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INFLUENCE OF THE TYPE OF CARBOHYDRATE BREAKFASTS ON METABOLISM AND ENDURANCE RUNNING CAPACITY IN MAN

by

Shiou-Liang Wee

A Doctoral Thesis

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

September 1999

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Abstract

Compared to the overnight fasted state, a high carbohydrate (CHO) breakfast 3-4 hours before exercise enhances endurance performance. However, the optimal type or composition of the pre-exercise meal to be consumed is less clear. Glycaemic and insulinaemic responses to a meal play a key role in subsequent metabolism during exercise. The investigations described in this thesis focused on the influence of 1) the composition and 2) glycaemic index (GI) of CHO breakfasts 3 hours before exercise on postprandial and exercise metabolism and endurance running capacity.

To examine the influence of the GI of a meal on postprandial metabolism, isoenergetic high and low GI meals of the same macronutrient composition were given to 10 healthy young men and women after an overnight fast. Although the meals contained the same amount of fat and CHO, overall CHO oxidation was higher and fat oxidation lower after the high than the low GI meal during the 3-hour postprandial period. To further elucidate the major influence on postprandial metabolism, the fat and CHO content of meals were considered. A high fat-low CHO meal (FM), a high CHO-low fat meal (CM), or a high fat-high CHO meal (HM) were given to 6 endurance trained men (aged 20 to 57) after an overnight fast. The CM and FM were isoenergetic while HM contained additional energy, in the form of fat or CHO compared to CM and FM respectively. During the 3-hour postprandial period, overall CHO oxidation was lowest following the FM and not different after the CM and HM. Overall fat oxidation was greatest after the FM and least after the CM. These results suggest that the type and amount CHO in a meal is more important than fat in determining postprandial substrate oxidation.

The influence of the breakfasts on metabolic responses and endurance running capacity was also examined during treadmill running at 70 % $\dot{V}O_2\text{max}$. Endurance capacity of the 6 trained runners was poorer after the FM than after the CM despite lower CHO oxidation during the first 80 min of exercise in the former. Moreover, running time to exhaustion was longer in the HM trial compared to FM but not different from the CM trial. Also, overall substrate oxidation during the first 80 min of exercise in the HM trial resembled that of CM rather than FM trial. Therefore, dietary CHO rather than fat availability before exercise also determines substrate oxidation during exercise and endurance running capacity. In another study (5 male and 3 female active subjects), compared to a high GI meal, ingestion of a low GI meal before exercise resulted in a reduction in CHO oxidation and an increase in fat oxidation during 80 min of exercise. In terms of substrate oxidation, similar results were
obtained in another study (7 physically active men) during 30 min of running period after high and low GI meals constituted of other foods. In addition, muscle glycogen concentration increased by 15% 3-hour after a high GI meal but did not change after a low GI meal. However, muscle glycogen utilisation and lactate accumulation were lower during 30 min of running in the low than the high GI trial. Despite the difference in metabolism, running times to exhaustion were similar three hours following the high or low GI meals.

In summary, the type and amount of CHO in a meal appears to be the dominant influence on postprandial and exercise metabolism. Furthermore, the amount of CHO rather than fat in, but not the GI of, pre-exercise meals influences subsequent endurance running capacity at 70 % \( \dot{V}O_2 \text{max} \) in the subjects of these studies.
Preface

Unless otherwise indicated by acknowledgement or reference to published literature the work contained herein is that of the author.

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

**Published paper**


**Conference communications**


This thesis is dedicated to my granny and parents
As for man, his days are like grass, he flourishes like a flower of the field; the wind blows over it and it is gone, and its place remembers it no more. But from everlasting to everlasting the Lord’s love is with those who fear him, and his righteousness with their children’s children with those who keep his covenant and remember to obey his precepts. (Psalm 103:15-18)
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8.2 Serum insulin concentration just before exercise and plasma FFA concentrations just before and at the end of exhaustive exercise in FM, CM and HM trials
Chapter 1
Introduction

A number of factors influence the contribution of carbohydrate (CHO) and fat to energy production at rest and during exercise, one of which is nutritional status. The importance of muscle glycogen and blood glucose as substrates during prolonged strenuous exercise is well documented. Depletion of glycogen in exercising muscles and a reduction of blood glucose concentration are associated with the onset of fatigue. Therefore, the nutritional and metabolic challenge is to maintain CHO supply to the muscles but to delay its depletion by using as much fat as possible for muscle metabolism. The metabolic influence of CHO in the days or an hour before, and during exercise has been well studied. There has also been interest in the ways of increasing fat metabolism during exercise. For example, caffeine has been used to stimulate the mobilisation of fatty acids, high fat diets and medium chain triglyceride have been used as a mean the concentration of fatty acids in plasma. The influence of the type of CHO of meals 3-4 hours before exercise on metabolism and performance has received less attention.

Even in well-nourished people, in the morning after an overnight fast, the liver will be low in glycogen. Pre-exercise CHO ingestion can increase liver (Nilsson and Hultman, 1973) and muscle glycogen concentrations (Coyle et al., 1985; Taylor et al., 1993) before exercise as well as provide an absorbable source of CHO as it empties from the stomach during exercise (Conus et al., 1996). However, pre-exercise CHO ingestion also increases muscle glycogen utilisation (Costill et al., 1977; Coyle et al., 1985) and muscle glucose uptake (Conus et al., 1996) during exercise. Nevertheless, compared to the fasted state, ingestion of a CHO meal 3-4 hours before exercise enhances endurance performance, regardless of whether CHO is also ingested during exercise (Neufer et al., 1987; Sherman et al., 1989; Wright et al., 1991; Chryssanthopoulos & Williams, 1997).
Many studies of CHO intake before exercise have been undertaken using glucose, fructose or glucose polymers. Nevertheless, people consume food rather than single nutrients in liquid form, and so these studies are less applicable to those who are preparing for training and competition. In addition to the provision of energy, CHOs also have a wide range of physiological effects including control of blood glucose and insulin metabolism as well as cholesterol and triglyceride metabolism. Furthermore, food choice depends not only on nutrition and health consideration but also on socio-cultural context and lifestyle. Carbohydrate rich foods have diverse functional, metabolic, and nutritional characteristics, and so it is difficult to provide athletes and non-athletes alike with simple advice about food choices for both performance and health benefits.

Carbohydrate foods can be classified according to their chemical composition. However, the chemical nature of the CHO in foods cannot be used to reliably indicate their actual physiologic effects. The concept of glycaemic index (GI) was conceived by Jenkins and co-workers (1981). The index provides a classification of foods based on their actual effects on blood glucose concentration. Glycaemic index can be used to compare foods of similar composition within food groups. There is some evidence that a low GI diet may confer some health benefits compared to a high GI diet (Chapter 2, Section 2.2.6). However, low GI foods can be high in fat and high GI can have high energy and nutrient content. Therefore, both glycaemic index and food composition need to be considered when choosing CHO foods.

The primary purpose of this thesis was to examine the influence of the glycaemic index and composition of CHO breakfasts on postprandial and exercise metabolism and endurance running capacity in man. A total of 33 subjects were involved in four separate studies in which their metabolic responses at rest and during exercise were measured after consumption of different breakfasts.
This thesis is presented in eight chapters. Chapter 2 provides a summary of the available literature on the metabolic inter-relationships between CHO and fat as substrates during exercise. In addition, it also covers the influence of the type of CHO on metabolism with relevance to health, the metabolic causes of fatigue during prolonged submaximal exercise and the influence of pre-event nutritional manipulation on metabolism and endurance performance. Chapter 3 describes the general methodology employed in the studies reported in this thesis.

Chapter 4 examines the influence I) GI and II) CHO and fat content of breakfasts on postprandial substrate oxidation at rest. Part I compares postprandial metabolism after high and low GI meals of the same energy content and macronutrient composition. Part II compares postprandial metabolic responses after three different meals: a high fat-low CHO meal, a high CHO-high fat meal and a high CHO-low fat meal. Chapter 5 tests the hypothesis that CHO rather than fat content of pre-exercise breakfasts determines substrate utilisation and endurance capacity during running at 70 \% \dot{V}O_2\text{max}. Chapter 6 compares the effect of high and low GI CHO breakfasts, containing 2 g CHO·kg\(^{-1}\) body mass, ingested 3 hours before exercise on subsequent endurance running capacity at 70 \% \dot{V}O_2\text{max}. Chapter 7 examines whether the GI of high CHO breakfast (2.5 g CHO·kg\(^{-1}\) body mass) affects muscle glycogen storage during the three hour postprandial period, and muscle glycogen utilisation during subsequent running exercise. Finally, Chapter 8 summarises the main findings of these studies and discusses their implications.
Chapter 2
Literature Review

2.1 Introduction

Glucose is the primary metabolic substrate for the central nervous system. Therefore, it is not surprising that the maintenance of glucose homeostasis is precisely regulated. This regulation takes place in spite of the demands for glucose by a wide range of tissues and cells. Exercise presents a significant physiological challenge to the body and has major effects on skeletal muscle, liver and adipose tissue metabolism. Although glucose requirement of the resting human skeletal musculature is small, glucose uptake is dramatically increased in the postprandial period and during exercise. Fat is also an important substrate for energy production in contracting skeletal muscles. Therefore, the regulation of fatty acid mobilisation in adipose tissue cells during periods of increased energy expenditure is critical for the maintenance of fuel homeostasis. A number of factors influence the contribution of carbohydrate (CHO) and fat to energy production during exercise, one of which is nutritional status. There has been considerable interest among sport nutritionists in ways in which the onset of fatigue may be delayed by influencing the contribution of CHO and fat to energy production. Furthermore, knowledge concerning the biochemical regulation of fuel homeostasis is especially important in order to devise pharmacological and dietary strategies aimed at improving the abnormalities in CHO and lipid metabolism associated with obesity and non-insulin dependent diabetes mellitus (NIDDM).

This chapter provides a review of the available literature on the influence of CHO on postprandial and exercise metabolism as well as endurance performance during prolonged strenuous exercise. The four main sections provide overviews of:

(i) Interaction of CHO and fat metabolism during exercise (Section 2.2)
(ii) Influence of type of CHO on metabolism (Section 2.3)
(iii) The metabolic causes of fatigue during prolonged submaximal exercise (Section 2.4)
(iv) Influence of pre-event nutritional manipulation on metabolism and endurance performance (Section 2.5)

In order to avoid unnecessary overlap, section 2.2 will focus on the mechanistic aspects of fat and CHO interaction, section 2.3 will focus on nutritional studies with relevance to health and section 2.5 will focus on studies with relevance to endurance performance.
2.2 Interaction of CHO and fat metabolism in skeletal muscle

The reciprocal relationship between CHO and fat metabolism during exercise is well known. Increasing the availability of CHO during exercise will increase CHO utilisation and reduce fat oxidation (Costill, et al., 1977). Conversely, increasing the availability of fat during exercise will increase fat oxidation and reduce CHO oxidation in rats (Rennie, et al., 1976) and in humans (Costill, et al., 1977). At the level of the muscle cell, the mechanisms governing the relationship between CHO and fat metabolism have not been fully elucidated. The classic glucose-fatty acid cycle (G-FAC) concept (Randle, et al., 1963) and more recently a ‘reversed’ G-FAC hypothesis (Sidossis, et al., 1996) was proposed to try to explain the CHO-fat interaction.

The G-FAC concept was proposed by Randle et al. (1963, 1981) to explain how, in a hormone-independent manner, increased FFA availability may directly discriminate against the uptake and oxidation of glucose in the rat heart and diaphragm muscle. The proposed mechanism is represented schematically in Figure 2.1. Increasing plasma free fatty acid (FFA) concentration can increase its delivery across the muscle sacrolemma and into the mitochondria. When this occurs, β-oxidation is enhanced and the concentrations of acetyl-CoA and citrate will increase. It has been proposed that the increase in acetyl-CoA activates pyruvate dehydrogenase (PDH) kinase, which in turn phosphorylates and inhibits PDH. Furthermore, the increase in citrate concentration will inhibit phosphofructokinase (PFK), thus reducing glycolysis. This will lead to an increase in glucose-6-phosphate (G6P) concentration, which inhibits hexokinase (HK), resulting in decreased glucose uptake (Randle, 1981; 1986). Since the G-FAC was initially described, numerous in vitro and in vivo studies have been conducted to examine its role in different tissues and under various metabolic conditions by increasing the supply of FFA. While considerable evidence confirms the existence of G-FAC in the heart and diaphragm muscle, the results of research on its operation in skeletal muscle and its effect on total body glucose metabolism remain inconclusive (Rodgers and Vranic, 1998; Spriet and Odland, 1999).
Figure 2.1 Proposed mechanism of operation of the glucose-fatty acid cycle
(Modified from figure 12.1, pp. 128, Spriet & Dyck 1996)
Some of the inconsistency concerning the presence of G-FAC in the skeletal muscle may be due to the different muscles or dominant muscle fibre types examined and the oxidative status of the muscle (rest vs exercise) (Rodgers and Vranic, 1998). Prevailing insulin (Vaag, et al., 1994), and glucose (Walker, et al., 1991) concentrations, as well as the concentration (Boden, et al., 1994) and duration (Boden, et al., 1991; Bonadonna, et al., 1994) of elevated FFA exposure also play a significant role in determining whether the G-FAC is expressed. Just as evidence in support of the existence of has been inconsistent, so has the data describing the mechanisms by which the G-FAC is induced (Rodgers and Vranic, 1998).

More recently, Sidossis and Wolfe (1996) proposed another hypothesis of a 'reversed' G-FAC which emphasises the importance of circulating glucose rather than FFA in controlling the reciprocal interplay between CHO and fat metabolism. Studies in support of this hypothesis have used isotopic tracers to determine fatty acid oxidation under conditions of high and normal glucose concentrations. For example, glucose infusion or ingestion has been shown to inhibit isotopic tracer determined long chain fatty acid oxidation in healthy man at rest (Sidossis, et. al., 1996) and during exercise (Coyle et. al, 1997) even when fatty acid concentration is maintained. Furthermore, combined hyperglycaemia-hyperinsulinaemia has also been shown to inhibit fatty oxidation measured across the leg and the splanchnic region even when plasma fatty acid uptake in these regions are maintained (Sidossis, et al., 1998a; 1999).

Interactions between CHO and fat metabolism also occur in the adipose tissue and in the liver. The pancreas is also involved because of insulin secretion. Together with the skeletal muscle, these interactions between these tissues are important for a clear understanding of the control of circulating fatty acid and glucose concentrations. Insulin may indirectly influence FFA metabolism by inhibiting lipolysis (Nurjhan, et al., 1986) while maintaining a constant rate of primary FFA reesterification (Campbell, et al., 1992). Furthermore, glucose may also suppress lipolysis independent of insulin (Carlson, et al., 1991). Elevated
plasma free fatty acid (FFA) concentrations can increase hepatic glucose production (Boden and Jadali, 1991; Ferrannini, et al., 1983; Saloranta, et al., 1993), stimulating pancreatic insulin secretion (Stein, et al., 1997). In this way, elevated plasma FFA concentrations may also indirectly affect glucose metabolism and their own production. However, an extensive review of the topic of fat and CHO metabolism is not within the scope of this thesis. Therefore, the following section shall be limited to the review of the possible mechanisms governing the interaction of CHO-fat metabolism in the skeletal muscle during exercise.

2.2.1 Effect of increased fat availability on CHO metabolism

Muscle can utilise fatty acids derived from adipose tissue triglyceride, intramuscular triglyceride and circulating triglyceride in the forms of very low-density lipoproteins (VLDL) and chylomicrons. The quantitative contributions of intramuscular and circulating triglyceride to lipid metabolism during exercise are not entirely clear (Turcotte, et al., 1995). For example, investigators have assigned major (Martin, 1997) or minor (Saltin and Astrand, 1993) quantitative roles to intramuscular triglyceride as supply of fuel to active skeletal muscles during prolonged submaximal exercise. In contrast, it is well established that the albumin-bound FFA in circulation derived from the adipocytes contribute significantly to lipid metabolism in trained skeletal muscle during exercise (Wolfe, 1998). The flux generating step for plasma FFA oxidation in muscle is the hormone sensitive lipase in adipose tissue, so increasing plasma FFA concentration increases the rate of FFA oxidation in muscle and other tissues (Randle and Tubbs, 1979).

Different approaches have been used to increase fat utilisation in exercising humans. These include fasting (Zinker, et al., 1990), high-fat diets (Helge, et al., 1996; Jansson and Kaijser, 1984; Lambert, et al., 1994; Putman, et al., 1993), acute high-fat feeding (Costill, et al., 1977; Vukovich, et al., 1993), Intralipid infusion (Dyck, et al., 1993; 1996), caffeine ingestion (Erickson, et al., 1987; Essig, et al., 1980; Spriet, et al., 1992) and aerobic training (Coggan, et al., 1993; Jansson and Kaijser, 1987). More recently, there have been
investigations on the influence of co-ingestion of fat and CHO before and during exercise (Jeukendrup, et al., 1995; 1996a; 1996b; Van Zyl, et al., 1996) on energy metabolism.

Following 3-14 days on a high-fat diet, plasma FFA concentration is higher and rate of muscle glycogen utilisation during exercise at 65-75 % $\dot{V}O_2$ max is lower compared to a high-CHO or normal mixed diet (see Table 2.4). Under resting conditions, PDH activity ($PDH_a$) is influenced by the fat or CHO fuels acting through the acetyl-CoA-to-CoASH ratio (Putman, et al., 1993). However, during exercise the activation of $PDH_a$ has been shown to occur independent of changes in the acetyl-CoA-to-CoASH ratio (Putman, et al., 1993). Furthermore, muscle citrate concentration may (Jansson and Kaijser, 1984) or may not (Putman, et al., 1993) increase but glucose uptake by skeletal muscle (Jansson and Kaijser, 1982a;1982b) remains unchanged following the different diets. Therefore increasing plasma FFA concentration during exercise does not seem to decrease muscle glucose uptake as proposed by the G-FAC (Randle, 1981; 1986). On the other hand the rate of muscle glycogenolysis is reduced. However, it is not known if this is the direct effect of the higher plasma FFA concentration or the effect of altered hormonal concentration and muscle glycogen concentration as a result of changes in diet.

