and the Control trial. Consuming high CHO diets with HGI and LGI for 5 days may be not long enough to influence insulin sensitivity in healthy individuals therefore changes in insulin sensitivity may require longer duration. In addition, insulin resistance was assessed using $HOMA_{IR}$ which is known as a good biomarker for insulin resistance at population level but not suitable at small number of participants.

We found that the concentration of circulating biomarkers of endothelial activation such as soluble VCAM-1 and ICAM-1 (Hackman et al., 1996; Sampietro et al., 1997) measured in the fasted state were similar after both diets with high CHO with HGI and LGI diets and were not different from the concentration measured after habitual diet. Thus, it may be assumed that our study did not confirm the hypothesis that the repeatedly increased post-meal glucose responses after a high CHO with HGI diet may be expected to impact on the expression of ICAM-1 and VCAM-1 and thus plasma concentration of these CAMs to a greater extent than a LGI diet. The present study also did not confirm the evidence that the concentration of circulating biomarkers of endothelial activation correlates with fasting TG and are increased under conditions of dyslipidemia (Abe et al., 1998; Hackman et al., 1996) and that a high CHO with LGI diet due to reduced hyperglycaemia and hyperinsulinemia decreases endothelial dysfunction (Hare-Bruun, 2008; Lavi et al., 2009; McCarty, 2004). It should be noted that the concentration of these CAMs was measured in the fasted state when glucose concentration was within the range of 4.78 – 4.98 mmol/L (Table 6.3). Thus, future studies investigating the impact of GI of high CHO diets on the concentration of the CAMs should aim to measure the concentration of these CAMs in postprandial state since evidence has shown that the circulating biomarkers of endothelial activation are increased under condition of hyperglycaemia (Lavi et al., 2009) and hyperlipidemia (Abe et al., 1998; Hackman et al., 1996) which are enhanced in the postprandial state.

It should be noted however that in the participants of the present study, fasting plasma concentrations of TG and HDL-cholesterol, glucose and insulin were within the range of recommended healthy values (AHA, 2000) even after consumption of

high CHO diets while in the above studies participants were patients with hyperlipidemia. Thus, in healthy moderately physical active individuals, increased intake of CHO has no detrimental impact on circulating biomarkers of endothelial activation.

Since plasma lipids and insulin sensitivity were not influenced by the GI of the diets prescribed, it can be assumed that dietary interventions applied in this study did not induce contrasting responses in glucose and insulin. However, prior to the main study, we conducted a pilot study which demonstrated that time-averaged incremental area under the glucose versus time curve was significantly higher when daily foods were based on HGI than LGI foods (see Chapter 3). Therefore, the findings that prescribed foods with contrasting GI did not induce difference in fasting plasma lipids, insulin, glucose and insulin resistance cannot be explained by no difference in metabolic responses during the 5 days leading up to the measurements of fasting values.

A study conducted on postmenopausal women demonstrated that participation in physical activity while consuming high CHO diet may diminish an increase in fasting plasma TG and a reduction in HDL-cholesterol (Koutsari & Hardman, 2001). Participants of our study were physically active and therefore it could be expected that high CHO diets should not increase plasma TG and decrease plasma HDL-cholesterol concentrations. However, it should be noted that during the dietary intervention they were asked to lead a sedentary lifestyle and totally eliminate structured physical activities during last 2 days of the dietary intervention. Therefore,

it is not surprising that in our participants both high CHO diets induced increase in plasma TG and a reduction in plasma HDL-cholesterol concentrations.

We conclude that in physically active individuals, consideration of GI while consuming high CHO diets for the duration of 5 days cannot prevent an increase in plasma TG and a reduction in HDL-cholesterol concentrations. On the other hand, there was no detrimental impact on insulin sensitivity and biomarkers of endothelial activation.

Chapter 7 General discussion

The aims of the studies in this thesis were to investigate the impact of high CHO diets with HGI and LGI consumed for 5 days on exercise energy substrate utilisation and exercise capacity, and fasting plasma lipids and insulin sensitivity in healthy physically active individuals. The rationale for the investigation whether lowering GI of high CHO diets may diminish the attenuation of fat oxidation and thus delay depletion of muscle glycogen comes from evidence indicating that the benefits of high CHO diets on endurance exercise performance maybe reduced due to the diminishing effect on fat oxidation (Brewer et al., 1988; Coyle et al., 2001).

In Chapter 4 and Chapter 5, the impact of a high CHO diets with HGI and LGI on energy substrate utilisation during exercise and running capacity were investigated in physically active men and women, respectively. In the present thesis the impact of GI of high CHO diets on energy substrate utilisation and exercise capacity was investigated in both men and women since exercise energy metabolism (Roepstroff et al., 2006; Steffensen et al., 2002; Tarnopolsky et al., 2008; Venables et al., 2005) and to some degree high CHO diets induced muscle glycogen loading (James et al., 2001; Sedlock, 2008; Tarnopolsky et al., 1995, 2001; Walker et al., 2000) were found to be gender specific. The rationale for the exercise studies came from the notion that high CHO diets consumed for a few days reduced fat oxidation during subsequent exercise and thus promoted the depletion of muscle glycogen (Arkinstall et al., 2004; Hargreaves et al., 1995; Wojtaszewski et al. 2003) leading to premature fatigue and that a high CHO with LGI diet consumed during a 24 hour recovery between bouts of prolonged strenuous exercise produced a greater availability of

plasma NEFA (Stevenson et al., 2009; Trenell et al., 2008) and a higher rate of total fat oxidation (Stevenson et al., 2005b) during subsequent exercise conducted in the fasted state.

In our studies, men (Chapter 4) and women (Chapter 5) performed three treadmill runs to exhaustion at 65% $\dot{V}O_2$ max: after a habitual diet (Control trial), after 5 days on a high carbohydrate-high glycaemic index diet (HC-HGI trial), and after 5 days on a high carbohydrate-low glycaemic index diet (HC-LGI trial) in randomised counterbalanced order. Runs were performed in the fasted state to maximise fat oxidation (Van Proeyen et al, 2010) and studies investigated whether exercise conducted after high CHO diets with HGI and LGI in the fasted state produce similar changes in substrate utilisation and exercise capacity as in running exercise conducted after breakfast (Chen et al., 2008). We found that during the 90 minutes of running, no significant differences were observed in the rates of fat and CHO oxidation, concentrations of plasma insulin, glucose, NEFA and glycerol between the HC-HGI and HC-LGI trials. In comparison to the Control trial, plasma glycerol concentration was significantly lower in both high CHO trials in both men and women. Fat oxidation however was significantly lower in both high CHO trials only in men, while in women fat oxidation was reduced only in HC-LGI trial in comparison to the Control trial. Thus, the findings when combining studies on men and women showed that the reduction in the rate of fat oxidation during running in the fasted state is not influenced by the GI of high CHO diets consumed for 5 days. These findings were similar with those of Chen et al. (2008) who reported that during running exercise conducted after breakfast, significant reduction in fat oxidation induced by high CHO diets consumed for 3 days was independent of the GI. Thus,

during both exercise in fasted and fed states (Chen et al., 2008) consideration of LGI diet cannot reduce attenuation of fat oxidation induced by high CHO consumption.

Our findings that in women, the HC-LGI trial reduced and HC-HGI trial had no impact on fat oxidation compared to the Control trial may be related to the higher reduction and thus oxidation of IMTG, the main contributor of non-plasma derived fatty acids during exercise (Roepstorff et al., 2005; van Loon et al., 2003) after a HC-HGI trial than after a HC-LGI trial. This is supported by evidence that the IMTG content was lower after consuming moderate CHO diet with LGI compared to a HGI (Kiens & Ritcher, 1996) and that a high CHO with LGI diet reduced IMTG utilisation and thus fat oxidation to a greater extent when compared to a HGI diet during exercise (Stevenson et al., 2009; Trenell et al., 2008). The responses of plasma glycerol and NEFA concentrations during 90 minutes of exercise found in our study also support this. We found no difference in plasma NEFA concentrations between all three trials while the glycerol concentration was lower in both high CHO trials with significant differences at more time points in the HC-HGI trial. More research is required to confirm the finding that a HC-HGI diet had no impact on fat oxidation in women.

The findings that there were no differences in fat oxidation between HC-HGI and HC-LGI trials in both men and women is unlikely to be related to the small number of study participants. Power calculations indicated that with 9 participants, a difference of 0.07 g/min in fat oxidation could be detected with a power of 85%. In addition, the similar rate of fat oxidation between the two high CHO trials cannot be explained by similar plasma glucose responses during 5 days of the dietary

interventions, since the pilot study demonstrated that the prescribed high CHO diets with contrasting GI induced significant differences in plasma glucose responses (Chapter 3). We conducted the pilot study since it has been suggested that the GI, based on tests of single foods, may not apply in the setting of mixed meals containing representative amounts of fat and protein (Coulston, 1984; Franz et al., 2002; Pi-Sunyer, 2002) and cannot predict the glycaemic response to that meal (Coulston et al., 1987). In addition, a recent study by Galgani et al. (2006) suggested that foods with contrasting glycaemic indexes do not always induce a proportionally comparable difference in plasma glucose when consumed in large amounts. We found that the time-averaged IAUC for glucose responses over 5 hours after breakfast, snack and lunch consumption was significantly higher ($P<0.05$) in the HC-HGI (3.29 ± 0.38 mmol/L) than HC-LGI (1.50 ± 0.42 mmol/L). However, it should be noted that the actual glycaemic responses to the meals with different GI were measured only for half day instead of all 5 days, while for food variability different menus were prescribed for each day.

We compared the rate of fat oxidation in men (Chapter 4) and fat oxidation found in women (Chapter 5). This comparison was possible since both men and women exercised at similar relative energy intensity which consisted of 65% $\dot{V}O_2$ max. The comparison showed that a higher proportion of energy from fat during exercise was obtained in women than men. During the Control, HC-HGI and HC-LGI trials, fat contributed approximately 56%, 49% and 42%, respectively towards exercise EE in women, which were significantly higher than in men (Control trial, 45%; HC-HGI trial, 35% and HC-LGI trial, 32%). Thus, our findings confirmed the notion that women oxidised a higher proportion of fat than men during endurance type of

exercise of similar relative intensity. For example, Wallis et al. (2006) showed that women and men oxidised 60% and 50% of energy from fat, respectively, during running in the fasted state at 67% $\dot{V}O_2$ max after 4 days on high CHO diets. In a study by Knechtle et al. (2004), women oxidised 51% energy from fat which was significantly higher than men, who oxidised 43% of fat during running at 65% $\dot{V}O_2$ peak. This may related to a higher protein content of the major enzymes involved in long and medium chain fatty acid oxidation found in women when compared to men (Maher et al., 2010). In addition, enhanced oxidation of fat during exercise in women may reflect a reduced rate of muscle glycogen (Tarnopolsky & Ruby, 2001), and plasma glucose utilisation (Devries et al., 2006; Horton et al., 2006; Tarnopolsky et al., 2007).

In the present study, the GI of high CHO diets consumed for 5 days had no impact on exercise capacity measured as time to exhaustion during an endurance run in the fasted state in both men and women. This finding is consistent with evidence suggesting that the GI of high CHO diets consumed for 3 days had no impact on the time taken to complete a 10-km run in men after 1 hour of steady state running conducted after breakfast consumption (Chen et al., 2008) and no impact of quality of CHO on time to complete a treadmill run to exhaustion at 70% $\dot{V}O_2$ max when the experienced runners consumed 70% of energy from CHO with either complex (synonymous with LGI) or simple (synonymous with GI) CHO for 3 days before running (Brewer et al.,1988). However, Stevenson et al. (2005) reported that a high CHO diet with LGI consumed the day before running to exhaustion in the fasted state, significantly delayed the onset of fatigue compared to a HGI diet, and this was

explained by an increase in the rate of fat oxidation in the LGI trial, thereby sparing muscle glycogen.