Several studies have tried to increase the availability of plasma FFA by a single fat meal or Intralipid infusion with heparin administration (Table 2.1). Compared to high fat diets, these methods can also serve to prevent confounding factors such as altered hormonal concentrations and CHO reserves. Such acute increase in FFA delivery to working muscle decrease CHO metabolism during running and cycling at exercise intensities equivalent to 65 to 85 % $\dot{V}O_2$ max. However, a shift in CHO metabolism is not observed when initial FFA level exceeds 0.5 mmol l$^{-1}$ (Hargreaves, et al., 1991; Ravussin, et al., 1986). It is possible that in these cases, increasing plasma FFA availability does not increase fat metabolism because a point of saturation of in the fatty acids’ transport system may have been reached. Nevertheless, the CHO sparing is due to reduced muscle glycogen utilisation (Dyck, et al., 1993; 1996; Odland, et al., 1998a) and not a reduction in muscle
glucose uptake during exercise (Odland, et al., 1998a; Ravussin, et al., 1986; Romijn, et al., 1995). Furthermore, when a glycogen sparing effect is observed, this is not related to a change in muscle acetyl-CoA or G6P concentrations (Dyck, et al., 1993; 1996; Odland, et al., 1998a). Therefore, these data strongly suggest that the interplay of CHO-fat metabolism during exercise is not regulated as proposed by the G-FAC hypothesis (Randle, 1981; 1986). The mechanism underlying the observed CHO-fat interaction may be dependent on the exercise intensity. This is supported by the observation that certain aspects of the classical hypothesis on the regulation of the fat-CHO interaction, which did not occur during exercise at 85 % \( \dot{V}O_2 \) max, was found to be present during exercise at 65 % \( \dot{V}O_2 \) max (Odland, et al., 1998b). Concentrations of muscle citrate and PDH\(_a\) activity were unchanged with elevated FFA concentrations during exercise at 85 % \( \dot{V}O_2 \) max (Dyck, et al., 1996), but increased and decreased respectively, with Intralipid infusion during exercise at 65 % \( \dot{V}O_2 \) max (Odland, et al., 1998a). An increase in total muscle citrate concentration may not necessarily reflect increased citrate concentration in the cytoplasmic compartment, where inhibition of PFK occurs. Nevertheless, observations from in vitro studies suggests that the small increase in total muscle citrate concentration (Odland, et al., 1998b) is not likely to influence PFK activity (Spriet, 1998). The mechanism behind the reduced PDH\(_a\) with increased fat oxidation remain to be elucidated.

Consistent with the observation that increasing fat oxidation reduces muscle glycogenolysis but not glucose uptake, it has been suggested that the regulatory site of fat-CHO interaction in the skeletal muscle may be at the level of glycogen phosphorylase (PHOS) (Spriet, 1998). Muscle glycogenolysis during contraction is a function of the transformation of PHOS \( b \) to \( a \) induced by Ca\(^{2+}\) release (Chasiotis, 1983). However, the transformation of PHOS \( b \) to \( a \) alone is not sufficient to bring about rapid glycogen degradation. A lack of Pi as substrate for the phosphorylation and low free AMP (allosteric regulator) concentration may also limit the action of PHOS (Ren and Hultman, 1990). Therefore, the rate of muscle glycogenolysis is controlled primarily by PHOS \( a \) and that accumulation of Pi and AMP are required for catalytic activity (Chasiotis, et al., 1983;
Ren and Hultman, 1990). In a recent study, 11 subjects received saline or Intralipid infusion during 30 min rest and 15 min cycling at 85 %\( \dot{V}O_{2\text{max}} \) (Dyck, et al., 1996). Reduced muscle glycogen breakdown (-46.7%) was observed in the Intralipid trial in 7 of these subjects (Dyck, et al., 1996). In these subjects, PHOS transformation into the \( a \) form was unaffected by high FFA concentration except for a non-significant reduction during the initial 5 min of exercise (Dyck, et al., 1996). However, when data with a previous study (6 subjects) (Dyck, et al., 1993) were pooled, the free ADP, AMP and Pi contents of subjects who spared glycogen were significantly lower at 15 min in Intralipid trial. It is suggested that the reduced glycogenolytic flux following Intralipid infusion is related to dampened increase in muscle free ADP, AMP and Pi and not reduced transformation of PHOS from \( b \) to \( a \) form (Dyck, et al., 1996; Spriet, 1998). Until the availability of more experiment evidence, the mechanism behind the observed reduction in muscle glycogenolysis in the face of increased fat metabolism remain largely unexplained.
<table>
<thead>
<tr>
<th>Study</th>
<th>Exercise</th>
<th>Initial [FAT]* (mmol·L⁻¹)</th>
<th>RQ or RER</th>
<th>muscle glycogen utilisation rate*</th>
<th>Glycogen PHOS activity</th>
<th>calculated free AMP</th>
<th>glucose uptake*</th>
<th>[citrate]</th>
<th>[PDH]</th>
<th>acetyl CoA to CoASH ratio</th>
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<tbody>
<tr>
<td>Costill et al, 1977</td>
<td>30 min TM (70% VO₂ max)</td>
<td>1.01 vs 0.21</td>
<td>↓</td>
<td>-40%</td>
<td>-</td>
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<tr>
<td>Ravussin et al, 1986</td>
<td>2.5 h cycle (44% VO₂ max)</td>
<td>1.12 vs 0.78</td>
<td>↓ 1st 30 mins</td>
<td>-</td>
<td>-</td>
<td>n.s.</td>
<td>n.s.</td>
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<tr>
<td>Hargreaves et al, 1991</td>
<td>1 h knee extensor (80% MWC)</td>
<td>1.1 vs 0.6</td>
<td>n.s.</td>
<td>n.s.</td>
<td>-</td>
<td>-</td>
<td>-33%</td>
<td>n.s.</td>
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<tr>
<td>Vukovich et al, 1993</td>
<td>1 h cycle (70% VO₂ max)</td>
<td>1.3 vs 0.4</td>
<td>↓</td>
<td>-28%</td>
<td>-</td>
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<tr>
<td>Dyck et al, 1993</td>
<td>15 min cycle (85% VO₂ max)</td>
<td>0.94 vs 0.21</td>
<td>↓</td>
<td>-44%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Romijn et al, 1995</td>
<td>30 min cycle (80% VO₂ max)</td>
<td>2.1 vs 0.3</td>
<td>↓</td>
<td>-15%</td>
<td>-</td>
<td>-</td>
<td>n.s.</td>
<td>-</td>
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<tr>
<td>Dyck et al, 1996</td>
<td>15 min cycle (85% VO₂ max)</td>
<td>1.00 vs 0.36</td>
<td>↓</td>
<td>-47% (in 7 of 11 subjects)</td>
<td>-</td>
<td>↓</td>
<td>-</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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<tr>
<td>Oland et al, 1998</td>
<td>1 h cycle (65% VO₂ max)</td>
<td>0.69 vs 0.25</td>
<td>↓</td>
<td>-23%</td>
<td>↓</td>
<td>↓</td>
<td>n.s.</td>
<td>↑</td>
<td>↓</td>
<td>n.s.</td>
</tr>
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*fat compared with control trial

'TM' denotes treadmill running and 'cycle' denotes cycle ergometer exercise.
2.2.2 Effect of increased CHO flux on fat metabolism

Glycogenolysis is reduced and fat metabolism increased during prolonged submaximal exercise when muscle glycogen concentration is low (Blomstrand and Saltin, 1999; Gollnick, et al., 1972; 1981; Hargreaves, et al., 1995; Van Hall, et al., 1995b; Weltan, et al., 1998). The picture is less clear concerning the effect of different concentration of pre-exercise muscle glycogen on glucose uptake. Gollnick et al (1981) and Blomstrand et al (1999) observed a greater glucose extraction in the leg that commenced exercise with low glycogen concentration as compared with the leg that started exercise with normal muscle glycogen stores. Whereas in another study, Hargreaves et. al. (1995) reported no effect of muscle glycogen concentration on glucose uptake.

The release of FFA from adipocytes appears to depend on regional blood flow and so may be reduced at high exercise intensities when there is a peripheral shift of central blood volume. The rate of appearance of FFA in the plasma was lower at an exercise intensity of 85 % $\dot{V}O_2$ max than at 65 % $\dot{V}O_2$ max (Romijn, et al., 1993; 1995). Furthermore, after exercise at the higher intensity, there was a marked increase in the rate of appearance of FFA, suggesting that FFA was trapped in the adipose tissue during the period of heavy exercise (Romijn, et al., 1993). When plasma FFA concentration was artificially increased during exercise at 85 % $\dot{V}O_2$ max, fat oxidation also increased (Romijn et al, 1995). However the increase in fat oxidation did not equal the rate at which physiological concentrations of plasma FFA were oxidised at 65 % $\dot{V}O_2$max (Romijn et al, 1993; 1995).

In a more recent study, pre-exercise glucose ingestion reduced FFA oxidation during exercise at 44 % $\dot{V}O_2$ max (Horowitz, et al., 1997). Combined with the same pre-exercise glucose ingestion, artificial elevation of circulating FFA increased fat oxidation. However, the increase in fat oxidation was still lower than that during exercise in the fasted state (Horowitz, et al., 1997). It seems that the various systems of the body coordinate to minimise the appearance of fatty acids that cannot be oxidised by skeletal muscle during exercise. These observations also suggest that fat oxidation is not merely limited by supply
of fatty acids. There seems to be other mechanism at work which depress fat oxidation rates during exercise after glucose ingestion or during heavy exercise.

In addition to the mobilisation of fatty acids, their transport and subsequent oxidation are determined by their chain length. Long chain fatty acids (LCFA) such as oleate and palmitate, are transported in chylomicrons through the lymphatic system while medium chain fatty acids (MCFA) such as octanoate can enter the blood stream directly through the portal system. Passive constraints on fatty acid utilisation include their transport from the plasma to mitochondria. This includes the (i) transport through the endothelium lining of the micro-vascular compartment, (ii) across the interstitial space; (iii) trans-saccosomal transport; (iv) transport of the fatty acids in the cytosol of the muscle cell; and finally (v) uptake and oxidation by the mitochondria. Unlike LCFA, the MCFA can cross the inner mitochondrial membrane in the muscle and liver independent of the acylcarnitine transferase system (Bremer, 1983).

Glycolytic flux during exercise may be increased by pre-exercise glucose ingestion (Coyle, et al., 1997) or increased workrate (Sidossis, et al., 1997). Using these means, the oxidation rates of plasma LCFA and MCFA can be compared under the influence of high and low glycolytic flux (Coyle, et al., 1997; Sidossis, et al., 1997). Sidossis et al. (1997) studied healthy subjects exercising at 40 and 80 % \( \dot{V}O_2 \)max during which small quantities of isotopically labelled LCFA (oleate) and MCFA (octanoate) were infused in order to determine their respective oxidation rates. Plasma FFA availability is lower during exercise at 80 than 40 % \( \dot{V}O_2 \)max, and so subjects also received Intrapid and heparin infusion during exercise at 80 % \( \dot{V}O_2 \)max so that plasma FFA concentrations were similar during exercise at both exercise intensities. The percentage of the LCFA taken up and oxidised decreased from 68 to 52% whereas the percentage of the MCFA oxidised was similar (85% vs 89%) during exercise at 40 and 80 % \( \dot{V}O_2 \)max respectively. Inhibition of fatty acid oxidation could not have been via the inhibition of \( \beta \)-oxidation because unlike the LCFA, oxidation of the MCFA increased slightly during exercise at 80 % \( \dot{V}O_2 \)max
(Sidossis, et al., 1997). In another study, pre-exercise glucose ingestion had no effect on the oxidation of a MCFA (octanoate) during exercise at 50 % $\dot{V}O_2$ max but oxidation of a LCFA (palmitate) was reduced (Coyle, et al., 1997). Octanoate (MCFA) oxidation is not limited by transport into mitochondria as is oleate or palmitate (both LCFA) (Bremer, 1983), and so these results suggest that fatty acid oxidation is limited during increased glycolytic flux because of direct inhibition of LCFA entry into the mitochondria (Coyle, et al., 1997; Sidossis, et al., 1997). In another recent study, the concentration of LCFA was measured directly in muscle samples (Kiens, et al., 1999). The intramuscular cellular concentration of LCFA was then estimated by deducting the calculated extracellular content from the measured muscle LCFA concentration (Kiens, et al., 1999). Both muscle and cellular LCFA concentrations decreased from rest after exercise at 65 % $\dot{V}O_2$ max and subsequently increased after exercise at 90 % $\dot{V}O_2$ max (Kiens, et al., 1999). This provides further support that the reduction of LCFA oxidation during high-intensity exercise is due to decrease in mitochondrial oxidation rather than an insufficient cellular availability (Kiens, et al., 1999).

Since increasing glycolytic flux reduces the oxidation of LCFA but not MCFA, it has been suggested that the limitation may be related to carnitine mediated entry of LCFA into the mitochondria (Sidossis, 1998; Coyle, 1999). Long chain fatty acids, for example palmitate, must bind to carnitine, a reaction catalysed by carnitine palmitoyl-transferase I (CPT-I) located on the inner surface of the outer mitochondrial membrane, to enter the mitochondria matrix (Fritz, 1959). The carnitine acyl-transferase system for the transport of a LCFA into the mitochondria is represented schematically in Figure 2.2 Increased pyruvate availability has been shown to increase malonyl-CoA formation via the acetyl-CoA carboxylase reaction (McGarry, et al., 1977; 1983):

$$\text{Acetyl-CoA} + \text{ATP} + \text{CO}_2 \xrightarrow{\text{ACC}} \text{Malonyl-CoA} + \text{ADP} + \text{Pi}$$
Figure 2.2 Carnitine acyl-transferase system for the transport of a LCFA into the mitochondria
(Modified from figure 14.2, pp.174, Winder, 1996)
In turn, malonyl-CoA inhibits CPT-I (McGarry, et al., 1977) but not carnitine octanoate-transferase (Saggerson, et al., 1992; Saggerson and Carpenter, 1981). In isolated rat hearts, increased glucose-derived acetyl-CoA concentrations have been reported to increase malonyl-CoA concentration leading to the inhibition of fatty acid oxidation (Saddik, et al., 1993). As high concentrations of malonyl-CoA in the cytosol can limit the transport of LCFA transport into the mitochondria, it has been proposed as a metabolic regulator in skeletal muscle (Winder, 1996). To explain the observed discrimination against LCFA metabolism (Coyle, et al., 1997; Sidossis, et al., 1997), the respective authors proposed that increased glycolytic flux, pyruvate formation and glucose derived acetyl-CoA lead to higher malonyl-CoA concentrations, which then reduce fat oxidation by inhibition of CPT-I and hence LCFA transport into the mitochondria (Coyle, 1999; Sidossis, 1998). However, malonyl-CoA and CPT-I activity were not measured in these studies (Coyle, et al., 1997; Sidossis, et al., 1997), and therefore this hypothesis remain to be confirmed. Berthon and co-workers (1998) reported that CPT-I activity in intact human mitochondria is higher in trained than sedentary subjects, and correlates with citrate synthase activity and \( \dot{V}O_2 \) max of subjects. This provides some support for the suggestion that the difference in fat oxidation between trained and untrained individuals at the same absolute exercise intensity may be explained by enhanced LCFA entry into the mitochondria in trained people (Sidossis, et al., 1998b). An alternative to this hypothesis is that accumulation of acetylcarnitine at the onset of exercise may impair subsequent LCFA transport into the mitochondria by reducing free carnitine availability. However, measurement of acetyl group concentrations revealed that this is probably not the case (Constantin-Teodosiu, et al., 1992). Constantin-Teodosiu and co-workers (1992) reported that at the end of exhaustive exercise, when muscle glycogen stores were depleted and the rate of fat oxidation was high, the acetyl group content in the cell remained the same as at the onset of exercise.

Some investigators have measured the concentration of malonyl-CoA in rat and human muscle. In rats, as with fat oxidation rate, skeletal muscle malonyl-CoA decreased with
prolonged exercise (Winder, et al., 1989). Moreover, this decrease in malonyl-CoA was attenuated by glucose infusion (Duan and Winder, 1993). However, human skeletal muscle malonyl-CoA concentration did not decrease during prolonged sub-maximal exercise at 35-40 and 65 % VO₂ max when fat oxidation increased (Odland, et al., 1996; 1998b). Furthermore, malonyl-CoA content did not increase during exercise at 90 % VO₂ max and hence could not contribute to the lower rate of fat oxidation at this power output (Odland, et al., 1998b). In this study, muscle acetyl-CoA was the same at rest and during exercise at 35 % VO₂ max but was elevated above rest after 10 min of exercise at 65 and 90 % VO₂ max (Odland, et al., 1998b). Therefore, these results do not seem to support that there is a correlation between exercise-induced decreases malonyl-CoA content and increases in fatty acid transport and oxidation. However, the possibility of malonyl-CoA induced inhibition of CPT-I cannot be dismissed because it is possible that overall measurement of tissue malonyl-CoA in these studies does not reflect the concentration of malonyl-CoA that interacts with CPT-I (Spriet and Odland, 1999). The concentration of malonyl-CoA required for 50% in vitro inhibition of human muscle CPT-I is 0.025μM (McGarry, et al., 1983). Although this is much lower than the 0.2 to 0.9 μM measured by Odland and co-workers (1996; 1998), the distribution of malonyl-CoA between the mitochondria and cytoplasm in the skeletal muscle is uncertain (Spriet and Odland, 1999). Furthermore, other regulators such as acetyl-CoA, free CoA and acetylcarnitine may affect the sensitivity of CPT-I inhibition by malonyl-CoA in vivo (Spriet and Odland, 1999). Hence, although the hypothesis proposed by Sidossis (1998) and Coyle (1999) seems to explain the reduction in long chain but not medium chain fatty acid oxidation (Sidossis et al 1997; Coyle 1997), there is as yet no definitive evidence to support it.
2.3 Influence of nature of CHO on metabolism

Carbohydrates are the single most important source of food energy in the world, comprising of 40 to 80 percent of total food energy intake. Apart from provision of energy, CHO have a wide range of physiological effects including control of blood glucose and insulin metabolism as well as cholesterol and triglyceride metabolism. Relationships between dietary fat intake and diseases such as coronary heart disease (CHD), non-insulin dependent diabetes mellitus (NIDDM) and obesity have been well researched; however this is not the case with dietary CHO. Following CHO ingestion, there are significant hormonal and metabolic changes. In the postprandial period, insulin prevents hyperglycaemia by promoting glucose uptake and its conversion into glycogen and restraining glucose release from the liver. Postprandial blood glucose and insulin play a key role in subsequent energy metabolism. The nature of dietary CHO and its subsequent metabolism, especially the glycaemic index is the focus of the following section of this literature review.