We found no difference in the time to exhaustion and distance covered between the high CHO trials and the Control trial in both men and women. This finding was not expected, since increased dietary CHO intake of more than 70% of energy promotes muscle glycogen storage in men (Burke & Hawley, 2006; James et al., 2001; Tarnopolsky et al., 2001) and women (James et al., 2001; Tarnopolsky et al., 2001; Walker et al., 2000) which prolongs time to exhaustion in trials lasting over 90 min (Brewer et al., 1988; Hawley et al., 1997; Sedlock, 2008). In our studies, men consumed CHO 7.3 and 7.4 g/kg BW/day and women consumed 7.9 and 7.7 g/kg BW/day in HC-HGI and HC-LGI diets, respectively. The CHO intakes were slightly below the amount required to optimally increase muscle glycogen stores (> 8-10 g/kg BW/day) for both men (Burke & Hawley, 2006; Bussau et al., 2002) and women (James et al., 2001; Walker et al., 2000). It should be noted that during the dietary interventions, participants were not refrained from their normal lifestyles, therefore the present study was conducted with no prior depletion of muscle glycogen and daily exercise could be performed by the participants except 2 days prior to the exercise trials. Previous evidence showed that a CHO-loading protocol that begins with a glycogen-depleting exercise resulted in significantly greater muscle glycogen than a CHO-loading protocol using only an exercise taper (Goforth et al., 2003), therefore the increased muscle glycogen content after high CHO diets in the present study may be not at an optimum level since the exhaustive exercise preceding the dietary interventions was not included in the experiment protocols. This may have

contributed to the finding of no significant difference in exercise capacity between control and high CHO trials.

It is important to note the difficulty in assessing exercise capacity. It has been found that exercise to exhaustion protocols have poor reproducibility with a large (26.6%) coefficient of variation (Jeukendrup et al., 1996). Although time trial test had much lower coefficient of variation (3.4%) (Jeukendrup et al., 1996) and is more reliable to determine exercise performance compared to run to exhaustion it would be difficult to adjust the speed of the treadmill since most of the participants were not trained athletes. Furthermore, it would be difficult to collect expired air using the Douglas bag technique and to obtain blood samples if the speed of the treadmill is high. It is possible that the validity of the assessment of exercise capacity was diminished by asking the participants to run at 65% rather than at 70% of $\dot{V}O_2$ max since it is well known that rate of muscle glycogen utilisation and thus the development of fatigue is very sensitive to exercise intensity. Thus, in our study psychological factors or muscle pain rather than reduced glycogen availability at the end of exercise could have been the cause of fatigue.

In the present study, muscle glycogen accumulation was not measured following the dietary interventions because the muscle biopsy technique is not available in our laboratory and the study design was already very demanding to the participants. However it can be assumed that the combined effect of a high CHO diet and two days rest before the exercise trials would have enhanced glycogen stores above of the control (Goforth et al., 2003; Sedlock, 2008). The increase in muscle glycogen stores most likely lead to greater rates of glycogenolysis during exercise which was

reflected as an elevation in the rate of CHO oxidation. Each participant was required to run until exhaustion at 65% of their $\dot{V}O_2$ max, which is a reasonably low intensity of exercise and is within the range identified that maximises fat utilisation (Achten et al., 2002). Therefore, even if the high CHO diets did enhance glycogen stores prior to exercise, the low intensity of the exercise enabled sufficient lipid oxidation. Thus, in our study psychological factors or muscle pain rather than reduced glycogen availability at the end of the exercise trial could have been the cause of fatigue.

Differences in exercise metabolic differences, most likely present during the 5 days consumption of HGI and LGI diets may be negated by fasting. Fasting overnight reduces the liver glycogen stores (Roden et al., 2001) and so decreases the availability of plasma glucose for muscle metabolism which results in lower plasma insulin and higher plasma epinephrine concentrations (Febrraio et al., 2000) leading to high availability of fatty acids for oxidation during exercise. Furthermore, exercise in the fasted state had found to increase reliance on muscle glycogen (Coyle et al., 1985) while another study had reported an increase in IMTG utilisation (De Bock et al., 2005) compared to exercise after CHO intake.

The number of participants who dropped out from these studies was quite high. Of the 30 men and 15 women recruited, only 12 men and 9 women completed the study. It was difficult to recruit participants based on the inclusion criteria, especially to get individuals who are recreationally active. The $\dot{V}O_2$ max measurements conducted on the participants during the preliminary test revealed that some of the participants did not reach the required fitness level i.e. minimum of 55% $\dot{V}O_2$ ml/kg/min and

50% $\dot{V}O_2$ ml/kg/min for men and women, respectively (McArdle et al., 2006) thus they could not be included in the study. There was one participant who did not pass the ECG measurement. It was also difficult to retain the participants in these studies, even when they only had one trial left to complete. Reasons for dropping out included work commitments, change of lifestyle, moving away, physical illness and could not stick to the highly demanding of the exercise protocols. We appreciate that changing from the moderate CHO intake of the Western diets to a high CHO intake in the prescribed diets may be difficult for some of the participants. Some of them found that it was more difficult to comply with the HGI diet than the LGI diet, and the women were found to be more particular about food choices than men. There were participants who admitted that they were not able to comply with the prescribed diets since they had difficulties with consumption of some foods or meals and thus they were not considered for further investigation.