2.3.1 The Glycaemic Index

For nutritional purposes, CHO has been classified according to its chemical structure and composition, digestibility or glycaemic index (Asp, 1995; Cummings, et al., 1995). Starches are the major storage polysaccharide in most higher plants consisting of two forms: amylose (20-30%) and amylopectin (70-80%) depending on the branching of the alpha bonds linking C₆H₁₀O₅ units. Glycogen or storage starch in animals, is similar in structure to amylopectin but is more highly branched and consists of shorter (C₆H₁₀O₅)ₙ units (n=12-18). Digestion of starch involves enzyme controlled hydrolysis into simpler molecules, ultimately becoming monosaccharides, which are then transported across the intestinal epithelium into the blood.

It has been shown that complex CHO results in lower glucose and insulin responses than equivalent amount of glucose (Crapo, et al., 1976; 1977), indicating that despite adequate amylase, the process of starch digestion takes place more slowly. However, treatment of
starch by gastrointestinal digestive and absorptive processes is more dependent on physical form rather than degree of polymerisation (Wahlqvist, et al., 1978; Wong and O’Dea, 1983). Jenkins and co-workers (1981) measured the blood glucose responses of healthy subjects after extensive feedings of commonly eaten foods containing 50g of CHO. There were significant differences in the glycaemic and insulinaemic responses to different complex CHO. Foods like white bread and potatoes elicited much higher glucose and insulin responses than foods like pasta and legumes (Jenkins, et al., 1981). A glycaemic index (GI) of foods was then proposed as a system of CHO classification (Jenkins, et al, 1981) and has since been used for some time in clinical nutrition and more recently in healthy, active population.

The GI is determined by measuring blood glucose response of subjects, in the fasted state, after the ingestion of a portion of food containing 50g of CHO. The area under the blood glucose curve over 2 hours is expressed as a percentage of the mean response to glucose or white bread (GI=100) containing an equivalent amount of CHO (Wolever, 1990; Wolever, et al., 1991). Wolever (1990) summarised three different methods used by different groups to calculate the area under the glucose curve, each giving different results for the same blood glucose data. The “incremental area” method ignores any area below the fasting blood glucose level (Wolever and Jenkins, 1986) while the “net incremental area” method subtracts the area below the fasting level from the area above it (Bantle, et al., 1983). Large differences are found between these two methods of calculations for normal subjects because it is common for blood glucose values to fall below fasting level following the ingestion of a large CHO load. The “total area” method considers the area under the blood glucose curve and above a blood glucose value of zero (Coulston, et al., 1987). This method is the least sensitive among the three for detection of blood glucose changes and it is also largely dependent upon the fasting glucose values. The use of GI has been criticised for the lack of agreement between different research laboratories and large individual variation in response mainly due to the different methods of calculation and use of both healthy and diabetic people respectively (Hollenbeck, et al., 1986).
Since then, the database on glycaemic responses of both healthy individuals and diabetic patients to different foods have been on the increase. Most of the laboratories have calculated GI using the 'incremental area' method (Wolever and Jenkins, 1986). From these, a comprehensive list of the glycaemic index values of about 500 different foods has been published (Foster-Powell and Brand Miller, 1995). Foods have been classified into those with high (GI > 85), moderate (60 < GI < 85) and low (GI < 60) glycaemic index (Coyle, 1992). Although the most common application of GI is in the area of clinical nutrition for glycaemic control (Brand-Miller, 1994; Smith, 1994), there is some recent interest in its application to sports performance (see section 4 of this review), appetite (Leathwood and Pollet, 1988) and its effects on energy balance (Flatt, 1995; Jequier, 1994). Low GI foods have aided in lowering serum cholesterol and triglycerides among both diabetics and non-diabetics, especially those with elevated triglyceride concentrations (Jenkins, et al., 1987). Furthermore, they are also more effective, per unit of energy, than most other foods in inducing satiety (Leathwood and Pollet, 1988).

2.3.2 Factors Affecting Glycaemic Responses

Variation of individual glycaemic response is subjected to the interaction of many factors. The GI is a function of the gastric emptying time and physical availability of the sugar or starch to hydrolytic enzymes. The former is influenced by coingestion of liquid (Hofman, et al., 1995; Noakes, et al., 1991), fat (Collier and O'Dea, 1983) and meal volume (Young and Wolever, 1998). For liquid feedings of equal volume, a more concentrated solution is found to induce a lower glucose response due to a lower gastric emptying rate (Hofman, et al., 1995). The latter is influenced by food structure, content and type of dietary fibre, method of cooking (Bjorck, et al., 1994; Thorne, et al., 1983) and amylose/amylopectin composition (Brand-Miller, et al., 1992). Amylopectin, being a much larger molecule (molecular weight $10^5$ to $10^6$), is more readily hydrolysed than amylose (molecular weight $10^4$). Differences in amylose to amylopectin content of foods is a major reason for differences in glycaemic responses to normal foods (Juliano and Goddard, 1986).
Legumes, notable for their uniformly low glycaemic responses they produced, contain 30 to 40% amylose and 60 to 70% amylopectin while most other CHO contain 25 to 30% amylose and 70 to 75% amylopectin (Thorne, et al., 1983). Legumes also have high viscous fibre and anti-nutrient content (Wong, et al., 1985). Viscous fibre reduces the rate of diffusion of glucose in the lumen of the small intestine (Jenkins, et al., 1986) and reduces the transit rate in small intestine (Blackburn, et al., 1984) and may lower glycaemic responses. However, insoluble fibre has no such effect and there is no difference between glycaemic response to white and wholemeal bread, white and brown spaghetti and white and brown rice (Jenkins, et al., 1983). Anti-nutrients are food components which, in large amounts, reduce the bioavailability of nutrients in foods by inhibiting the enzyme amylase (Wolever, 1990). The GI of foods is closely related to their content of anti-nutrients such as lectins (Rea, et al., 1985), phytate and polyphenols (Thompson, et al., 1984).

Cooking or food processing affects the postprandial glycaemic responses to starchy foods. During cooking, starches undergo disruption and swelling known as gelatinisation (Cummings & Englyst, 1995). On cooling, gelatinised starch recrystallises, a change known as retrogradation. Retrograded amylose is more resistant to enzymatic hydrolysis (Cummings & Englyst, 1995). Different processing of rice (Panlasigui, et al., 1991) or wheat (Ross, et al., 1987) of similar amylose contents results in different physiochemical (gelatinisation) properties which in turn influence starch digestibility and glycaemic response. Modern methods of food processing like grinding, flaking and popping and increased gelatinisation have been associated with increased glycaemic responses compared to more traditional methods like parboiling of wheat and rice and use of whole grains in rye breads (Wolever, 1990).

The addition of fat and protein will also affect the glycaemic response to foods. Protein stimulates insulin secretion (Rabinowitz, et al., 1966). Fat delays gastric emptying by
increasing the viscosity of the effluent through gastrointestinal tract and interferes with the action of digestive enzymes on starch (Collier and O'Dea, 1983; Welch, et al., 1987). Addition of both fat and protein slightly reduce the glycaemic response of a HGI food like potato (Gulliford, et al., 1989). However, the effect may be quantitatively smaller in foods with lower GI values (Collier and O'Dea, 1983; Gulliford, et al., 1989). Addition of protein greatly increased the insulin response to potato and spaghetti (Gulliford, et al., 1989) and a liquid glucose polymer drink (Spiller, et al., 1987). However, there is no evidence that if the same amount of fat and protein is added to foods with different glycaemic responses, the fat and protein affect the glycaemic responses to the foods differently so that the difference between their glycaemic response is abolished (Wolever, 1990). Wolever & Jenkins (1986) proposed a method of calculating the GI of mixed meals which is as follows:

\[
\text{meal GI} = \text{GI}_a (g_a/g) + \text{GI}_b (g_b/g) + \text{GI}_c (g_c/g)
\]

\[g = g_a + g_b + g_c\]

where \( a, b \) and \( c \) represents individual foods and total meal CHO, \( g = g_a + g_b + g_c \). Retrospective analyses of published data using this method showed that the observed glycaemic response of mixed meals and whole diets are proportional to the predicted GI if the meals are equivalent in proportion of fat, protein and CHO (Wolever, 1990).

In addition, a protein-starch complex may be digested more slowly than starch alone. Considerable amounts of ingested CHO may escape digestion in the small intestine and are available for microbial fermentation in the colon. Because colonic fermentation is the only source of breath hydrogen, hydrogen measured in breath can give an estimate of the amount of undigested CHO. Breadth hydrogen studies have shown that removal of the protein found in wheat resulted in a reduction in CHO malabsorbed from an estimated 10 to 20% found with white bread to zero found in gluten-free bread (Anderson, et al.,

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Legumes contain twice as much protein as cereals and legume protein isolates have been found to contain tightly bound CHO (Alii and Baker, 1980).

The relative importance of the various factors outlined above for the consistently low GI of legumes is still not known (Thorne, et al., 1983; Wolever, 1990). Trout et al (1993) examined the associations between GI and chemical components of 18 starchy foods by regression analysis. It appears that the method of preparing foods and the characteristics of starch and starch granules are more important in predicting GI among legumes, grains and tuber than is the content of fat, protein, total dietary fibre and phytate (Trout, et al., 1993).

It has also been suggested that glycaemic control could be improved when a CHO load is eaten slowly as opposed to being gorged (Jenkins, 1997). In a study to assess the effect of spreading CHO load over time, 50g of glucose solution were either taken as a single bolus or sipped at an even rate over 210 min (Jenkins, et al., 1990). A greater than 50% reduction in insulin area was observed in the 4-hour postprandial period in the non-diabetic subjects when the solution was sipped.
2.3.3 Thermic Effect of Food (TEF)

The thermic effect of food (TEF), previously called the specific dynamic action of food, refers to the elevated metabolic rate due to food consumption and accounts for about 10% of the total energy expenditure in sedentary individuals (Poehlman, 1989). The thermic effect of macronutrients is 0-3% for fat, 5-10% for CHO and 20-30% for protein (Tappy, 1996). In adult humans, the TEF is related to the stimulation of energy-requiring processes during the postprandial period. The intestinal absorption of nutrients, the initial steps of their metabolism and the storage of the absorbed, but not immediately oxidised nutrients, all require ATP hydrolysis. The TEF can be divided into obligatory and facultative components (Acheson, et al., 1984). The former accounts for energy requirements for digestion, absorption, resynthesis and storage of substrates. Antagonists of the beta-adrenoreceptors decrease the thermic effect of glucose. The part of glucose induced thermogenesis which is eliminated by beta-adrenergic antagonists has been called 'facultative thermogenesis' and takes place, at least in part, in skeletal muscle. Insulin-induced stimulation of muscle sympathetic nerve activity which releases catecholamines to the β1-adrenergic receptors may be involved in this facultative thermogenesis (Acheson, et al., 1983). Physically active people are reported to have higher and obese and insulin resistant people lower TEF (Tappy, 1996; Tataranni, et al., 1995).

2.3.4 Metabolic Disposal of Carbohydrates

The amount of glucose in a typical breakfast (~100 g) has the potential to raise the plasma glucose concentration about 8-fold. In normal healthy people, however, peak plasma glucose concentration after such a meal does not exceed 7-8 mmol·l⁻¹ from a fasting value of 5 mmol·l⁻¹. By the end of the absorptive period (5-6 hours), part of the dietary CHO would have been oxidised in various tissues and the rest stored as glycogen. Exercise and other factors like illness and stress can influence the deposition of CHO. Carbohydrate uptake and lipid synthesis also occurs in the liver and adipose tissues. In the postprandial state, only about 10g of glucose is oxidised per hour (Flatt, 1995). The CHO load is mainly disposed of by converting the absorbed glucose into liver and muscle glycogen.
(Nilsson and Hultman, 1974). The liver glycogen is very sensitive to diet. One day of a CHO poor diet has been shown to reduce the liver glycogen by ~80% whilst a CHO rich diet can double the liver glycogen (Nilsson and Hultman, 1973). Two studies have directly quantified muscle glycogen storage following a high CHO meal in non-glycogen depleted and overnight fasted subjects and reported increase ranging from 10-20% in 3-5 hours (Chryssanthopoulos, et al., 1998; Taylor, et al., 1993) (see Section 2.4.3). Since protein stimulates insulin secretion, a protein-CHO complex like baked potato with tuna may increase the rate of muscle glycogen storage by increasing clearance of glucose into muscles (Hofman et al., 1995). It is suggested that after a typical meal supplying 50-150g CHO, one-quarter to one-third of the CHO is converted to liver glycogen and one-third to one-half to muscle glycogen and the rest is oxidised during the postprandial hours (Flatt, 1995).

Glucose enters the muscle cell by facilitated diffusion. Of the facilitative glucose transporters, the GLUT-4 isoform is responsible for contraction and insulin stimulated glucose transport (Mueckler, 1994). Stimulation of muscle glucose transport by insulin and contraction is mediated by translocation of the GLUT-4 isoform of the glucose transporter from intracellular sites into the plasma membrane (Wardzala and Jeanrenaud, 1981; Cartee, et al., 1989; Goodyear, et al., 1991). Upon entering the muscle cell, glucose is rapid converted to glucose-6-phosphate (G-6-P) by hexokinase. The G-6-P is then converted to glucose-1-phosphate which combines with uridine triphosphate to form uridine diphosphate-glucose (UDP-glucose). The glucose is then transferred from UDP-glucose (catalysed by glycogen synthase) to an amylose chain for glycogen formation (Danforth, 1965). Glycogen synthase (GS) activity was once thought to limit the rate of muscle glycogen formation (Ivy, 1991). More recent evidence suggests that glucose transport is the rate limiting step (Ren, et al., 1993; 1994; Tsao, et al., 1996). The increase in circulating insulin following CHO ingestion not only increases muscle glucose uptake but also keep glycogen synthase activity high independent of glycogen concentration. Therefore ingesting foods that elicit high glucose and insulin response may result in
greater muscle glycogen storage compared to low GI foods. Indeed, glucose and sucrose (high GI) resulted in greater muscle glycogen storage during 6 hour of post-exercise recovery than equal amounts of fructose (low GI) (Blom, et al., 1987). Other investigators also reported greater muscle glycogen during 12 (Joszi, et al., 1996) or 24 (Burke, et al., 1993) hour recovery from exercise, when subjects consumed CHO based on high GI foods or drinks compared to an identical amount of CHO based on low GI foods or drinks. Furthermore, data from a study of chronic exposure (4 weeks) to high and low GI diets also observations of increased muscle glycogen storage when on a high than low GI diet (Kiens & Ritcher, 1996). However, it is not clear if the differences in glycogen storage can be explained by the different glucose and insulin responses. When a ‘nibbling’ as opposed to a ‘gorging’ eating pattern is adopted, glucose and insulin responses can be reduced even when on a high GI diet (Jenkins, et al., 1989). Burke and co-workers (1996) reported that despite marked differences in the glucose and insulin responses over 24 hour recovery period, muscle glycogen storage was similar between ‘nibbling’ and ‘gorging’ trials.

Hepatic glycogen synthesis after a glucose load occurs as a result of a combination of the ‘direct pathway’ (glucose uptake; G-6-P formation; and glycogen synthesis) and the ‘indirect pathway’ (uptake of 3-carbon gluconeogenic substrates, particularly lactate; formation of G-6-P; and glycogen synthesis). The flux-generating steps for gluconeogenesis are the release of alanine, glycerol and lactate from extra-hepatic tissues. Increased concentrations of lactate and alanine will raise the concentration of pyruvate in the liver mitochondria thereby increasing the activity of pyruvate carboxylase and, in turn, by internal regulation, the activities of the other enzymes of the pathway. Elevated blood lactate concentration is often observed during the initial postprandial period after a high CHO load (Chryssanthopoulos, 1995; Radziuk, 1989a, 1989b; Segal, et al., 1990). The lactate may be converted glycogen in the liver (Katz and McGarry, 1984; Radziuk, 1989b). Using an isotopic approach, Radziuk (1989a, 1989b) examined hepatic glycogen formation in humans after 100g oral and intravenous glucose load. There was no
difference between the oral or intravenous route of delivery. A total of 25g of the glucose load contributed to liver glycogen formation in the overnight fasted subjects (Radziuk, 1989a, 1989b). The direct pathway was estimated to contribute 10g (40%) (Radziuk, 1989a) and the indirect pathway 15g (60%) (Radziuk, 1989b). However, the contribution of the direct pathway increased to 70% when an oral glucose load is taken in the fed state (Shulman, et al., 1990).

Lipogenesis from CHO is insignificant in adult humans consuming a normal mixed diet (Flatt, 1995; Sjostrom, 1972). Even after the ingestion of 500g CHO, the non-protein respiratory quotient does not rise above 1.0, which indicates that fat are being synthesized at a rate which exceeds the concomitant rate of fat oxidation (Acheson, et al., 1982). Deliberate and sustained over-consumption of large amounts of CHO to achieve large increase in glycogen stores (Acheson, et al., 1988) or greater than 2 times energy expenditure (Aarsland, et al., 1997) is required to induce significant lipogenesis. Both the liver and adipose tissue contain the necessary enzymes for the conversion of CHO into fat and this process is stimulated by insulin (Newsholme and Leech, 1983). The relative contribution of the liver and adipose tissue in converting surplus CHO into fat remains a controversial issue. Earlier studies have assigned either a minor (Sjostrom, 1972) or moderate (Chascione, et al., 1987) lipogenic potential to the adipose tissue. However, a more recent study has concluded that the adipose tissue is the main site for fat synthesis from surplus CHO with minor hepatic contribution (Aarsland, et al., 1997).

### 2.3.5 Influence of GI and dietary fat on postprandial metabolism

The GI of CHO foods have been shown to influence postprandial glucose disposal and this effect may be time dependent (Ritz, et al., 1991; Tappy, et al., 1986; Wursch, et al., 1988). When bean and potato flake meals of similar CHO, fat and protein content were consumed, the increase in energy expenditure and glucose oxidation was significantly higher for the potato meal during the first 2-3 postprandial hours. This has been attributed to the blunted glucose and insulin responses after the bean compared to the potato meal.
(Tappy, et al., 1986; Wursch, et al., 1988). In another study, resting metabolic responses were measured for 6-hours after the consumption of 50g of manioc starch or glucose (Ritz, et al., 1991). Blood glucose oxidation reached a higher peak but returned to baseline values faster after the glucose feeding. Overall, glucose oxidation was similar from 0 to 3-hour but higher from 3 to 6-hour postprandial after the starch than glucose load. This increased CHO disposal was associated with reduced plasma FFA and elevated insulin concentrations which was likely to be due to the continued clearance of the starch load 3 hours after its ingestion (Ritz, et al., 1991).

Flatt et al. (1985) reported that addition of 50g of fat to a breakfast providing 75g of CHO caused no alteration in insulin response, FFA concentrations and the oxidation of individual macronutrients over the subsequent 9-hour. Similar findings have been reported from other studies which have monitored postprandial metabolic responses for up to 24 hours (Bennett, et al., 1992; Shutz, et al., 1989).The implication is that the extra dietary fat is stored and increased dietary fat did not lead to increased fat oxidation. In these studies additional fat was added to CHO and so the diets were not isoenergetic.

When normal weight healthy subjects are in energy balance, short term (1-7 days) variations in fat and CHO intake in the diet lead to changes in their relative oxidation rates (Stubbs, et al., 1993; Thomas, et al., 1992; Verboeket-van de Venne, et al., 1994). That is, a high fat-low CHO diet caused a suppression of CHO oxidation and a reciprocal elevation in fat oxidation (Stubbs, et al., 1993; Thomas, et al., 1992; Verboeket-van de Venne, et al., 1994). This is in contrast to the reports that fat oxidation is insensitive to its intake (Bennett, et al., 1992; Flatt, et al., 1985; Schutz, et al., 1989). Furthermore, while lean subjects demonstrated a significant positive relationship between fat intake and oxidation, obese subjects did not (Thomas, et al., 1992).

Although both CHO and fat balance are related to their respective intake, overall CHO balance is zero while fat balance is positive in response to overfeeding or underfeeding
(Jebb, et al., 1996), a weight maintenance diet (Abbott, et al., 1988) or isoenergetic diets of varying fat and CHO compositions (Stubbs, et al., 1993; Thomas, et al., 1992; Verboeket-van de Venne, et al., 1994). That is, shifts in CHO oxidation in response to dietary CHO are greater than changes in fat oxidation in response to dietary fat. Jebb et al. (1996) demonstrated an oxidative hierarchy from CHO, protein to fat; fat being the most easily stored and least readily oxidised during the postprandial period. Changes in fat balance accounted for the majority (>70%) of the energy imbalance during overfeeding and underfeeding and metabolic fuel selection seemed to be dominated by the need to maintain CHO balance (Jebb, et al., 1996).

There are also reports of a close inter-relationship between CHO and fat metabolism following single isoenergetic meals of varying proportions of CHO and fat (Babbioni-Harcsh, et al., 1996; Griffiths, et al., 1994; Whithley, et al., 1997). Compared to a high CHO-low fat meal, there was a lesser suppression of plasma FFA from postabsorptive values and reduced insulin concentrations after a meal with increased fat but lower CHO content (Whithley, et al., 1997). Insulin is known to activate adipose tissue lipoprotein lipase (LPL) (Sadur and Eckel, 1982). Griffiths et al. (1994) suggest that after ingestion of combinations of CHO and fat, the action of LPL on chylomicron-triglyceride leads to direct release of fatty acids into the blood and increased fat oxidation. A recent study demonstrated that the rate of action of LPL increased with both oral and intravenous fat load, in adipose tissue and skeletal muscle, without any increase in insulin concentration (Evans, et al., 1999). However, there was increased escape of LPL-derived fatty acids into the circulation from adipose tissue but not from skeletal muscle (Evans, et al., 1999). Overall fatty acids release from the adipose tissue into the circulation may not change with a high fat-low CHO meal because increased LPL-derived fatty acids may counter the effect of insulin suppression of hormone sensitive lipase.
2.3.6 Low GI diets - potential clinical applications?

In the clinical setting, diet and exercise therapy are recognised to be the cornerstone in the management of NIDDM (Henry and Genuth, 1996; Tan and Nelson, 1996). The goal of intensive glycaemic control and therapy in both IDDM and NIDDM is to reduce microvascular, macrovascular and neuropathic complications (Gonzales Barranco, et al., 1998; Henry and Genuth, 1996; Tan and Nelson, 1996). One of the major aims of diabetes therapy is to normalise the blood glucose profile, including both the fasting and postprandial blood glucose concentrations. In 1986, the US National Institutes of Health consensus conference on diet and exercise in NIDDM recommended against the use of GI in the dietary management of diabetes (National Institutes of Health, 1986). The main concerns against adopting such an approach were that no difference was apparent when individual CHO foods were eaten as part of a mixed meal, and there was no study showing long-term benefits.

These issues have since then been addressed. Brand-Miller (1994) reviewed 11 studies that have used the GI approach to determine clinical gains in diabetes or lipid management. In these studies, there were a total of 156 subjects, including 63 with NIDDM, 45 with IDDM, and 42 with hyperlipidaemia and 6 healthy individuals (Brand-Miller, 1994). Then intervention trials in these studies lasted between 2 to 12 weeks with an average of 4.5 weeks. All but one of the studies showed improvements in CHO or lipid metabolism or both following a low GI diet (Brand-Miller, 1994). Other and subsequent studies have also reported similar benefits in normal and hyperinsulinaemic subjects (Behall and Howe, 1995; Behall, et al., 1989; Jarvi, et al., 1999). A recent study reported that a low GI diet (achieved by altering structure of starchy foods), in addition to improving glycaemic and lipid profile, also reduced plasminogen activator inhibitor-1 activity (a fibrinolytic inhibitor) (Jarvi, et al., 1999). Although an association between optimal glycaemic control and reduced risks of macrovascular complications in diabetic patients has not yet been proven, it is recognised to be a desirable goal (Mann, 1997). The use of low glycaemic index foods has been recognised by some as one of the best nutritional means to achieving satisfactory blood glucose concentration (Brand-Miller, et
al., 1996; Mann, 1997). This view, however, is not shared by other authorities such as the American Diabetes Association and a number of questions remain unresolved (Wolever, 1997).

There is some evidence that the hypolipidaemic effect of a low GI diet is not due to fibre content alone but the ability to flatten postprandial glycaemic and insulinaemic responses (Behall and Howe, 1995; Brand-Miller, 1994; Jarvi, et al., 1999). Nibbling, as opposed to gorging has also been shown to simultaneously reduce insulin and triglyceride responses (Jenkins, et al., 1989). Acarbose is a bacterially derived α-glucosidase inhibitor which exerts its activity in the gastrointestinal tract. It reduces starch, sucrose and maltose digestion and flattens glucose and insulin responses (Coniff and Krol, 1997; Frati Munari, et al., 1998). Acarbose has also been reported to be as effective as lipid lowering drugs in lowering plasma triglyceride (Walther, et al., 1981). These studies further support the hypothesis that diets that minimise increases in postprandial glucose and insulin concentrations also reduce triglycerides concentrations.

Hyperinsulinaemia is a common finding in obesity (DeFronzo and Ferrannini, 1991; Meylan, et al., 1987) and sustained hyperinsulinaemia is a factor in the development of hyperlipidaemia and atherosclerosis (DeFronzo and Ferrannini, 1991). Both enhanced secretion and reduced clearance of insulin can result in hyperinsulinaemia (Meistas, et al., 1983; Bonora, et al., 1985). A number of studies have examined the effect of 3-12 week high and low GI diets on insulin stimulated glucose uptake or fasting insulin concentration in obese women (Slabber, et al., 1994), women at risk of coronary heart disease (CHD) (Frost, et al., 1998), CHD patients (Frost, et al., 1996) as well as normal healthy (Behall and Howe, 1995; Kiens and Ritcher, 1996) and well-trained subjects (Tegelman, et al., 1996). In these studies, the low GI diet comprised of CHO with low GI and foods that contain high GI CHO such as potatoes and white bread were specifically avoided. In a crossover design, Slabber et al. (1994) compared the effect of a 12-week low GI and energy-restricted diet with a balanced normal energy-restricted diet in hyperinsulinaemic
obese females. The low GI diet resulted in lower fasting insulin concentration these subjects (Slabber, et al., 1994). It is also interesting to note that both diets resulted in significant weight loss, but mean weight loss was greater after the low GI diet (Slabber, et al., 1994). However, the mechanism by which the low GI diet caused more loss and weight and a lower fasting insulin concentrations is not known. Using a independent group design, Frost and co-workers reported the effects of low vs high GI diet on whole body and adipocyte insulin-stimulated glucose uptake in patients with advanced coronary heart disease (CHD) (Frost, et al., 1996) or women at risk of CHD (Frost, et al., 1998). Insulin response after oral glucose tolerance test and insulin stimulated glucose uptake in isolated adipocytes was reduced after 4 weeks in the LGI group but not in control subjects who consumed their normal diets (Frost, et al., 1996). A 3-week LGI diet also improved both visceral and subcutaneous adipocyte insulin sensitivity in women at risk of CHD and in vivo insulin sensitivity in both normal and at-risk women (Frost, et al., 1998).

In healthy subjects, fasting insulin concentration may be the same (Kiens and Ritcher, 1996) or lower (Tegelman, et al., 1996) after prolonged low GI than a high GI diet. However, fasting insulin concentration is only a crude reflection of whole body insulin sensitivity. Behall et al. (1989) did not find any difference in insulin response in healthy men to a standard glucose load 4 weeks after a diet containing 34% of energy as 70% amyllose or 70% amylopectin starch. In a study involving healthy lean young men, Kiens & Ritcher (1996) reported that switching CHO from high to low GI sources for 4 weeks decreased whole body glucose disposal at a high but not at physiologic plasma insulin concentrations (Kiens and Ritcher, 1996). They chose to use a randomised crossover design and used the hyperinsulinaemic-euglycaemic clamp (gold standard) to assess whole body insulin sensitivity. In this study, plasma glucose and insulin concentrations were lower during part of the day (post-lunch) 3 days after the LGI compared to the HGI diet. However, this difference diminished after 4 weeks, suggesting that adaptation in terms of digestion and absorption to a low GI diet may take place. Furthermore, plasma fatty acid concentration during part of the day (post-lunch) was higher with the LGI compared to
the HGI diet throughout the 4-week dietary period. Kiens & Ritcher (1996) suggest that the decreased insulin action at high plasma insulin concentration following the low GI diet may be related to higher plasma fatty concentration. This finding is also in contrast with that of Wolever et al. (1996) who reported a greater suppression of postprandial FFA following prolonged low GI compared to high GI diet (Wolever, et al., 1996). In Kiens & Ritcher’s study (1996), apart from the post-lunch period, differences in concentrations of glucose, insulin and FFA in the blood were not observed at any other times 3 or 30 days after either dietary treatment. It unlikely that the different findings are related to diurnal differences in metabolite responses because of greater glucose and insulin responses have been reported after consuming the same foods at breakfast than at lunch (Frape and Jones, 1995; Wolever and Bolognesi, 1996; Frape, et al., 1997).

Using isotopic tracer method, a recent study measured splanchnic fatty acid oxidation by femoral artery and hepatic vein catheterization (Sidossis, et al., 1998a). Fifteen hours of moderate hyperglycaemia (~8 mmol·l⁻¹) reduced fatty acid oxidation in the splanchnic region even when fatty acid availability was maintained by infusion of lipid and heparin (Sidossis, et al., 1998a). There was also a significant increase in the hepatic triglyceride secretion and plasma VLDL-triglyceride concentration (Sidossis, et al., 1998a). Similar effect was observed after a 2 week high CHO diet. Compared to a high fat diet (55% fat, 30% CHO), a 2 week high CHO diet (70% CHO, 10% fat) decreased hepatic fatty acid oxidation and increased VLDL-triglyceride secretion (Sidossis and Mittendorfer, 1999). The increase in VLDL-triglyceride secretion rate results in elevation of plasma VLDL- and total-triglyceride concentrations. The link between lower fasting and/or postprandial insulin concentrations and hypolipidaemia may involved reduced hepatic glucose production. Fermentation of undigested CHO produces short-chain fatty acids, which may reduce hepatic glucose production (Venter, et al., 1990). Thorburn and co-workers (1993) reported that low GI foods that contain a significant amount of slowly absorbed fermentable CHO ingested the evening before an oral glucose tolerance test enhanced the suppression of hepatic glucose production by oral glucose. Overnight fasting serum FFA
concentration was also reduced following a low GI-fermentable CHO meal the evening before. Therefore, apart from the acute postprandial small-bowel effects after the ingestion of GI foods, the link between lower fasting and/or postprandial insulin concentrations and hypolipidaemia may involved reduced hepatic glucose production. It is not known if hepatic lipogenesis may be reduced by lowering glucose and insulin concentrations. The exact mechanism by which blood lipid concentrations may be decreased via glycaemic and insulinaemic control remain to be elucidated.
2.4 The metabolic causes of fatigue during prolonged submaximal exercise

Fatigue has been defined as the failure to maintain a required or expected force leading to reduced performance of a given task (Edwards, 1981). The command chain for voluntary muscular activity involves numerous steps from motor centres in the brain to the actin-myosin cross bridge formation within the muscle fibres. Fatigue can result from the functional failure at any one of the link (Enoka and Stuart, 1992). A loss of force can occur as a result of inadequate activation by the neural network (central) and/or as a result of deterioration in excitation-contraction coupling within the muscle itself (peripheral). Discomfort and pain associated with prolonged exercise may also influence motivation (central fatigue) and mechanisms of such fatigue during exercise are even more complex. However, central fatigue may at least, under some circumstances, be related to metabolic demands induced by the exercise.

Several determinants have been suggested to explain the causes of fatigue during contractile activity (Green, 1990; 1995). In contrast, there are two prominent theories about the contribution of substrate availability and fatigue during prolonged submaximal exercise: the energy crisis hypothesis and the central fatigue hypothesis. The following section will focus on the biochemical concerns as it is directly relevant to problems concerning fuel supply and hence offer possible nutritional strategies which may delay the onset of fatigue.

2.4.1 Peripheral Fatigue - The Energy Crisis Hypothesis

Fatigue during prolonged exercise at intensities ranging from 60-85% $\% \text{VO}_{2\max}$ in comfortable ambient temperatures occurs when muscle glycogen concentrations are reduced to low values (Ahlborg, et al., 1967; Gollnick, et al., 1973a; 1973b; Hermansen, et al., 1967; Saltin and Karlsson, 1971). The association between low muscle glycogen concentration and fatigue can be explained as the inability of alternative substrates (e.g., blood glucose, FFA and muscle triglyceride) in providing the required amounts of acetyl-CoA to sustain mitochondria ATP production. However, it has now been demonstrated
that muscle acetyl-CoA and acetylcarnitine concentrations do not fall below resting values at exhaustion (Spriet, et al., 1992), even when muscle glycogen concentration decreases by 90% (Constantin-Teodosiu, et al., 1992). This suggests that availability of acetyl group was not limiting exercise performance despite the near total depletion of the glycogen store. Therefore, fatigue in these cases may be related to a reduced capacity to oxidise acetyl group and not the lack of acetyl group. When ATP cannot be regenerated at a sufficient rate to meet its demand, this creates an energy crisis in the muscle cell.

Observations that substantial amount of muscle glycogen are present at the end of exhaustive running (Costill, et al., 1973; Madsen, et al., 1990) and cycling (Coggan and Coyle, 1987; Coyle, et al., 1986; Hawley, et al., 1997) exercise suggest that whole-muscle glycogen depletion may not be the exclusive cause of fatigue. Histochemical analysis of glycogen in individual fibres showed that type I fibres were almost glycogen depleted at the end of exercise in some (Coggan and Coyle, 1987; Costill, et al., 1973; Sherman, et al., 1983) but not all (Madsen, et al., 1990) studies. In these studies, type II fibres appear to have used relatively little glycogen. However, the histochemical methods used in these studies have provided at best, a semi-quantitative estimation of glycogen content. In contrast, a more recent study using a quantitative biochemical method, shows clear selective glycogen depletion in type I muscle fibres of the vastus lateralis after prolonged exhaustive running (Tsintzas, et al., 1996). On the basis of these results, a possible cause of fatigue during prolonged exercise may be related to the selective impairment of the contractile process in type I fibres. This may be due to the energy crisis created by a decreased rate of ATP resynthesis or reduced capacity to oxidise acetyl-CoA in these fibres (Broberg and Sahlin, 1989; Conlee, 1987).