The last study of the present thesis investigated the hypothesis that in physically active individuals, detrimental changes in relation to plasma lipid profiles induced by high CHO diets may be diminished by consuming high CHO diets based on LGI foods. High CHO diets are commonly consumed during endurance training or prior to endurance events with an aim to increase muscle glycogen content and thus exercise performance (Brewer et al., 1988; Burke & Hawley, 2008; Rauch et al., 1995; Tarnopolsky et al., 2001). Previous studies have shown that in endurance athletes, high CHO diets induced significant increases in TC, TG and LDL-cholesterol and decreases in HDL-cholesterol (Brown & Cox, 1998; Thompson et 1984). Since the high TG concentration enhances the opportunity for the reciprocal transfer of cholesterol and TG between HDL-cholesterol and TRL by the action of

CETP (Davidson, 2010), the increase in plasma concentration of TG leads not only to lower concentration of HDL-cholesterol, but also to increased preponderance of small dense LDL_3 particles. Thus, consumption of high CHO diets for a short duration may be detrimental in relation to the development of atherosclerosis even in physically active individuals.

It should be noted that in the above studies (Brown a Cox, 1998; Thompson et 1984) CHO were represented mostly by simple CHO, which in most cases were HGI. Thus, we aimed to investigate whether in physically active individuals, detrimental changes in relation to lipid profile induced by high CHO diets may be diminished by consuming CHO meals based on LGI foods. We found that there were no differences in plasma concentrations of TG and HDL-cholesterol between the two high CHO diets. Thus it seems that plasma lipids were influenced by the amount of CHO rather than quality. Our finding is in consistency with studies which observed no effect of LGI diet on plasma lipids and insulin sensitivity in sedentary healthy individuals (De Rougemont et al., 2007; Ells et al., 2005; Kiens & Ritcher, 1996; McMillan-Price et al., 2006; Sloth et al., 2004). On the other hand, our data differs from studies in patients with type-2 diabetes, impaired glucose tolerance and risk of CHD, in which CHO diet with LGI improved insulin sensitivity, decreased plasma concentrations of TC, LDL-cholesterol and TG, and increased concentration of HDL-cholesterol (Frost et al., 1996; Frost et al., 1998; Jarvi et al., 1999; Jebb et al., 2010; Luscombe et al., 1999; Rizkalla et al., 2004; Wolever & Mehling, 2002). Thus, it seems that only those with impaired metabolism benefit from consideration of GI. This may be related to the possibility that responses of glucose and insulin while consuming CHO with different GI may induce more profound differences in

individuals with metabolic disorders than healthy individuals. This requires further investigation. Further investigation is also needed when considering the impact of GI on both fasting and postprandial concentrations of plasma TG. Most likely GI may modify postprandial TG concentration even when the test meals consumed are the same for LGI and HGI diets when conducted in the fasted state.

It should be noted that in the present study, the increase in plasma TG and the reduction in HDL-cholesterol concentrations induced by high CHO consumption for 5 days, regardless of being significant were very small. Indeed, even after high CHO diets with both HGI and LGI, plasma concentration of TG (1.02 mmol/L and 0.92 mmol/L, respectively) and HDL-cholesterol (1.37 mmol/L and 1.42 mmol/L, respectively) were within healthy (TG, <1.69 mmol/L) and within medium (HDL-cholesterol, 1.03-1.55 mmol/L) level (AHA, 2009). This may be related to the fact that participants in the present study were healthy and physically active and had initially very good lipid profiles. The addition of a brisk walk for 60 min/day during 3 days on a high CHO diet leads to fasting plasma TG concentration being similar to that in a high fat diet (Koutsari et al., 2001). Combining our data with those of Koutsari et al. (2001), it could suggest that in healthy physical active individuals, consumption of high CHO diets even when CHO intake provides approximately 70% of energy or available CHO between 7.3 and 7.9 g/kg BW/day for 5 days are not detrimental to cardiovascular health. Thus, when physically active individuals reduce their activity levels while inducing glycogen loading strategies, changes in plasma TG and HDL-cholesterol can be expected to be negligible and safe.

We also found that there was no difference in plasma concentrations of glucose and insulin and $HOMA_{IR}$ between high CHO trials and when compared to the Control trial. This confirmed the notion that consuming LGI diets may be more advantageous for individuals with type-2 diabetes (Jarvi et al., 1999; Luscombe et al. 1999; Wolever et al., 1992), and individuals with impaired glucose tolerance (Wolever & Mehling, 2002) than healthy individuals. Thus benefits of LGI diets in relation to insulin sensitivity may be only be achievable in those who are either predisposed to, or already have some degree of insulin resistance. We note that in participants of the present study, insulin resistance estimated as $HOMA_{IR}$ values were very low (Control, 0.8; HC-HGI, 0.74; HC-LGI, 0.85), which is consistent with other findings on physically active participants (Hamzah et al., 2009).

The very important part of the experimental work was developing high CHO diets with HGI and LGI. For all the experimental studies, both high CHO diets were developed to be isoenergetic to the habitual diet. Since dietary records are known to involve a risk of underreporting (Hills & Davies, 2002), the estimation of energy intake was based on the participants' habitual energy requirements. Prescribed high CHO diet aimed to provide 70% of energy from CHO or 8 g/kg BW/day of CHO which is recommended for muscle glycogen loading (Burke & Halwey, 2006; James et al., 2001; Walker et al., 2000). Energy intake from fat was aimed to provide 15% of energy and the remaining 15% was from protein. For the dietary prescription, a list of CHO containing foods with HGI and LGI was discussed with each participant to make sure the foods would be acceptable. The GI of each CHO food in the HC-HGI and HC-LGI diets was taken from the GI tables available in the literature (Aston et al., 2008; Foster-Powell et al., 2002; Henry et al., 2005) and the GI of the meals

was estimated using methods described by Wolever & Jenkins (1986). Diets were aimed to provide a similar amount of fibre, protein and fat since these are known to influence postprandial glucose responses by slowing gastric emptying and reducing the absorption rate of glucose from the small intestine (Bornet et al., 1987; Welch et al., 1987). When prescribing high CHO diets with HGI and LGI, we aimed to achieve a GI of 70 and 35 as being high and low, respectively. To increase compliance, participants were provided with all foods, menus and digital scales and they were instructed how to cook the meals, how much to eat and when to eat during the dietary interventions. It has been shown that cooking the CHO-containing foods can affect the particle size and the integrity of the starch granules (Jenkins et al., 1988) making the CHO portion more accessible to digestive enzymes (Collins et al., 1981; Wolever, 1990). Therefore, to eliminate any influence of cooking methods on glycaemic response and increase compliance, the menus were based on processed and easy-to prepare foods. Any deviations from these instructions were recorded by the participants.