Rapid tricarboxylic acid cycle (TCA) activity during submaximal exercise results in cataplerotic losses such as loss of α-ketoglutarate to form glutamate and glutamine (Brooks and Gasser, 1980). Pyruvate derived from glycogen or glucose may compensate for these losses by providing anaplerotic substrates for the TCA cycle. Many of the
reactions that can lead to net increase of TCA intermediates (TCAI) are directly or indirectly dependent on the level of pyruvate, including those catalysed by alanine aminotransferase (α-ketoglutarate), malate dehydrogenase (malate), pyruvate carboxylase (oxaloacetate) and phosphoenolpyruvate carboxylase (oxaloacetate). Free-fatty acids and triglycerides are not known to possess anaplerotic capabilities and the flux of anaplerotic substrates to the TCA cycle from amino acids may be too low to offset the cataplerotic losses. The purine nucleotide cycle also has an anaplerotic function (Flanagan, et al., 1982). A recent review suggests that the alanine aminotransferase reaction is the most important anaplerotic reaction (Graham and Gibala, 1998). Several mechanisms have been proposed to explain the association between low muscle glycogen and impairment in ATP regeneration. A low muscle glycogen content may be associated with reduced TCAI concentrations (Gibala, et al., 1997; Sahlin, et al., 1990) or an increased degradation of adenine nucleotides (Broberg and Sahlin, 1989) and increased oxidation of branched-chain amino acids (BCAA) (Wagenmakers, et al., 1991) which may also drain the TCA cycle of its intermediates.

A number of studies reported a substantial increase in inosine 5'-monophosphate (IMP) in glycogen depleted muscles following exhaustive exercise (Broberg and Sahlin, 1989; Norman, et al., 1987; Parkin, et al., 1999). This suggests that fatigue is related to a failure of chemical processes to rephosphorylate ADP at a sufficient rate (Broberg and Sahlin, 1989). Green (1991) argued that this increase in IMP could be attributed to a shift in fibre recruitment pattern from type I towards type II (Gollnick et al, 1973b; Vollestad et al, 1984 cited in Green, 1991) since type II fibres are known to have a higher AMP dianaminase activity (Terjung, et al., 1986). However, Norman (1988) reported that IMP increment was only observed in glycogen depleted fibres and the IMP content was not significantly different in two populations of fibres. The finding that CHO supplementation attenuates muscle IMP accumulation (Spencer, et al., 1991) as well as glycogen utilisation in type I fibres (Tsintzas, et al., 1995; 1996) provides further support for the inverse relationship between IMP formation and glycogen depletion. This indicates that recruitment of type II
fibres does not in itself induce an increase in IMP accumulation unless the fibres are glycogen depleted (Broberg and Sahlin, 1989).

Although acetyl-CoA concentration may not be limiting during prolonged exhaustive exercise, pyruvate and sum of TCAI have been reported to decrease from 5 or 15 min to the end of exercise (Gibala, et al., 1997; Sahlin, et al., 1990). This reduction of TCAI may be related to the decrease in the availability of pyruvate, which is essential for anaplerotic processes (Sahlin, et al., 1990). As pyruvate provides essential precursors for the generation of TCAI, glycogen depletion may possibly reduce the flux through the TCA cycle and hence the rate of oxidative phosphorylation (Sahlin, et al., 1990).

Wagenmakers (1997) hypothesised that an increase in oxidation of the BCAA (valine, isoleucine and leucine) during the latter stages of prolonged strenuous exercise is primarily responsible for the decline of TCAI. Low glycogen content augments the exercise-induced branched-chain α-keto acid dehydrogenase (BCKADH) activation (the rate determining step in BCAA oxidation) (Van Hall, et al., 1996; Wagenmakers, et al., 1990; Wagenmakers, et al., 1991). Muscle extraction of the BCAA from the blood also increases with prolonged duration of exercise (Van Hall et al, 1995;1996). In glycogen-depleted muscles, regeneration of α-ketoglutarate via transamination of alanine by pyruvate may be compromised. It is proposed that, in the low glycogen state, the transamination of the BCAA leucine to form glutamate drains the TCAI α-ketoglutarate and reduces TCA cycle activity (Wagenmakers, 1997). Observations of patients with McArdle's disease, whose maximum oxygen consumption is 40-50% of predicted values, provides further support to this hypothesis because the glycogen breakdown defect in these patients significantly reduces their anaplerotic capacity (Wagenmakers et al, 1990).

Behind the proposed association between pyruvate availability and rate of ATP regeneration is the assumption that TCAI pool size regulates the TCA cycle flux (Sahlin, et al., 1990; Wagenmakers, 1997). However, the TCAI pool size may not actually
represent an important regulatory signal for TCA flux as has been assumed. There is almost complete activation of pyruvate dehydrogenase enzyme complex (PDC) during prolonged submaximal exercise corresponding to a 4-5 fold increase from the resting level (Constantin-Teodosiu, et al., 1992). This occurs at the onset of exercise and is maintained until exhaustion (Constantin-Teodosiu, et al., 1992). Activation of PDC by dichloroacetate infusion may reduce rather than expand the TCAI pool size (Constantin-Teodosiu, et al., 1999). The TCAI pool expansion is principally a reflection of increased muscle pyruvate availability (Constantin-Teodosiu, et al., 1999). Furthermore, tremendous increase in estimated TCA cycle flux can occur in skeletal muscle despite a relatively small change in TCAI pool size (Gibala, et al., 1998). Therefore, the TCAI pool size may be of little functional significance to TCA cycle flux (Constantin-Teodosiu, et al., 1999; Timmons, et al., 1996). It can be suggested that reduced pyruvate availability and TCA pool size may not necessarily reduce TCA cycle flux and hence ATP regeneration as long as PDC remains activated. If so, the link between low glycogen concentrations and rate of ATP regeneration remains to be clearly established.

A lack of change in ATP concentrations at fatigue does not support the theory that fatigue is due to the inability of muscle to maintain energy supply to the contractile process (Green, 1991). Immediately following prolonged exhaustive exercise, mixed muscle ATP concentration either remains unchanged (Ball-Brunett, et al., 1991; Norman, et al., 1987; Tsintzas, et al., 1996) or is reduced (Broberg and Sahlin, 1989; Green, et al., 1989; Tsintzas, et al., 1996). Tsintzas et al (1996) reported a 6.3% decline in mixed fibre ATP at the point of fatigue during a 70% $\dot{V}O_2_{max}$ treadmill run during which no CHO supplementation was given. However, in a CHO supplementation trial in the same study, ATP concentration did not decline at the point of exhaustion although type I muscle glycogen concentration was reduced to similar extent in both trials −30 mmol (kg dry wt)$^{-1}$ (Tsintzas, et al., 1996). More recently, Sahlin (1997) reported that although ATP concentration remained relatively high in all single fibres following fatiguing exercise at −75% $\dot{V}O_2_{max}$, inter-fibre variation in PCr was large and some fibres were depleted to

41
the same extent as after sprint exercise. It is suggested that PCr depletion may contribute to the large increase in Pi and free ADP despite ATP being relatively stable (Sahlin, et al., 1997). The local increases in ADP may reach inhibitory levels for the contraction process (Sahlin, et al., 1998). If so, the impairment of the contractile process may be due to a failure of the machinery to utilise ATP (Cooke and Pate, 1990) rather than a reduced capacity to regenerate ATP as is proposed by the energy crisis hypothesis. At this point, whether peripheral fatigue during submaximal exercise is due to dysfunction the muscle cell for ATP regeneration (substrate availability) or ATP utilisation (by-product inhibition, see section 2.4.3) remain unresolved. Both may be instrumental in the reduction of power output during prolonged submaximal exercise and at present, there is no simple way to control for the variables responsible for either case in vivo in order to separate their effects.
2.4.2 Central Fatigue - The Central Fatigue Hypothesis

Central fatigue has been defined as fatigue that is associated with specific alterations in the central nervous system (CNS) function and which cannot be reasonably explained by peripheral markers of muscle fatigue (Davis and Bailey, 1997). A number of studies have supported a role of the CNS in exercise fatigue (Assmussen, 1979; Bigland-Ritcher, et al., 1986; Davis and Bailey, 1997; Secher, 1992). Central fatigue may have a metabolic basis, either as a result of metabolic changes in the muscle altering reflex feedback or by direct effects of the by-products released from the muscle carried by the blood and acting at one or more central locations (Green, 1987). Christensen and Hansen (1939) first suggested that hypoglycaemia could cause fatigue by affecting the CNS. However, Felig et al (1982) have shown that cycling exercise at 60-65 % $\dot{V}O_2$ max can be continued in the presence of hypoglycaemia (blood glucose concentration <2.5 mmol.l$^{-1}$). Moreover, hypoglycaemia seldom occurs during prolonged running to volitional fatigue (Tsintzas, 1993; Chryssanthopoulos, 1995).

2.4.2.1 Central fatigue hypothesis

Recent research on brain 5-hydroxytryptamine (5-HT) (serotonin) and fatigue has generated the most interest. Tiredness and sleep may be, in part, influenced by the concentration of serotonin in the brain (Young, 1986). The rat model has been used to study the effects of fatigue on regional brain concentrations of 5-HT and metabolites. Research in humans have been limited to nutritional and pharmacological effects on blood tryptophan concentration and some indirect markers of central fatigue, like prolactin. The central fatigue hypothesis states that an increased concentration of brain serotonin can impair CNS function and thereby cause a deterioration in exercise performance (Newsholme, et al., 1987). The brain uptake of tryptophan (Trp), the precursor of serotonin, is an important factor in the regulation of brain serotonin concentration. Tryptophan is transported bound to plasma albumin, at the same sites as FFA. There is a strong correlation between concentrations of plasma free-tryptophan and FFA (Blomstrand, et al., 1988; Davis, et al., 1992; Zanker, et al., 1997). The concentration of
free-Trp governs the rate of entry of Trp into the brain (Blomstrand and Newsholme, 1996). In addition, both the large neutral amino acids (LNAAs), including branched-chain amino acids (BCAAs), and free-Trp enter the brain by the same amino acid carrier and there is competition between the two types of amino acid. Therefore, the central fatigue hypothesis further suggests a link between plasma free-Trp to BCAA ratio and the disinclination for exercise (Blomstrand and Newsholme, 1996).

Some (Blomstrand, et al., 1988; Davis, et al., 1992) but not all (Pitsiladis, et al., 1998; Wilson, 1994) studies reported that the plasma free-Trp to BCAA ratio increase after exhaustive endurance exercise. Two recent studies reported that the free-Trp to BCAA ratio increased after 60 min of cycling (Blomstrand, et al., 1997) or running (Zanker, et al., 1997) at 70 \% \textit{V}O_2\text{max} in subjects with low pre-exercise muscle glycogen concentrations. In the Blomstrand et al (1997) study, no increase in the free-Trp to BCAA ratio was observed when BCAA supplements were used during exercise. Nevertheless, the results of an endurance performance test after the 60 min exercise in this study were similar regardless of the free-Trp to BCAA ratio (Blomstrand, et al., 1997). This increase in the ratio of free-Trp to BCAA has been attributed to the increased BCAA oxidation (Wagenmakers, et al., 1991) or displacement of tryptophan from albumin by increasing concentration of FFA (Davis, et al., 1992; Zanker, et al., 1997). This may result in an increased serotonin synthesis in the brain, triggering the onset of fatigue (Blomstrand and Newsholme, 1996; Chaouloff, 1997; Davis and Bailey, 1997). In one study, however, an estimated 20-fold increase in free-tryptophan influx into the brain does not influence cycling time to exhaustion at 70-75 \% \textit{V}O_2\text{max} (Van Hall, et al., 1995a). The latter result suggests that manipulation of tryptophan supply to the brain either has no additional effect upon serotoninergic activity or that serotoninergic activity does not contribute to the mechanism of fatigue (Van Hall, et al., 1995a).

The central fatigue hypothesis is supported by a study which showed that endurance cycling capacity of well-trained cyclists at 70 \% \textit{V}O_2\text{max} was impaired when an oral dose
of serotonin agonist (20mg Paroxetine) was taken before exercise compared to a placebo (Wilson and Maughan, 1992). However, other studies did not find any difference in endurance capacity when other serotonin agonist (70 mg Fluoxetine) (Davis, et al., 1993) or antagonist (1mg Pizotifen) (Pannier, et al., 1995) were administered compared to placebo. This may be partly due to the complexities of the drugs like non-selectivity of drug action and inter-individual sensitivity of subject response, making it very difficult to control the degree of agonist or antagonist action. In conclusion, there seems to be a lack of experimental support for the role of tryptophan in explaining the disinclination to exercise in humans.

2.4.2.2 Prolactin - a marker of serotonergic activity

Prolactin is important for the synthesis of milk constituents during lactation and may have other functions similar to GH as well as being secreted during stress (Norman and Litwack, 1987). There is evidence that serotonin is involved in the control of prolactin secretion. In rats, serum prolactin concentration increased after administration of serotonin and the serotonin reuptake inhibitor, fluoxetine (Clemens, et al., 1977). In addition, the serotonin agonist quipazine also elevates serum prolactin concentration (Clemens, et al., 1977). In humans, oral and intravenous administration of tryptophan lead to increases in circulating prolactin concentrations (Charney, et al., 1982; Woolf and Lee, 1977). Furthermore, the increase in plasma prolactin concentration during exercise is blunted by serotonergic antagonism (DeMeirleir, et al., 1985b). Elevated concentration of prolactin in plasma, as seen during or after acute muscular exercise, are caused by increased pituitary secretion, rather than decreased elimination (DeMeirleir, et al., 1985a). Therefore, some investigators have chosen prolactin an indirect endocrine marker of serotonergic activity during endurance exercise (Fischer, et al., 1991; Marvin, et al., 1997; Struder, et al., 1997).

Fisher et al (1991) reported that both plasma free-Trp and prolactin increased during 1-hour of cycling exercise and that concentrations of plasma prolactin and free-Trp
concentrations during exercise were correlated. In another study, administration of BCAA was shown to lower circulating prolactin concentration during 60 min of running (Carli, et al., 1992). Struder et al (1997) studied changes in plasma prolactin, FFA and amino acids during prolonged cycling exercise at 55 and 75 % $\dot{V}O_2$max. Plasma FFA concentration increased with exercise at both intensities but was higher during the last 2-hours of exercise at the higher intensity. Furthermore, plasma free-Trp to BCAA ratio and prolactin concentration increased only during exercise at 75 % $\dot{V}O_2$ max (Struder, et al., 1997). However, another study by the same group reported that elevations of plasma FFA or LNAA concentrations had no effect on plasma prolactin concentration compared to saline infusion during 90 min of treadmill exercise which elicited a blood lactate concentration of 2 mmol·l$^{-1}$ (Struder, et al., 1996). In yet another study, serum prolactin concentration was also elevated following buspirone (5HT1A agonist) administration, indicating increased hypothalamic 5HT1A receptor stimulation and endurance cycling capacity was reduced compared to a control trial (Marvin, et al., 1997). However, not all studies have observed the same pattern of plasma prolactin response to exercise. Therefore, there is some support for the direct relationship between the concentrations of plasma prolactin and free-Trp as well as the use of prolactin as a surrogate marker of serotonergic activity during endurance exercise.

2.4.2.3 Dopamine and Acetylcholine

Other neurotransmitters that have been proposed as having an influence on CNS mediated fatigue are dopamine and acetylcholine (Davis and Bailey, 1997). Studies have been conducted using rats where dopaminergic activation was achieved by amphetamine administration. In one study, swimming endurance was improved following administration of amphetamine doses from 10 to 20 mg.kg$^{-1}$ (Bhagat & Wheller, 1973 cited in Davis & Bailey, 1997). However, another study reported that doses greater than 2.5 mg.kg$^{-1}$ resulted in a reduction of endurance performance (Gerald, 1978 cited in Davis & Bailey, 1997). Furthermore, increased brain dopaminergic activity is known to inhibit brain serotonin synthesis and metabolism (Chaouloff, et al., 1989). It has been suggested that
when dopaminergic activity is reduced during prolonged exercise, fatigue may be precipitated by a loss of co-ordination (i.e. reduced efficiency) and/or a reduction in motivation (Davis and Bailey, 1997). However, the mechanism by which dopaminergic activity may influence fatigue during prolonged exercise remains largely unexamined in humans. Acetylcholine is employed by preganglionic sympathetic fibres and neurones within the CNS. Its synthesis, release and reuptake are also essential for muscular contraction. It has been hypothesised that fatigue during prolonged exercise may be initiated by a reduction in cholinergic activity subsequent to the depletion in the availability of its precursor, choline (Conlay, et al., 1992; Wurtman, 1988). However, a more recent study reported that serum choline was not depleted following prolonged cycling to exhaustion at 70 % V\textsubscript{O\textsubscript{2}} max and choline supplementation did not result in improved performance (Spector, et al., 1995).

Investigations into central fatigue in humans are limited by the general failure to provide plausible neurological mechanisms. Experimental support for a specific role of CNS fatigue is also limited due to lack of objective measures. The available evidence does not always support the hypothesis that there is a central component to fatigue which is mediated by the serotonergic or other neurons. Nevertheless, it seems that fatigue during exercise at ~70 % \textsubscript{V}O\textsubscript{2} max is at least partly mediated by the central component in some of these studies. Therefore, elucidation of how the CNS influences fatigue and how nutrition can help to delay the onset of such fatigue is relevant to endurance performance.

2.4.3 Other considerations

Metabolic factors play an important role in muscle fatigue \textit{in vivo} but conditions exist where fatigue cannot be explained by metabolic changes (Green, 1990; Hultman, et al., 1990). The possibility that fatigue during prolonged exercise can be attributed to failure in the contraction apparatus to utilise ATP has been suggested by Green (1987; 1990; 1995). Using \textit{in vitro} systems designed to simulate conditions of moderate activity, Cooke and Pate (1990) showed that elevations in AMP, IMP and ammonia had no effect on
contractile activity. Their findings implicated the products of ATP hydrolysis: Mg-ADP, H+ and phosphate as the modulators of actomyosin interaction (Cooke & Pate 1990).

It has been hypothesised that changes in intracellular environment, mediated by metabolic factors, if allowed to persist for a critical period, may induce structural or compositional changes in membranes and proteins at selected intracellular sites (Green 1995). Non-metabolic fatigue, characterised by a persistent weakness after metabolic recovery, commonly occurs after eccentric exercise where energy demands are low but the force production is high (Clarkson et al 1992). This may be a consequence of oxidative damage due to increased free-radical activity. It remains difficult to determine whether performance decreases as a result of muscle fatigue or as a result of muscle damage (Fitts, 1996).
2.5 Pre-event nutritional manipulation - effects on metabolism and endurance performance

Research in the 1960s established that the capacity of man to exercise at 70-75% $\dot{V}O_2\text{max}$ is determined by the glycogen concentrations in the active muscles before exercise (Astrand, 1967; Bergstrom, et al., 1967; Bergstrom and Hultman, 1967; Karlsson and Saltin, 1971). Since then, a considerable amount of research has been conducted in search of the optimal dietary strategy to elevate muscle glycogen concentrations prior to competition, to rapidly replenish muscle glycogen stores between exercise bouts as well as to reduced the rate of muscle glycogen utilisation during exercise (Burke, 1996; Costill and Miller, 1980; Hargreaves, 1996; Hawley, et al., 1998; Ivy, 1991; Williams, 1989).

Athletes who train or compete after an overnight fast may fatigue prematurely due to reduced liver glycogen stores (Nilsson and Hultman, 1973) leading to an ultimate decline in blood glucose (Coyle and Coggan, 1984). The beneficial effects of CHO supplementation during endurance exercise are well documented (Coggan and Swanson, 1992; Tsintzas, 1993; Valeriani, 1991). However, the high ventilatory rates of elite athletes during endurance races can impede the absorption of large volumes of fluid, and can cause gastrointestinal discomfort. Under such circumstances pre-exercise CHO ingestion may be advantageous.

The blood glucose and insulin responses following CHO ingestion play a key role in subsequent exercise metabolism. In a pivotal paper, Costill et al (1977) reported that compared to a fasted condition, glucose ingestion before exercise induced hyperinsulinaemia, leading to increased rate of muscle glycogenolysis during subsequent 30 min of exercise at 70% $\dot{V}O_2\text{max}$. In contrast, pre-exercise fat ingestion which caused an elevation of circulating plasma fatty acids, resulted in reduced rate of muscle glycogen utilisation compared to the fasted condition (Costill, et al., 1977). Following this, many pre-exercise feeding studies have used different methods to achieve different glycaemic responses. These include using different monosaccharides, whole foods with different GI,
foods that are processed differently, and the addition of other macronutrients to a CHO source. The benefits of CHO ingestion 30-60 min prior to exercise, however, are not entirely clear (Burke, et al., 1999; Coyle, 1992). Compared to exercise in the fasted state, a high CHO meal 3-4 hours before exercise enhances endurance performance (Brooks, et al., 1996). Nevertheless, pre-exercise CHO feeding 3-6 hours before exercise still causes a shift in blood-borne fuels from fat to CHO (Coyle, 1992; Hultman and Harris, 1988).

There has also been recent interest on nutritional strategies which may promote fatty acid oxidation, reduce the rate of muscle glycogen utilisation and improve exercise capacity. These include the ingestion of caffeine, medium chain triglyceride (MCT) as well as the consumption of high fat meals and diets (Hawley, et al., 1998).

Studies that have examined the effects of various dietary interventions on endurance performance have used different exercise protocols to assess performance. The studies reported in this review have used either ergometer cycling or treadmill running exercise. Testing methodologies can generally be classified into those that measure endurance capacity or endurance performance (Williams, 1989). Endurance capacity is defined as the time to volitional fatigue during submaximal exercise (at a fixed percentage (65-85%) of VO_{max} ) (Williams, 1989). Endurance performance has been measured using time trials during which subjects have to complete a pre-set amount of work as fast as possible (Jeukendrup, et al., 1997; Starling, et al., 1997) or by having subjects perform as much work as possible during a fixed time period (Bishop, 1997). In a number of cycling studies, subjects are required to exercise at a certain submaximal intensity for a fixed duration and then complete an endurance performance test (Febbraio and Stewart, 1996; Jeukendrup, et al., 1997).

The following section of this review considers the relevant literature on the influence of the type and composition of pre-exercise CHO meals on metabolism and endurance performance. It is sub-divided into five sub-sections.
2.5.1 Glucose and fructose ingestion in the hour before exercise

Pre-exercise CHO feeding can result in variable effects on metabolism and performance. The underlying factors could be the timing of ingestion, type and amount of CHO (Coyle, 1992; Hawley and Burke, 1997), the relative exercise intensity (Montain, et al., 1991) and state of fast/fed (Chryssanthopoulos, 1995). These factors influence the duration and magnitude of insulin release, which may be a dominant factor influencing substrate metabolism during subsequent exercise.

Whether the ingestion of 50-100 g glucose 30-60 min before exercise affects performance remains equivocal, with negative (Foster, et al., 1979; Keller and Schwarzkopf, 1984), neutral (Braun, et al., 1994; Hargreaves, et al., 1987; McMurray, et al., 1983; Williams, et al., 1995) and positive (Gleeson, et al., 1986; Millard-Stafford, et al., 1994; Sherman, et al., 1991) effects being reported.

The combined effects of hyperinsulinaemia induced by the feeding and increased glucose uptake due to muscle contraction during exercise invariably results in a transient decline in blood glucose early in exercise but this usually returns to pre-exercise values after 30 minutes of exercise (Sherman et al., 1991; Williams et al., 1995; Foster et al., 1979; Levine et al., 1983; Gleeson et al., 1986; Hargreaves et al., 1987; Koivisto et al., 1985; Chryssanthopoulos et al., 1994b). Of the studies on this topic, blood glucose concentration did not fall below 3.5 mmol.l\(^{-1}\) (Gleeson et al., 1986; Felding et al. 1987; Sherman et al., 1991; Chryssantopoulos et al. 1994b; Seifert, et al., 1994; Williams, et al., 1995). Since symptoms of hypoglycaemia for different individuals are apparent at different blood glucose concentrations, any detrimental effect is more likely to be associated with individuals who are sensitive to a reduction of blood glucose during exercise (Coyle et al., 1983). Feeding induced hyperinsulinaemia also has a long lasting inhibitory effect on lipolysis and the rate of fat oxidation but depression of plasma FFA concentrations has been reported in some (Costill, et al., 1977; Koivisto, et al., 1985) but not all (Alberici, et
al., 1993; Calles-Escandon, et al., 1991) studies. It has been suggested that the decline in blood glucose may be avoided by inhibiting the insulin response when feedings are ingested 5 min before exercise (Wright, et al., 1991). The rationale for this strategy is that during exercise, the increase in epinephrine inhibits the release of insulin.

Some (Costill et al., 1977; Hargreaves et al., 1985) but not all (Levine et al., 1983; Koivisto et al., 1985; Fielding et al., 1987; Hargreaves et al., 1987) studies have reported increased muscle glycogenolysis during exercise following glucose ingestion. The increase in muscle glycogenolysis is accompanied by a large decline in blood glucose concentration, which may have prevented further increases in glucose uptake by muscle (Coyle, 1992). The lack of increase in muscle glycogenolysis observed in some studies has been attributed to enhanced insulin sensitivity of trained subjects (Fielding, et al., 1987; Hargreaves, et al., 1987).

Three studies have compared the effect of different doses of glucose feedings during the hour before exercise on glycaemic responses during exercise at 60-70 % $\dot{V}O_2$ max. All have reported that despite the different dosage (22 to 75g or 78 and 156g), blood glucose at the onset of exercise and 15-20 min into exercise were similar among all treatments (Seifert, et al., 1994; Sherman, et al., 1991; Short, et al., 1997). A relative hypoglycaemic response occurred shortly after the start of exercise when subjects ingested either 78g or 156g of a maltodextrin and glucose mixture (Sherman, et al., 1991). Similarly, when either 22 or 75g of CHO was consumed before cycling, blood glucose concentration was below fasting values 20 min into exercise (Seifert, et al., 1994). Comparing four smaller feedings to a large single bolus, Short et al., (1997) reported that although the insulin response was greater with a larger dose, blood glucose concentrations were similar throughout exercise in both conditions.

Fructose can be taken up by the muscle (Bergstrom and Hultman, 1967) and liver (Nilsson and Hultman, 1974). Compared to glucose, fructose ingestion results in smaller changes in blood glucose and insulin (Jenkins et al., 1981). However, its effect on subsequent
exercise performance and muscle glycogen utilisation is not clear. Lower muscle glycogen utilisation during exercise after ingestion of fructose compared with ingestion of glucose or placebo reported by Levine et al. (1983), has not been observed in other studies (Koivisto et al., 1985; Hargreaves et al., 1985; 1987; Fielding et al., 1987). It is likely that the slow gastrointestinal absorption and extensive hepatic metabolism of fructose may have resulted in relatively stable blood glucose concentrations and so do not alter glycogen metabolism (Hargreaves et al., 1987). The studies that have reported muscle glycogen sparing (Levine et al., 1983) and improvement in endurance capacity (Okano et al., 1988) have used subjects in the fed state compared to other studies where subjects have fasted overnight (McMurray et al., 1983; Koivisto et al., 1985; Hargreaves et al., 1987; Fielding et al., 1987). Because fructose is more important for liver glycogen replenishment than glucose (Sonne and Galbo, 1986), the majority of the fructose, if consumed after an overnight fast, will be available to the liver and not muscle. Furthermore, the use of fructose alone, especially in too great a concentration, can cause gastrointestinal distress (Craig, 1993), making its use undesirable.

2.5.2 Carbohydrates of different GI in the hour before exercise

More recent studies have used other CHO sources in an attempt to alleviate the hypoglycaemic response during exercise (Table 2.2). Different sugars or starches ingested can produce the same glucose and insulin responses and hence metabolic responses. For example, the use of corn starch did not seem to present any advantage over glucose as a pre-exercise meal because glycaemic and hence metabolic profile presented by both feedings were similar during the 1-hour postprandial period and during subsequent exercise (Gueuzennec, et al., 1989). Subsequent studies confirmed that it is the physical characteristics or glycaemic index of the CHO rather than the biochemical composition of the starch which determines postprandial glycaemic response and subsequent exercise metabolism (Table 2.2). It is therefore not surprising that when different starch forms ingested produced the same glycaemic and insulinaemic responses, endurance performance was also not different (Goodpaster, et al., 1995).
When comparing the influence of GI of a pre-exercise meal on subsequent performance, various experimental protocols have been used (Table 2.2). Some (DeMarco, et al., 1999; Febbraio and Stewart, 1996; Kirwan, et al., 1998; Sparks, et al., 1998; Thomas, et al., 1991) but not all (Paul, et al., 1996b; Thomas, et al., 1994) studies have included a control or fasting trial during which no CHO is given. Some studies measured endurance capacity in terms of cycling time to exhaustion (DeMarco, et al., 1999; Kirwan, et al., 1998; Thomas, et al., 1991; Thomas, et al., 1994), while others measured work output during a set time (Febbraio and Stewart, 1996; Sparks, et al., 1998) or time taken to complete a set distance (Paul, et al., 1996b) after a prolonged bout of submaximal exercise.

A slow releasing CHO meal taken before exercise has been reported to enhance subsequent endurance cycling capacity when compared to high GI foods (DeMarco, et al., 1999; Kirwan, et al., 1998; Thomas, et al., 1991, see Table 2.2). However, the glycaemic index of foods in the hour before exercise did not influence endurance cycling performance (Sparks, et al., 1998; Febbraio and Stewart, 1996; Paul, et al., 1996b, see Table 2.2). The increased exercise capacity was accompanied by less postprandial hyperglycaemia and hyperinsulinaemia (DeMarco, et al., 1999; Thomas, et al., 1991; Thomas, et al., 1994). In the same studies, plasma lactate concentration was lower throughout exercise and plasma glucose and FFA concentrations higher during the latter stages of exercise in the low than high GI trials (DeMarco, et al., 1999; Thomas, et al., 1991; Thomas, et al., 1994). In most studies, compared to high GI foods, a low GI pre-exercise meal prevented the decline in blood glucose concentration during exercise (Table 2.2). This prevailing glucose, insulin and FFA concentrations were associated a lower rate of CHO oxidation in the first 90-120 minutes during exercise after the low GI compared to a high GI meal (Guezennec, et al., 1993; Thomas, et al., 1991; 1994; DeMarco, et al., 1999; Sparks, et al., 1998). In studies where there were no differences in blood glucose and FFA concentrations during exercise, substrate oxidation was also not different between the respective high and low GI trials (Febbraio and Stewart, 1996; Kirwan, et al., 1998; Paul, et al., 1996b).
The underlying mechanism of improved endurance capacity has been suggested to be related to a slower rate of CHO oxidation (Guezenneec, et al., 1993; Thomas, et al., 1994), which may spare muscle glycogen (Guezenneec, 1995; Thomas, et al., 1991). However, Febbraio et al. (1996) reported that the GI of pre-exercise CHO foods had no effect on the rate of muscle glycogenolysis during 120 min cycling exercise at 70 % \( \dot{V}O_2 \text{max} \). However, their subjects had very high pre-exercise muscle glycogen concentration (>600 mmol (kg dw)\(^{-1}\)) and muscle glycogen was not depleted at the end of 120 min of exercise (>200 mmol (kg dw)\(^{-1}\)). It is, therefore, not surprising that results of the endurance performance tests were not different between the high, low GI and control trials in this study (Table 2.2).

Horowitz & Coyle (1993) reported that both high and moderate GI feedings 30 minutes before exercise caused plasma glucose to decline to equally low concentrations early during exercise and CHO oxidation were similar during exercise at 50-70 % \( \dot{V}O_2 \text{max} \). They suggest that a large difference in GI may be necessary to elicit significant differences in substrate utilisation during exercise (Horowitz and Coyle, 1993). Well-trained subjects are particularly insulin sensitive, and so Febbraio & Stewart (1996) speculated that glucose homeostasis in their subjects after the meals may have been restored before the commencement of exercise. It is likely that in some studies, insulinaemic and glycaemic perturbation after the respective meals and/or early during exercise, if any, made little difference to muscle glycogen metabolism and hence endurance performance (Burke, et al., 1998; Febbraio and Stewart, 1996; Goodpaster, et al., 1995; Paul, et al., 1996b).

Ingestion of CHO throughout or late in exercise has been shown to increase cycling times to exhaustion (Coyle, et al., 1983, 1986; Coggan & Coyle, 1989). This strategy is thought to delay the decline in blood glucose concentration and fatigue especially during cycling. A low GI pre-exercise meal may confer similar benefits by favouring higher concentration of circulating glucose towards the end of prolonged exercise compared to a high GI meal.
(DeMarco, et al., 1999; Thomas, et al., 1994). Thomas et al. (1994) reported that plasma glucose and FFA concentrations after 90 minutes of cycling exercise at 65-70 % $\dot{V}O_2$ max correlated inversely with GI of food consumed before exercise. If the pre-exercise CHO feeding continue to be absorbed into the bloodstream during exercise, performance may either be unaffected or improved via enhanced glucose oxidation (Sherman et al., 1991, Thomas et al., 1991, 1994), in a way similar to glucose infusion during exercise (Coggan & Coyle, 1987). Moreover, during the early recovery period after 90 mins of exercise, plasma glucose and insulin concentrations were higher with low GI than high GI foods (Thomas, et al., 1994). It has been suggested that glucose is still being released from the gut to the blood 3 hours after consumption (Thomas, et al., 1994).

In summary, depending on the degree of hyperinsulinaemia, pre-exercise CHO ingestion of especially high GI foods results in increased CHO and decreased fat oxidation during subsequent exercise. In contrast to high GI foods, CHO oxidation during exercise may be lower after ingestion of low GI foods which may also result in a greater endurance capacity (Kirwan, et al., 1998; Thomas, et al., 1991; DeMarco, et al., 1999). However, the mechanism for the enhanced exercise capacity remained to be elucidated. The hypothesis that a low GI pre-exercise meal may enhance subsequent endurance exercise capacity by sparing muscle glycogen (Guezzennec, 1995; Thomas, et al., 1991) has yet to be substantiated.
Table 2.2 - Studies comparing the effects of GI of pre-exercise food on exercise metabolism and performance

<table>
<thead>
<tr>
<th>Studies</th>
<th>Amount</th>
<th>Type</th>
<th>Time before exercise</th>
<th>Exercise Protocol</th>
<th>Blood glucose during exercise</th>
<th>Plasma FFA during exercise</th>
<th>CHO oxidation during exercise</th>
<th>Effect on performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guezenec et al., 1989</td>
<td>100g</td>
<td>glucose (G), fructose (F), and corn starch (CS)</td>
<td>1-h</td>
<td>120 min cycling at ~60% VO₂max</td>
<td>G, CS &lt; F during 1st hour</td>
<td>G, CS &lt; F during 1st hour</td>
<td>G, CS &gt; F</td>
<td>-</td>
</tr>
<tr>
<td>Thomas et al., 1991</td>
<td>1.08</td>
<td>lentils (L), potato (P), glucose (G), water (W)</td>
<td>1-h</td>
<td>cycling to exh at 65-70% VO₂max</td>
<td>P, L &gt; G, W at 75 and 90 min</td>
<td>W &gt; L &gt; G, P</td>
<td>G &gt; L, W</td>
<td>L &gt; P: no difference between the rest</td>
</tr>
<tr>
<td>Guezenec et al., 1993</td>
<td>i) 836kJ</td>
<td>spaghetti, rice, bread, potato, glucose⁷</td>
<td>1-h</td>
<td>120 min cycling at ~60% VO₂max</td>
<td>glucose, potato &lt; rice, spaghetti after 30 min into exercise</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ii) 836kJ</td>
<td>¹³C labelled glucose and spaghetti</td>
<td>1-h</td>
<td>120 min cycling at ~60% VO₂max</td>
<td>-</td>
<td>-</td>
<td>glucose &gt; spaghetti at 30, 60, 90 min during exercise</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>iii)1672kJ</td>
<td>4 combinations of amylose/amylopectin, crude/gelatinised and glucose</td>
<td>1-h</td>
<td>120 min cycling at ~60% VO₂max</td>
<td>-</td>
<td>-</td>
<td>% oxidised: glucose (82), gel.amylopec. (85) &gt; crude amylopec (49), crude amylose (38)</td>
<td>-</td>
</tr>
</tbody>
</table>