Even with a good compliance to the prescribed meals, there is uncertainty whether the GI of CHO foods determine the glycaemic effect of prescribed mixed meals and whether the meals with different GI induces significant differences in plasma glucose responses during days leading up to main trials. With large intra and inter-subject variations in the glycaemic response to a meal, the value of the calculated GI of the meals in the present study may not have reflected the true glycaemic responses. Therefore, a pilot study was conducted to confirm this. Using a crossover design, the plasma glucose responses to high CHO with HGI and LGI meals consisting of breakfast, morning snack and lunch were measured over 5 hours in 8 physically

active healthy participants. We found that the time-averaged incremental area under the glucose versus time curve for glucose responses over 5 h was significantly higher ($P<0.05$) in the HC-HGI (3.29 ± 0.38 mmol/L) than the isocaloric HC-LGI (1.50 ± 0.42 mmol/L).

In all of the present studies high CHO diets with HGI and LGI were isocaloric. In real life situations, appetite regulation by HGI and LGI meals may be different (Burton-Freeman & Keim, 2008) and thus lead to differences in caloric intake. Since energy balance is known to impact plasma lipids (Maraki et al., 2010), the impact of GI could be expected to be seen under conditions of *ad libitum* intake. In addition, in women, muscle glycogen accumulation (McLay et al., 2007; Oosthuyse & Bosch, 2010), exercise metabolism and performance (Oosthuyse & Bosch, 2010), plasma lipids and insulin concentrations (Gill et al., 2005) could be affected by menstrual cycle where controlling of menstrual cycle was achieved in some but not all women in the present studies. Investigations on the impact of GI on postprandial TG concentrations and changes in LDL particle distribution are needed to obtain full information.

Conclusions

HC-HGI and HC-LGI meals prescribed using GI values of foods available from the existing GI tables in the literature produces significant differences in the postprandial plasma glucose responses and thus can be used in studies investigating impact of the GI of high CHO diets.

The consideration of the GI of high CHO diets consumed by the physically active healthy men and women for 5days had no impact energy substrate utilisation during running conducted in the fasted state and the GI of high CHO diets commonly consumed during days leading up to an athletic endurance event had no impact on exercise capacity.

In women compared to habitual diet, 5 days high CHO diet with LGI reduced the rate of fat oxidation during moderate intensity running conducted in the fasting state while high CHO diet with HGI has no impact on fat oxidation. This may reflect different impact of high CHO diets with HGI and LGI on IMTG content.

During exercise at the intensity providing maximal fat oxidation (65% $\dot{V}O_2$ max) contribution of fat towards energy expenditure is higher in women than men. This may be related to higher content of IMTG and lower content of muscle glycogen in women.

In physically active individuals, consideration of GI while consuming high CHO diets for the duration of 5 days to increase muscle glycogen content cannot prevent the increase in fasting plasma TG and reduction in HDL-cholesterol concentrations. Further studies should consider whether GI of high CHO diets has impact on postprandial lipaemia and proportions of LDL and HDL subfractions.

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APPENDICES

Appendix I

TO RUNNERS……

HUMAN NUTRITION DEPARTMENT OF GLASGOW UNIVERSITY IS CONDUCTING A STUDY ON 'THE ROLE OF HIGH CARBOHYDRATE DIET ON RUNNING PERFORMANCE'.

We need males and females volunteers who are:

- Age between 18-35 years
- Healthy, stable body mass for at least 2 months
- Physically active (involved in running training at least three times per week)

Taking part would involve:

- Cardio respiratory fitness testing ($\dot{V}O_2$ max)
- Consuming high carbohydrate diet with high and low glycaemic index diets for 5 days (food will be provided)
- Exercise trials (involving blood sampling)

We will cover your travelling expenses and at the end of this study we will provide you with information on how diet with different types of carbohydrates influence running performance.

If you are interested or would like more information, please email
Sareena Hamzah s.hamzah.1@research.gla.ac.uk
or Dr. Dalia Malkova dm88n@clinmed.gla.ac.uk

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Appendix II

SUBJECT INFORMATION SHEET

Study title

The effect of high carbohydrate diet with high and low glycaemic index diets on energy substrate utilisation and exercise performance

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?

The main purpose of this study is to investigate the effect of a 5-day diet which provide approximately 70% of energy intake from carbohydrate with either a high or low glycaemic index (GI) meals on metabolic responses and contribution of fat and carbohydrate towards energy expenditure at rest and during submaximal exercise conducted in fasted state. This study also aims to examine the impact of these diets on exercise performance.

Why am I eligible?

You are eligible to take part in this study because you are aged between 18-35 years, you are recreationally active (exercise at least 3 times a week), healthy female and your body weight has stable for at least a month. We intend to recruit at least twelve healthy women to take part.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be provided with this information sheet in advance so that you have a minimum of 24 hours to consider participation. If you decided to take part you will be asked to sign consent form. However, you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

You will be involved in this study for approximately two weeks although this time may be spread over five or six weeks. During this time, you will be required to come to the laboratory for four study visits. An explanation of what will happen at each of these visits is given below. This is a randomised counterbalanced study and you will start with any of these dietary intervention i.e. habitual, high glycaemic index or low glycaemic index diet.