⁷ g CHO(kg.bw)⁻¹, in order of observed GI.
<table>
<thead>
<tr>
<th>Studies</th>
<th>Amount</th>
<th>Type</th>
<th>Time before exercise</th>
<th>Exercise Protocol</th>
<th>Blood glucose during exercise</th>
<th>Plasma FFA during exercise</th>
<th>CHO oxidation during exercise</th>
<th>Effect on performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horowitz &amp; Coyle, 1993</td>
<td>0.7g</td>
<td>rice (R), potatoes (P), rice + magarine (RF), potatoes + magarine (PF), sucrose (S), confectionery bar (SF), control (C)</td>
<td>30 min</td>
<td>1-h cycling: 30min at 60%, 15min at 50% VO$_2$max, 15 min at 70% VO$_2$max</td>
<td>decrease in plasma glucose for RF, PF &lt; P, S and SF at 20 min</td>
<td>after 30 min: P, S, SF &lt; C but no difference between C and the rest</td>
<td>P, S &gt; C during 1st 30 min, P, S, SF, PF &gt; C 30-45 min, P&gt;C during 45-60 min</td>
<td>no difference in RPE</td>
</tr>
<tr>
<td>Thomas et al., 1994</td>
<td>1.0g</td>
<td>potato (100), rice (73), lentils (36), bran (30)*</td>
<td>1h</td>
<td>cycling to exhaustion at 65-70% VO$_2$max</td>
<td>correlate inversely with observed GI after 90 mins of exercise</td>
<td>correlate inversely with observed GI after 90 min</td>
<td>potato &gt; lentils, rice, bran</td>
<td>no difference</td>
</tr>
<tr>
<td>Goodpaster et al., 1996</td>
<td>1.0g</td>
<td>waxy starch (WS), resistant starch (RS), glucose (G) and placebo (PL)</td>
<td>30 min</td>
<td>90 min cycling at 66% VO$_2$max followed by 30 min performance ride</td>
<td>similar in all trials</td>
<td>serum glycerol PL &gt; GL, WS, RS</td>
<td>GL, WS, RS &gt; PL</td>
<td>GL, WS, RS &gt; PL</td>
</tr>
<tr>
<td>Febbraio &amp; Stewart, 1996</td>
<td>1.0g</td>
<td>control, lentils and potato^1</td>
<td>45 min</td>
<td>120 min cycle at 70% VO$_2$max and maximal performance trial.</td>
<td>no difference</td>
<td>potato &gt; lentils, control</td>
<td>similar CHO oxidation and muscle glycogenolysis</td>
<td>no difference</td>
</tr>
</tbody>
</table>

<sup>g CHO(kg.bw)^-1, *(observed GI during 1 hour postprandial period) cont...</sup>
...cont Table 2.2

<table>
<thead>
<tr>
<th>Studies</th>
<th>Amount</th>
<th>Type</th>
<th>Time before exercise</th>
<th>Exercise Protocols</th>
<th>Blood glucose during exercise</th>
<th>Plasma FFA during exercise</th>
<th>CHO oxidation during exercise</th>
<th>Effect on performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paul et al., 1996b</td>
<td>isocaloric, ~1540kJ for male, ~1176kJ for female</td>
<td>Fasted (F), oat (O), wheat (W), corn (C)</td>
<td>90 min</td>
<td>120 min cycling at ~60% VO₂max followed by 6.4 km performance ride.</td>
<td>F &gt; O &gt; W, C at 20 min into exercise</td>
<td>F &gt; O, W, C.</td>
<td>O, W, C &gt; F</td>
<td>no difference</td>
</tr>
<tr>
<td>Kirwan et al., 1998</td>
<td>75g CHO</td>
<td>water (CON), rolled oat (RO), oat flour (OF)</td>
<td>45 min</td>
<td>cycling at 60% VO₂max to exh</td>
<td>no difference</td>
<td>RO, OF &lt; CON for first 90 mins</td>
<td>RO &gt; CON at 90 and 120 mins, no difference for OF (225 min).</td>
<td>RO (267 min) &gt; CON (225 min), no difference for OF (250 min).</td>
</tr>
<tr>
<td>Sparks et al., 1998</td>
<td>1.0⁵</td>
<td>potato, lentils, diet drink (control)</td>
<td>45 min</td>
<td>50 min cycle at 70% VO₂max, 15 min performance ride</td>
<td>lower in potato at 30 mins into exercise</td>
<td>control &gt; lentils &gt; potato</td>
<td>potato &gt; lentils, control</td>
<td>no difference</td>
</tr>
<tr>
<td>DeMarco et al., 1999</td>
<td>1.5⁶</td>
<td>HGI, LGI meal or water</td>
<td>30 min</td>
<td>2-h cycle at 70% VO₂max, then 100% VO₂max till exh</td>
<td>LGI, HGI &lt; water at 20 min</td>
<td>HGI &gt; LGI, water during 1st 100 mins</td>
<td>LGI(206s) &gt; HGI(130s) and water (120s)</td>
<td></td>
</tr>
</tbody>
</table>

⁵ g CHO(kg.bw)⁻¹, ⁻¹ in order of observed GI.
2.5.3 Effect of a high CHO meal 3-4 hours before exercise

Some studies delayed the start of exercise by 3-4 hours after a CHO meal in order for insulin to return to fasting values in attempt to prevent the decline in blood glucose and FFA early during exercise (Table 2.3). In contrast to studies employing feedings in the hour before exercise (Table 2.2), these studies have used larger amounts of CHO (2 to 5g CHO.kg\(^{-1}\) body mass). This, together with a longer postprandial rest period before exercise, has resulted in some muscle glycogen synthesis (Chryssanthopoulos, et al., 1998; Taylor, et al., 1993). Although hepatic glycogen content was not measured, it is likely some of the CHO was stored within the liver because the meals were consumed after an overnight fast (Nilsson and Hultman, 1974).

Two studies have directly quantified muscle glycogen storage following a high CHO meal in non-glycogen supercompensated and overnight fasted subjects and reported increase ranging from 10-20% in 3-5 hours (Chryssanthopoulos, et al., 1998; Taylor, et al., 1993). Coyle et al. (1985) compared data from two separate trials and reported that 4-hour following a meal containing 142 g CHO, muscle glycogen concentration increased by 42%. Upon closer examination, the magnitude of increase in glycogen (49 mmol·(kg ww\(^{-1}\))\(^{1}\) in this study seems unlikely. Assuming muscle mass to be 40% of body mass, even if all the 142 g of CHO (789 mmol) ingested were to be stored as muscle glycogen, one would expect muscle glycogen to increase by only about 28 mmol·kg\(^{-1}\) ww. Estimations of glycogen storage from the difference in values in separate fasted and fed trials are less reliable (Coyle, et al., 1985; Neuffer, et al., 1987; Schabort, et al., 1999) since subjects may not have identical muscle glycogen concentrations before the meal.

The meal resulted in an increase in plasma insulin and glucose concentrations which returned to fasting values prior to exercise in some studies (Coyle, et al., 1985; Neuffer, et al., 1987; Sherman, et al., 1989) but not in others (Chryssanthopoulos and Williams, 1997; Whitley, et al., 1998; Wright, et al., 1991). When plasma insulin remained higher than fasting values, it is likely that some portion of the meal still remained in the stomach or gut.
(Chryssanthopoulos and Williams, 1997; Wright, et al., 1991). Even when plasma insulin concentration had returned to fasting values, the persistent effect of insulin on peripheral tissues and insulin-like effect of muscle contraction (Ivy, 1987) at the start of the exercise still caused a transient drop in blood glucose during the early stage of exercise (Table 2.3). However, it seems that this transient decrease in blood glucose concentration do not limit performance because studies in which pre-exercise meals was provided reported improvement in subsequent exercise performance (Table 2.3). Furthermore, the normal increase in plasma glycerol and FFA were suppressed throughout exercise (Chryssanthopoulos, 1995; Coyle, et al., 1985) and CHO oxidation was elevated (Table 2.3). The increase in CHO oxidation was greater during the first hour of exercise (Chryssanthopoulos, 1995; Coyle, et al., 1985) and with increased exercise intensity (Neufer, et al., 1987). This shift in substrate utilisation from fat to CHO increased with the amount of CHO provided (Sherman, et al., 1989). Coyle et al. (1985) reported that glycogen utilisation during 105 min exercise was greater in the fed then fasted condition. However, this was not observed another study (Neufer, et al., 1987). This may be due to the ingestion of a confectionary bar just before exercise, which also prevented the drop in blood glucose concentration early during exercise (Neufer, et al., 1987).

Wright et al., (1991) reported that when the CHO oxidation fell to below 2 g.min⁻¹ during exercise, the subjects fatigued even when fed CHO. In another study, subjects were able to continue cycling at 70 % \( \text{\dot{V}O}_2 \text{max} \) with glucose infusion at a rate of 1 g.min⁻¹ even when muscle glycogen concentrations were very low (Coggan and Coyle, 1987). It has been suggested that improvements in performance after a pre-exercise meal is the result of enhanced CHO oxidation, especially during later stages of exercise (Wright et al., 1991; Coyle, 1992). The increased CHO availability from the pre-exercise feeding probably countered the effects of insulin action. The feeding-induced increase in muscle glycogen synthesis (~15 mmol·kg⁻¹·ww) during the hours before exercise (Chryssanthopoulos, et al., 1998; Taylor, et al., 1993) could not account for either the greater CHO oxidation or the improved performance. It is likely that the higher rate of CHO oxidation is due to the
greater availability and utilisation of blood glucose as a significant portion of the ingested CHO may continue to be emptied from the stomach and be available for intestinal absorption during exercise (Chryssanthopoulos, 1995; Wright, et al., 1991). It has also been suggested that a considerable amount of CHO from the feeding could be converted into liver glycogen and that the pre-exercise CHO meal may also caused liver glycogen sparing by reducing the rate of liver glycogenolysis at the beginning of exercise (Chryssanthopoulos, 1995; Neufer, et al., 1987). During the latter stages of exercise, blood glucose concentration may be maintained by increased splanchnic glucose output and reduced stimulation of glucose uptake by insulin. Contrary to the findings of Coyle et al (1985), it has also been suggested that such a pre-exercise meal may actually reduce muscle glycogen utilisation during subsequent exercise (Schabort, et al., 1999; Tsintzas and Williams, 1998). The exact mechanism for improvement in endurance capacity remains to be elucidated.

Either a pre-exercise CHO feeding (2-5g CHO.kg⁻¹ body mass) or drinking a CHO-electrolyte solution during exercise offered similar benefits in terms of performance during 30km self paced treadmill run (Chryssanthopoulos, 1995) or endurance cycling to exhaustion (Wright, et al., 1991) compared to placebo feedings. Even when CHO was ingested during exercise, a pre-exercise meal has been reported to improve endurance running (+18%) (Chryssanthopoulos and Williams, 1997) and cycling (+9%) (Wright, et al., 1991) capacities. In a recent study, although time trial performance were similar in fed and fasted trials when CHO was ingested during exercise, five out of six subjects achieved better performance in the fed trials (Burke, et al., 1998).
<table>
<thead>
<tr>
<th>Studies</th>
<th>Amount*</th>
<th>Type</th>
<th>Time before exercise (hrs)</th>
<th>Muscle glycogen synthesis before exercise</th>
<th>Exercise Protocol</th>
<th>Estimated increased in CHO oxidation</th>
<th>Muscle glycogen utilisation</th>
<th>Effect on performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coyle et al., 1985</td>
<td>2.0*</td>
<td>mainly HGI food</td>
<td>4</td>
<td>30-50% (not measured directly)</td>
<td>105 min cycling at 70% VO₂ max</td>
<td>45% greater in the first hour, 32% greater overall</td>
<td>Greater in fed trial (−51%)</td>
<td>-</td>
</tr>
<tr>
<td>Neufer et al., 1987</td>
<td>−2.5* and 43g sucrose</td>
<td>mainly HGI foods and candy bar</td>
<td>4 hrs and 5 min</td>
<td>~15% (n.s.) (not measured directly)</td>
<td>45 min cycling at 77% VO₂ max then 15min performance</td>
<td>−40% greater</td>
<td>no difference</td>
<td>meal: 22% greater power than fasted</td>
</tr>
<tr>
<td>Sherman et al., 1989</td>
<td>312g</td>
<td>20% glucose polymer solution</td>
<td>4</td>
<td>not measured</td>
<td>95 min cycling at 52-70% VO₂ max then performance trial (−50mins)</td>
<td>51% greater</td>
<td>not measured</td>
<td>meal: 48mins &lt; fast: 56 mins</td>
</tr>
<tr>
<td>Wright et al., 1991</td>
<td>5.0*</td>
<td>25% glucose polymer solution I) with and II) without CHO ingestion during exercise</td>
<td>3</td>
<td>not measured</td>
<td>exhaustive cycling at 70% VO₂ max with high intensity intervals every 45min</td>
<td>I) +18% II) +46%</td>
<td>not measured</td>
<td>I) +9% II) +18% endurance time to exh</td>
</tr>
<tr>
<td>Taylor et al, 1993</td>
<td>289g</td>
<td>mainly HGI foods</td>
<td>0-7</td>
<td>+20% at 4.9 hrs</td>
<td>no exercise</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cont...</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
### Table 2.3

<table>
<thead>
<tr>
<th>Studies</th>
<th>Amount of CHO</th>
<th>Type</th>
<th>Time before exercise (hrs)</th>
<th>Muscle glycogen synthesis before exercise</th>
<th>Exercise Protocol</th>
<th>Estimated increased in CHO oxidation</th>
<th>Muscle glycogen utilisation</th>
<th>Effect on performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I) Chryssanthopoulou et al., 1997</td>
<td>2.5*</td>
<td>mainly HGI food</td>
<td>3</td>
<td>III) +11%</td>
<td>I, II) run 70% VO₂ max till exh</td>
<td>significantly higher in both I and II</td>
<td>Not measured</td>
<td>I: +18% time to exh</td>
</tr>
<tr>
<td>II) Chryssanthopoulou, et al., 1994</td>
<td></td>
<td>I) with and II) without CHO ingestion during exercise</td>
<td></td>
<td></td>
<td>III) no exercise</td>
<td></td>
<td></td>
<td>II: +9% time to exh</td>
</tr>
<tr>
<td>III) Chryssanthopoulou et al., 1998</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whitley et al., 1998</td>
<td>215g</td>
<td>mainly HGI food</td>
<td>4</td>
<td>not measured</td>
<td>90 min cycling at 70% VO₂ max and 10 km time trial (~14min)</td>
<td>Fast 227g vs meal 274g during 90 mins</td>
<td>not measured</td>
<td>fast: 874s vs meal: 878s (n.s.)</td>
</tr>
<tr>
<td>Burke et al., 1998</td>
<td>2*</td>
<td>HGI, LGI (CHO ingestion during exercise in all trials)</td>
<td>2</td>
<td>not measured</td>
<td>2h min cycling at 70% VO₂ max and time trial (~16min)</td>
<td>HGI 403 g/2h LGI 376 g/2h CON 373 g/2h (n.s.)</td>
<td>not measured</td>
<td>HGI 946s LGI 954s CON 976s (n.s.)</td>
</tr>
<tr>
<td>Schubort et al., 1999</td>
<td>100g</td>
<td>HGI foods</td>
<td>3</td>
<td>-25% (n.s.) (not measured directly)</td>
<td>cycle 70% VO₂ max till exh</td>
<td>no difference</td>
<td>not directly compared</td>
<td>Fed: 136 min &gt; Fast: 109 min</td>
</tr>
</tbody>
</table>

* g CHO(kg.bw)²
2.5.4 Nutritional interventions to promote fat utilisation during exercise

Comprehensive reviews of strategies which may increase fat utilisation during exercise have been recently published (Hawley, et al., 1998; Jeukendrup, et al., 1998). Therefore, the following section will focus on the effects of pre-exercise dietary manipulations of fat intake on exercise metabolism and endurance performance.

2.5.4.1 Short and long term high fat and low CHO diets

A high fat-low CHO (>50% fat, 5% CHO) diet for 3-6 days will result in lower muscle glycogen stores and reduced exercise capacity at ~70 % $\dot{V}O_2$ max compared to a high CHO (>70% CHO) or normal mixed (50% CHO and 30% fat) diets (Bergstrom, et al., 1967; Christensen and Hansen, 1939; Galbo, et al., 1979). However, several reports have demonstrated that a prolonged high fat diet is not necessarily detrimental to endurance exercise performance in well-trained athletes, in spite of reduced pre-exercise muscle glycogen stores (Lambert, et al., 1994; Phinney, et al., 1983). The mechanism which may explain this phenomena that contradicts the well established beliefs about muscle glycogen and exercise endurance, is a biochemical adaptation to dietary fat, which shifts substrate use from glycogen to fatty acids. Lambert et al. (1994) studied the effects of 14-days of either a high-fat (67% fat and 5% CHO) or high-CHO (74% CHO and 12% fat) diet in 5 endurance-trained cyclists. The cyclists then undertook Wingate test, followed by rides to exhaustion at ~90% and then at 60 % $\dot{V}O_2$ max with short rest periods in between. Times to exhaustion were not different at 90 % $\dot{V}O_2$ max (8.3 vs 12.5 mins for the high fat and high CHO diets respectively) but was enhanced at 60 % $\dot{V}O_2$ max following the high-fat diet (42 vs 80 mins). The finding that cycling time to exhaustion at 60 % $\dot{V}O_2$ max in the high fat trial was twice that in the high CHO trial is highly surprising because pre-exercise muscle glycogen concentration was actually twice as high in the CHO (73 mmol·kg$^{-1}$ ww) than fat trial (32 mmol·kg$^{-1}$ ww) (Lambert, et al., 1994). That fuel metabolism are likely to be different during exercise at 60% compared to 75-85 % $\dot{V}O_2$ max (Romijn, et al., 1993; 1995) may provide some explanation to this result. Unfortunately, muscle glycogen
utilisation was not measured during exercise at 60 % $\dot{VO}_2$ max in this study (Lambert, et al., 1994).