<u>First,second and third visit</u>

At the first study visit, you will be asked to come to the laboratory after an overnight fast (you can drink water). We will measure your resting metabolic rate (RMR) and body composition. We will then take your height, weight and after that a standard 12-lead exercise electrocardiogram (ECG) test will be conducted. Also at this visit, you will be asked to sign a consent form and complete questionnaires about yourself, physical activity and general health. Then you will be asked to participate in submaximal running test to determine the relationship between running speed and oxygen uptake ($\dot{V}O_2$ max). You will be given drink water ad libitum during and immediately after the test. You will rest for ~ 30 minutes and then you will participate in maximum oxygen consumption ($\dot{V}O_2$ max). During $\dot{V}O_2$ max test, you will be asked to exercise up to the point of fatigue which will be the point at with you will be unable to maintain the running speed for 20 seconds consecutively. During this test, the treadmill speed will be kept constant while the inclination of the treadmill will be increased by 3-5 % every three minutes from an initial grade of 4-8 %. We will measure your heart rate, collect your expired air samples and record your rate of perceived exertion at every stage of the test. During this visit you will also be advised and instructed of how to comply with the dietary intervention with either

high or low glycaemic index and to keep with this diet for 5 days. Finally, at this visit, we will arrange for a suitable day for you to come back for your main exercise trials. Please take note that you may have to do a familiarisation trial before you start the main exercise trial (third visit).

Fourth, fifth and sixth visit

On these visit (after each dietary intervention – Control, High GI and Low GI), you will be asked to come to the laboratory after 12 hours overnight fast. You will take part in the main exercise trial, in which you are required to run a minimum 90 minutes and run to exhaustion thereafter on a treadmill at 65% $\dot{V}O_2$ max. Blood samples and expired air will be collected before and during the exercise trial. We will also record your rating of perceive exertion and your heart rate will be monitored at the same period. The trial will continue until you indicate that you manage to continue 1 more minute and the final expired air will be collected.

What do I have to do?

If you decide to take part in this study, you will be asked to change your normal diet to comply with the high and low glycaemic index diet for two 5-days periods. We will explain how to do this and will provide you with the main foods that you are required to eat based on the carbohydrate foods that you normally eat. You will also be asked to avoid alcohol and strenuous physical activity (i.e. sport other than the exercise tests performed as part of the study) for three days before each of the four study visits. There are no other lifestyle restrictions as part of this study.

What are the possible disadvantages and risks of taking part?

There are no major risks involved in taking part in this study. You will be asked to follow a both high and low glycaemic index diet for two 5-day periods. The high glycaemic index diet may increase your triacylglycerol levels (main type of fat in your blood stream). If this happened, it will not be lasting effect and your triacylglycerol levels will normalise within a few weeks. Following the diets may be inconvenient for you. However, we will explain very clearly what you will have to do and we will also supply you with the main foods that you would be asked to eat.

During the main exercise trials, the intensity will be moderate and comfortable to maintain for healthy people. You will be asked to exercise up to the point of fatigue which will be the point at with you will be unable to maintain a particular running speed (or level of intensity) for 20 seconds consecutively. This however would have no adverse effect on your health as you will not become dehydrated as you can drink as much water during the test as you like. Your blood sugar levels may drop at the very end of this test but we will provide energy rich drinks and food to replace your blood sugar levels immediately after you have finished the test. Also during the exercise trial, we will take a number of blood samples using a cannula (tiny plastic tube). Blood sampling can cause minor bruising. Very rarely it may cause inflammation of the vein. A qualified and experienced person will take the blood samples and they will make every effort to avoid this happening. Medical doctor who has advanced life support training will be immediately available for any exercise testing protocol.

What are the possible benefits of taking part?

There are no direct benefits to you taking part in this study. However, we will add to knowledge on how diet and in particular different types of carbohydrates influence performance and the fuels that the body uses during exercise. You will also obtain information about your fitness level and profile of your habitual diet.

Will my taking part in this study be kept confidential?

All information, which is collected, about you during the course of the research will be kept strictly confidential. You will be identified by an ID number, and any information about you, will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

The results of this study will be used by the investigators involved in carrying out the study in the project write-ups. The results will hopefully also be published in a scientific journal and presented at a scientific conference. You will not be identified in any of these reports or publications.

Who is organising and funding the research?

This study is being organised by the Human Nutrition Section of the Division of Developmental Medicine at the University of Glasgow, which is based at Yorkhill Hospital in Glasgow. The research is funded by University of Malaya, Kuala Lumpur, Malaysia.

Contact for further information.

If you have questions about the study, you may contact Sareena Hamzah, Human Nutrition, Division of Developmental Medicine, University of Glasgow at 07765994151 or by email s.hamzah.1@research.gla.ac.uk. You also may contact the supervisor Dr Dalia Malkova at 0141 2010648 or by email dm88n@clinmed.gla.ac.uk

Thank you for taking the time to read the information sheet.

Appendix III

Centre Number:
Study Number:
Subject Identification Number for this trial:

CONSENT FORM

Title of Project:
The effect of high carbohydrate diet with high and low glycaemic index diets on energy substrate utilisation and exercise performance.
Name of Researcher: Sareena Hanim Hamzah, Dr Dalia Malkova

Please initial box

1. I confirm that I have read and understand the information sheet dated …… for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected. ☐

3. I agree to take part in the above study. ☐

Name of subject	***Date***	***Signature***
Name of Person taking consent (if different from researcher)	***Date***	***Signature***
Researcher	***Date***	***Signature***

1 for subject; 1 for researcher

Appendix IV

HEALTH SCREEN FOR STUDY VOLUNTEERS

Name: …….

Date: ……

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 required 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) Hearing problems	yes [ ]	no [ ]
(i) Problems with bones or joints	yes [ ]	no [ ]
(j) Disturbance of balance/co-ordination	yes [ ]	no [ ]
(k) Numbness in hands or feet	yes [ ]	no [ ]
(l) Disturbance of vision	yes [ ]	no [ ]
(m) Thyroid problems	yes [ ]	no [ ]
(n) Kidney or liver problems	yes [ ]	no [ ]
(o) Chest pain or heart problems	yes [ ]	no [ ]
(p) Any other health problems	yes [ ]	no [ ]

4. **Have any of your immediate family** ever had any of the following: (if yes please give details including age of first diagnosis)

(a) Any heart problems	yes [ ]	no [ ]
(b) Diabetes	yes [ ]	no [ ]
(c) Stroke	yes [ ]	no [ ]

(d) Any other family illnesses yes [ ] no [ ]

5. Do you currently smoke yes [ ] no [ ]
Have you ever smoked yes [ ] no [ ]

If so, for how long did you smoke and when did you stop? ……

6. How many units of alcohol do you typically drink in a week? …….

If YES to any question, please describe briefly if you wish (e.g. to confirm whether problem was short-lived, insignificant or well controlled.) (Use a separate sheet if necessary)

……
……
……
……
……

Name and address of GP
…….....
.....
.

Blood pressure measured at screening……..mm Hg

Appendix V

PHYSICAL ACTIVITY QUESTIONNAIRE

Name: **Date:**

During one week, how many times on average do you do the following kinds of exercise for more than 15 minutes?

(a) Strenuous Exercise (heart beats rapidly)

For example; running, jogging, squash, vigorous swimming, vigorous long distance cycling.

_____ times per week.

(b) Moderate Exercise (not exhausting)

For example; fast walking, tennis, easy cycling, badminton, easy swimming, dancing.

_____ times per week.

(c) Mild Exercise (minimal effort)

For example; yoga, archery, fishing, bowling, golf, easy walking.

_____ times per week.

Appendix VI

Standardising Gas Volumes

Gas volumes vary with temperature and pressure. Comparison of respiratory gas volumes over days is therefore only valid if volumes are corrected to standard conditions. Standard pressure is universally defined as 760 mm Hg and standard temperature as 273 K (0^oC). Gas volumes are measured under ambient conditions, i.e. those prevailing at the time of experiment, and subsequently corrected to STPD (standard temperature and pressure for a dry gas), using the gas laws.

Boyle's Law (PV = constant) and Charles' Law (V/T = constant) can be combined to yield an expression which predicts the volume change that results from changes in temperature and pressure of a given mass of gas.

$$\frac{PV}{T} = \text{constant} \quad \text{OR} \quad \frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$$

Where P is pressure, T is temperature and V is volume.

An additional correction is required when adjusting expired air volumes to standard conditions because expired air is saturated with water vapour. The water vapour reduces the gas pressure by an amount related to temperature.

$$\text{So } \dot{V}_{E\,STPD} = \dot{V}_{E\,ATPS} \times \frac{(BP - SWVP)}{760} \times \frac{273}{273 + t}$$

Where: BP is barometric pressure in mmHg
SWVP is saturated water vapour pressure in mmHG at ambient* temperature
t is ambient temperature in degree Celsius
ATPS is ambient temperature and pressure, saturated with water vapour

* Ambient temperature and pressure describe the condition as the volume of the expired air sample is measured.

NB. Saturated water vapour pressure depends only on temperature. This relationship is linear within the range used for the present purposes and SWVP may be predicted from temperature using the following equation:

SWVP = (1.1001 x t) – 4.19

Where t is the temperature of the gas in ^{0}C as its volume is measured.
SWVP is the saturated water vapour pressure in mm Mercury.

Appendix VII

Haldane Transformation to derive $\dot{V}_I$

This method uses the concentration of nitrogen (assumed to be metabolically inert) in the inspired and expired air to derive the volume of air inspired from direct measurements of the volume expired.

$$\text{i.e.} \quad \text{As concentration} = \frac{\text{mass}}{\text{volume}}$$

$$\text{mass } N_2 \text{ inspired} = F_IN_2\%$$

$$\frac{\text{mass } N_2 \text{ inspired}}{\dot{V}_I} = F_IN_2\%$$

and

$$\text{mass } N_2 \text{ expired} = F_EN_2\%$$

$$\frac{\text{mass } N_2 \text{ expired}}{\dot{V}_E} = F_EN_2\%$$

But mass of N_2 inspired = mass of N_2 expired

Therefore $F_IN_2\% \times \dot{V}_I = F_EN_2\% \times \dot{V}_E$

$\dot{V}_I$ can therefore be derived, if $\dot{V}_E$ is measured and $F_IN_2\%$ and $F_EN_2\%$ are known. $F_IN_2\%$ and $F_EN_2\%$ are found by subtracting the concentrations of carbon dioxide and oxygen from 100%.

Appendix VIII

Calculation of oxygen uptake and carbon dioxide production

All gas volumes referred to below are STPD

Introduction:

Volume of O_2 taken up = Volume O_2 inspired – Volume O_2 expired

$$\text{Vol. } O_2 \text{ inspired} = \dot{V}_I \times \frac{F_I O_2\,\%}{100}$$

$$\text{Vol. } O_2 \text{ expired} = \dot{V}_E \times \frac{F_E O_2\,\%}{100}$$

Where F_I = inspired fraction, F_E = expired fraction.

Similar equations may be derived for carbon dioxide production ($\dot{V}CO_2$)

Thus:

Vol. of CO_2 produced = vol. CO_2 expired – vol. CO_2 inspired.

$$\dot{V}CO_2 = \dot{V}_E \times \frac{F_E CO_2\,\%}{100} - \dot{V}_I \times \frac{F_I CO_2\,\%}{100}$$

F_IO_2% can be assumed to be 20.93% and F_ICO_2% to be 0.03% as the composition of inspired air is very stable.

F_EO_2%, F_ECO_2% and $\dot{V}_E$ can be measured in an expired air sample.

$\dot{V}_I$ is obtained by the HALDANE TRANSFORMATION

Appendix IX

5 DAYS EXERCISE DAIRY

Name : _____ Date : _____

1 Instructions: Please tick the box which best corresponds to your occupation.

1. Low Activity
(e.g. working mostly at a desk) ☐

2. Medium Activity
(e.g. mostly standing but low intensity, e.g. barwork) ☐

3. High Activity
(e.g. manual labour, e.g. bricklaying) ☐

B. Please list all activities (i.e. exercise undertaken) that you will perform during 5 days leading up to main trials (during 5 days of intervention). There is no need to record work related activities. Remember that you will be required to replicate the activities before your each main trial. Please note that the duration of exercise should minus rest periods. Where possible please note distances. Also please rate activity with following intensity annotations.

(L) - Low intensity; able to hold a conversation

(M) - Medium intensity; unable to hold a conversation but able to communicate short sentences

(H) - High intensity; unable to communicate verbally

Example

Day 1: Monday

Time	Activity	Intensity (L/M/H)	Duration (minute/hour)	Distance (m/KM)
0730	Jogging	L	35 minutes	7 km
1300	Squat	H	3 min	
	Pull up	H	3 min	
	Lunges	H	3 min	
1900	Green bowling	L	1 hour	
2100	Billiards	L	1 hour	

5 days prior to main trial (1st day of dietary intervention)

Time	Activity	Intensity (L/M/H)	Duration (minute/hour)	Distance (m/KM)

4 days prior to main trial (2nd day of dietary intervention)

Time	Activity	Intensity (L/M/H)	Duration (minute/hour)	Distance (m/KM)

3 days prior to main trial (3rd day of dietary intervention)

Time	Activity	Intensity (L/M/H)	Duration (minute/hour)	Distance (m/KM)

2 days prior to main trial (4th day of dietary intervention)

Time	Activity	Intensity (L/M/H)	Duration (minute/hour)	Distance (m/KM)

NO STRENOUS PHYSICAL ACTIVITY/EXERCISE PLEASE!

1 day prior to main trial (5th day of dietary intervention)

Time	Activity	Intensity (L/M/H)	Duration (minute/hour)	Distance (m/KM)

NO STRENOUS PHYSICAL ACTIVITY/EXERCISE PLEASE!
NO ALCOHOL TOO!

Day 0 MAIN TRIAL / RUN TO EXHAUSTION

Thank you for your time.

Appendix X

FOOD INVENTORY INSTRUCTIONS

It is important that you weigh and record everything that you eat and drink for the **two** days prior to each oral fat tolerance test (OFTT). Please do not take any alcohol on these days. Your last food and drink should be taken 12 hours before your OFTT appointment.

Please (i) start a separate page for each day.

(ii) start a separate line for each item.

Column 1

Record meal and time and place of eating.

Column 2

Describe each item as accurately as possible, stating where relevant:

(i) type and brand

(ii) whether food is fresh, dried, canned, frozen, salted, smoked, etc.

(iii) whether food is cooked, if so give method of cooking e.g. fried, baked, etc.

Column 3

Record the weight of each item after cooking:

(i) place scales on a level surface

(ii) place plate or container on top of scales

(iii) press 'ON/Reset' button to turn on scales

(iv) once zero appears, add first item of food

(v) record weight displayed

(vi) press reset button before weighing next item

Wherever possible, record weights in grams. If this is not possible, record weights in household measures (e.g. sugar or jam in teaspoons, stating whether level, rounded, or heaped).

Column 4

Record the weight of any leftovers, such as food remaining on plate, weight of container in which food has been weighed, apple cores, etc.

Columns 5 and 6

Please leave blank.

If food consists of several items, please list each on a separate line i.e. instead of writing 'one cheese sandwich', record separately the weights of bread margarine, cheese, etc.

Please remember to record all drinks, as well as food, giving weights where possible, or volumes if these are known. Record separately the weights of added milk and sugar.

An example is shown overleaf.

Food Inventory - Example

Name_____ **Date** _____

1. Time/Place	2. Description of food/drink	3. Weight food/drink (g)	4. Weight container/ leftovers	Leave Blank	
Breakfast	Cornflakes (Kelloggs)	28			
8:30am	Milk (Sainsbury's virtually fat-free)	48			
Home	Bread (Mothers Pride, large white	76			
	sliced, toasted)				
	Flora margarine	7			
	Robinsons lemon marmalade	12			
	Coffee (instant)	2			
	Milk (whole pasteurised)	10			
Lunch	Cheese (Cheddar)	55			
1:00pm	Bread (white, crusty)	76			
Pub	Butter	4			
	Chutney (2 teaspoons)				
Snack	Coffee (instant)	2			
3:30pm	Coffee-mate	6			
Office	Mars Bar	35			
	Apple	76	8 (core)		
Dinner	Turkey Fillet (frozen, grilled)	102			
6:30pm	Potatoes, old, boiled	320	74		
Home			(leftover)		
	Peas (Birds Eye, frozen, boiled)	50			
	Heinz tomato ketchup	14			
	Yoghurt (Ski strawberry thick and	162	10		
	creamy)		(carton)		
	Coffee, filter	148			
	Milk (Sainsbury's virtually fat-free)	8			
Snack	Banana	107			
7:45pm	Orange Tango (can)	330			
Home					

Food Inventory

Name_____ **Date** _____

1. Time/Place	2. Description of food/drink	3. Weight of food/drink (g)	4. Weight of container/ leftovers (g)	Leave Blank	