Apart from its influence on substrate availability, diet may also influence substrate selection in the muscle. Following 3-14 days on a high fat diet, muscle glycogen concentration is lower and plasma FFA concentration higher compared to a mixed- or high-CHO diet (Table 2.4). The rate of muscle glycogen utilisation during exercise also tends to be lower following a high-fat diet (Table 2.3). Peters et al. (1998) reported that short term (3-6 days) severe deficiency of dietary CHO combined with a two-fold increase in dietary fat and protein caused a rapid increase in skeletal muscle PDH kinase activity and decreased CHO metabolism. However, activities of enzymes in fatty acid oxidation did not change (Peters, et al., 1998). Another study also did not find any change in the activities of citrate synthase and $\beta$-hydroxyacyl-CoA dehydrogenase ($\beta$-HAD) after 4-weeks on a modestly-increased fat diets in trained subjects (control diet: 43% fat vs high-fat: 54% fat) (Kiens, et al., 1987). It has been suggested that longer term exposure to a high-fat diet may induce mitochondrial adaptations and increase muscle capacity for fat oxidation (Lambert, et al., 1994). Indeed, irrespective of physical training, $\beta$-HAD activity increased by 25% after 7 weeks following a high-fat (60% fat) compared to a control diet (37% fat) (Helge and Kiens, 1997).

It is well known that endurance training improves the capacity of the muscle for fat metabolism. To examine the interaction of diet and training on metabolism and performance, Helge et al. (1998) assigned two groups of untrained males to a high-fat (62% fat) or high-CHO (65% CHO) diet while following an endurance training programme. Endurance capacity at 80% pre-training $\dot{VO}_2$ max, was improved to the same extent after 2 and 4 weeks on both diets although pre-exercise muscle glycogen concentration and CHO oxidation during exercise were lower in the high-fat group (Table 2.5). In another study, a similar experimental design was used except that the dietary and training regimen was extended to 7 weeks (Helge, et al., 1996). Then, for the 8th week of
training, both groups followed the high-CHO diet. After 7 weeks, endurance capacity at 80% pre-training $\dot{V}O_2$max was improved in both the CHO (35 to 102 min) and fat (36 to 65 min) groups. After 8 weeks, endurance capacity remained unchanged in the CHO group but increased to 77 min in the fat group, which was still less than that of the CHO group. Rate of muscle glycogen breakdown during exercise was reduced equally in both groups (Table 2.5). It seems that in these subjects, adaptation to a high fat diet up to 4-weeks has no consistent impact on endurance performance, whereas beyond 4-weeks, a high-fat diet has a detrimental effect on endurance performance (Helge, et al., 1998). It should, however, be noted that the training regimen used in Hedge's study did not elicit any increase in fat contribution in the energy requirement of exercise performed at the same absolute intensity (Table 2.4) and therefore, these subjects cannot be considered as well-trained.

Regardless of the effects on performance, substrate utilisation during submaximal exercise can be modified substantially by variation in dietary composition of fat and CHO. Current literature suggests that chronic high-fat diets may enhance endurance performance in some well-trained athletes during moderate intensities exercise (Hawley, et al., 1998). However, these results are of practical relevance only to a small select group of athletes.
**Table 2.4 Effect of short term high-fat vs high-CHO or normal mixed diets and fuel metabolism**

<table>
<thead>
<tr>
<th>Study</th>
<th>Diet regimen</th>
<th>Exercise</th>
<th>Initial [FFA] (mmol l⁻¹)*</th>
<th>RER*</th>
<th>Initial muscle glycogen concentration*</th>
<th>Muscle glycogen utilisation rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergstrom, et al., 1967</td>
<td>4 days high fat vs mixed diet</td>
<td>cycle to exh (75% V̇O₂ max)</td>
<td>-</td>
<td>↓</td>
<td>-64%</td>
<td>-37%</td>
</tr>
<tr>
<td>Galbo, et al., 1979</td>
<td>4 days high fat vs high CHO</td>
<td>TM run to exh (70% V̇O₂ max)</td>
<td>~0.6 vs -0.35</td>
<td>↓</td>
<td>-60%</td>
<td>-57%</td>
</tr>
<tr>
<td>Jansson &amp; Kaijser, 1982a</td>
<td>5 days high fat vs high CHO</td>
<td>25min cycle (65% V̇O₂ max)</td>
<td>1.02 vs 0.59</td>
<td>↓</td>
<td>192 vs 437*</td>
<td>-22% (n.s.)</td>
</tr>
<tr>
<td>Jansson &amp; Kaijser, 1982b</td>
<td>5 days high fat vs high CHO</td>
<td>6min cycle (65% V̇O₂ max)</td>
<td>~0.9 vs -0.45</td>
<td>↓</td>
<td>239 vs 445*</td>
<td>-57% (n.s.)</td>
</tr>
<tr>
<td>Jansson &amp; Kaijser, 1984</td>
<td>5 days high fat vs high CHO</td>
<td>25min cycle (65% V̇O₂ max)</td>
<td>~0.85 vs -0.6</td>
<td>↓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Putman et al., 1993</td>
<td>3 days high vs high CHO</td>
<td>~47min cycle (75% V̇O₂ max)</td>
<td>1.26 vs 0.56</td>
<td>↓</td>
<td>185 vs 655*</td>
<td>-63%</td>
</tr>
<tr>
<td>Lambert et al., 1994</td>
<td>2 wks high fat vs high CHO</td>
<td>cycle to exh 90% V̇O₂ max followed by 60% V̇O₂ max</td>
<td>~0.55 vs -0.4</td>
<td>↓</td>
<td>before test 1: 68 vs 121* n.s.</td>
<td>test 1: n.s. 68 vs 121* n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>before test 2: 32 vs 73*</td>
<td>test 2: not measured</td>
</tr>
</tbody>
</table>

*Comparison are made for fat vs CHO or fat relative to CHO diets, *mmol·(kg dw)⁻¹ or *mmol·(kg ww)⁻¹
<table>
<thead>
<tr>
<th>Study</th>
<th>Diet regimen</th>
<th>Exercise</th>
<th>Endurance capacity or performance</th>
<th>RER</th>
<th>Initial muscle glycogen concentration (mmolkg⁻¹dw)</th>
<th>Muscle glycogen utilisation rate (mmol kg⁻¹min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helge et al., 1996</td>
<td>7 weeks high HF vs HC groups with training</td>
<td>cycle to exh at 80% pre-training VO₂max</td>
<td>Improvement in endurance capacity is 55% greater in HC group</td>
<td>no change in HC group, ↓ in HF compared to pre-training</td>
<td>HF 511 vs HC 611</td>
<td>pre-training HF 6.0 vs HC 6.5</td>
</tr>
<tr>
<td></td>
<td>as above</td>
<td>as above</td>
<td>Only further improvement in HF but still lower than HC group</td>
<td>similar in both groups</td>
<td>HF/HC 738 vs HC 561</td>
<td>post-training HF 3.1 vs HC 3.0</td>
</tr>
<tr>
<td></td>
<td>as above</td>
<td>as above</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helge et al, 1998</td>
<td>4 weeks HF vs HC groups with training</td>
<td>cycle to exh at after 2- &amp; 4- wks at 80% pre-training VO₂max</td>
<td>2 wk - +62% in HF (n.s.), +87% in HC 4 wk - +150% in HF, +166% in HC</td>
<td>no change in HC group, ↓ in HF compared to pre-training</td>
<td>HF 460 vs HC 651</td>
<td></td>
</tr>
</tbody>
</table>
2.5.4.2 High-fat vs high-CHO pre-exercise meals

Some studies have compared the effects of a single high fat-low CHO (FM), to an isocaloric high CHO-low fat or normal mixed (CM) meal on subsequent endurance performance (Table 2.6). Although heparin was not used in these studies, there was still a modest but significant elevation in plasma FFA concentrations after the FM compared to the CM (Okano, et al., 1996; 1998; Whitley, et al., 1998). This increased plasma FFA availability resulted in a higher fat oxidation rate during the first hour of exercise (Okano, et al., 1996; 1998; Whitley, et al., 1998). Despite this CHO sparing effect of the FM compared to the CM, endurance capacity (Okano, et al., 1996; 1998) and performance (Whitley, et al., 1998) were not different.

The difference in the amount of CHO provided between the isoenergetic CM and FM in studies by Okano et al. (1996) and Whitley et al. (1998) were 136g and 165g respectively. In the study by Okano, subjects cycled for 2 h at 65 \% \( \dot{V}O_2 \text{ max} \) before pedalling to exhaustion at 80 \% \( \dot{V}O_2 \text{ max} \) (~5 mins) (Okano, et al., 1996). In the study by Whitley, subjects cycled at 70 \% \( \dot{V}O_2 \text{ max} \) for 90 mins and then performed a 10 km time trial (~14 mins) (Whitley, et al., 1998). At the commencement of the respective performance tests, it could be estimated that subjects’ CHO stores in the CM trials must have exceeded the respective FM trials by at least 50g. It may be that such a difference in body CHO stores does not determine performance of short duration-high intensity exercise tests used in studies reported by Okano, et al., (1996) and Whitley, et al., (1998). In this respect, it is also puzzling that Whitely did not find any difference in endurance performance between the CM, which provided 215g CHO, and the fasted trial. This is in sharp contrast which the results of other studies (refer to Table 2.3). Unfortunately, none of these studies provided any information on muscle glycogen metabolism.

In another study, trained subjects consumed isoenergetic (3600 kcal) high-CHO (83\% CHO) or high-fat (68\% fat) meals during a 12-hour period after 2 hours of cycling at 65 \% \( \dot{V}O_2 \text{ max} \) (Starling, et al., 1997). After another 12-hour overnight fast, endurance
performance during a ~2-hour self-paced ride was better in the high-CHO then the high-fat trial (Starling, et al., 1997). This is not surprising since muscle glycogen concentration was higher before exercise in the high-CHO trial (549 vs 327 mmol·kg⁻¹ dw). Therefore, such a glycogen sparing effect after high-fat meal(s) may not translate to better exercise capacity or performance compared to high-CHO meal(s).
Table 2.6 Effects of high fat-low CHO (FM) and high CHO-low fat (CM) pre-exercise meals on metabolism and endurance performance

<table>
<thead>
<tr>
<th>Studies</th>
<th>Total energy intake (kcal)</th>
<th>CHO/fat % energy contribution</th>
<th>Time before exercise (hrs)</th>
<th>Exercise Protocol</th>
<th>Plasma FFA concentration</th>
<th>RER or total CHO oxidation</th>
<th>Endurance capacity or performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sherman et al., 1989</td>
<td>733</td>
<td>FM: 26/60</td>
<td>4</td>
<td>95 min cycling at 52-70% $\text{VO}_{2\text{max}}$ then time trial</td>
<td>no difference</td>
<td>CHO oxidation during 95 mins FM: 142g CM: 156g (n.s.)</td>
<td>no difference in time to complete fixed amount of work</td>
</tr>
<tr>
<td>Okano et al., 1996</td>
<td>1120</td>
<td>FM: 30/61</td>
<td>4</td>
<td>cycling at 65% $\text{VO}<em>{2\text{max}}$ for 2h and at 80% $\text{VO}</em>{2\text{max}}$ till exh</td>
<td>FM &gt; CM throughout exercise</td>
<td>RER during 1st 40 mins CM &gt; FM</td>
<td>cycling capacity: CM: 128 vs FM: 122 mins (n.s.)</td>
</tr>
<tr>
<td>Okano et al., 1998</td>
<td>1150</td>
<td>FM: 30/61</td>
<td>4</td>
<td>cycling at 67% $\text{VO}<em>{2\text{max}}$ for 2h and at 78% $\text{VO}</em>{2\text{max}}$ till exh</td>
<td>FM &gt; CM throughout exercise</td>
<td>RER during 1st 60 mins CM &gt; FM</td>
<td>cycling capacity: CM: 138 vs FM: 141 mins (n.s.)</td>
</tr>
<tr>
<td>Whitley et al., 1998</td>
<td>970</td>
<td>FM: 20/74</td>
<td>4</td>
<td>cycling at 70% $\text{VO}_{2\text{max}}$ for 90 min and 10 km time trial</td>
<td>FM &gt; CM throughout 90 min exercise</td>
<td>CHO oxidation during 90 mins FM: 238g CM: 274g (n.s.)</td>
<td>no difference in time to complete fixed amount of work</td>
</tr>
</tbody>
</table>
2.5.5 Pre-exercise meals and brain serotonin synthesis

The relationship between diet, plasma LNAAs concentration and brain serotonin synthesis was first demonstrated more than 25 years ago (Fernstrom et al, 1971; 1972). Compared to the fasted state, a CHO meal increased the tryptophan-LNAAs ratio through insulin-stimulated skeletal muscle uptake of BCAAs (Fernstrom and Wurtman, 1971; Fernstrom and Wurtman, 1972). Consumption of a protein-free glucose solution has been reported to increase plasma Trp-LNAA ratio by 20% at rest (Martin-Du Pan, et al., 1982). However, the scenario is different during exercise. Carbohydrate ingestion before or during exercise, which depressed plasma FFA concentration, has been reported to attenuate the increase in plasma free-Trp to BCAA ratio during exercise (Davis, et al., 1992; Zanker, et al., 1997).

In one study, breakfasts of corn, wheat, oat cereal or no breakfast were provided 90 min before cycling exercise at 60% \( \dot{V}O_2 \) max (Paul, et al., 1996a). The meals were isoenergetic but had different CHO and protein contents. Postprandial plasma insulin concentration was lowest after oat feeding but plasma Trp-LNAAs ratio for corn was the lowest before and during exercise (Paul, et al., 1996a). The authors concluded that when a CHO meal is not protein free, the direct effect of the protein content may exert a greater effect on changes in plasma tryptophan-LNAAs ratio than the indirect effect of insulin (Martin-Du Pan, et al., 1982; Paul, et al., 1996a). However, the small difference in plasma Trp:LNAAs ratio caused by the different feedings was insufficient to influence physical and cognitive performance (Paul, et al., 1996a).

In another study, either no meal or a high CHO meal (80% CHO, 10% protein, 10% fat) was provided to subjects who had undergone a prior glycogen depletion regimen 3 hours before 60-mins treadmill running exercise at 75% \( \dot{V}O_2 \) max (Zanker, et al., 1997). The consumption of the pre-exercise meal resulted in attenuation of the increase in plasma free-Trp to BCAA ratio during exercise. This increase of plasma free-Trp to BCAA ratio reflected increases in free-Typ rather than the decline in BCAAs concentrations (Zanker, et al., 1997). Davis et al., (1992) reported that cycling endurance capacity at \( \sim 68 \)
\% \dot{V}O_2\text{max} was enhanced when subjects received 6\% of 12\% CHO solution during exercise compared to placebo. The five to seven-fold increase in plasma free-Trp to BCAA ratio during the placebo trial was attenuated in a dose related manner when subjects received CHO. Moreover, plasma free-Trp and plasma FFA concentrations were highly correlated. The authors of the last two studies suggested that the CHO feedings not only reduced the exercise induced rise in plasma FFA concentration but also attenuated the increase in plasma free-Trp concentrations (Davis et al., 1992; Zanker et al, 1997). However, the lack of assessment of muscle glycogen and specific measures of central fatigue prevents the further delineation of the mechanism of performance enhancement in Davis et al.'s (1992) study.

In the postprandial resting period, consumption of high GI CHO may result in a greater serotonergic effect a low GI CHO (Lyons and Truswell, 1988). This can be attributed to the greater insulin response following the high GI meal, stimulating BCAA uptake and hence lowering the ratio of plasma free-Trp to BCAA. Furthermore, the coingestion of fat or protein may neutralise this effect (Lyons and Truswell, 1988). The influence of GI of pre-exercise meals on the ratio of plasma Free-Trp to BCAA during exercise has not been studied. During exercise, plasma FFA concentration is lower following consumption of high than low GI CHO (DeMarco, et al., 1999; Thomas, et al., 1991; Thomas, et al., 1994). Because concentrations of plasma FFA and free-Trp are correlated, one might expect plasma free-Trp concentration to be higher after pre-exercise ingestion of low than high GI CHO.
CHAPTER 3
GENERAL METHODS

3.1 Introduction
This chapter describes the equipment and procedures used during the preliminary and main experimental tests employed by the four studies described in this thesis. Ethical approval was obtained from Loughborough University Ethical Advisory Committee for all procedures described in this thesis. The ‘Code of Practice for Workers having Contact with Body Fluids’ was strictly adhered to. All subjects were informed of the nature, purpose and possible risks of the each experiment before their written consent for participation was obtained. Subjects were also required to complete a questionnaire providing information of their medical history and current training status.

3.2 Experimental design
In the four studies in this thesis, breakfasts containing different types and amounts of carbohydrate (CHO) were consumed by subjects in the morning, after 12 to 16 hours overnight fast. In all studies, the resting metabolic responses of subjects were monitored for 3 hours after the meals. In Study 2 (Chapters 4 and 5) and 3 (Chapter 6), after the three hour postprandial rest period, subjects ran on the treadmill at 70 \% \dot{V}O_2 \text{ max} until volitional fatigue. In Study 4 (Chapter 7), after the 3-hour postprandial period, the subjects were only required to perform a 30 min of treadmill running at 70 \% \dot{V}O_2 \text{ max}. The experimental protocols are summarised in Fig 3.1. The order of the trials was randomised and also counterbalanced when possible. The interval between the main tests for each subject was three to seven days for study 1 (Chapter 4) and one week to two weeks for studies 2 (Chapters 4 and 5), 3 (Chapter 6) and 4 (Chapter 7).

3.3 Preliminary measurements
Body mass of the subjects was measured using a calibrated balance (Avery 3306 ABV) and their height was measured using a stadiometer (Holtain Ltd). A motorised treadmill (Quinton or Marquette, both Seattle, USA) was used in all exercise tests. The
treadmill was connected to a microcomputer (BBC Master series) which was interfaced with two channel 40/80 track single disc drives (Akher Instrument Ltd, Type DS80TK) and a printer. The speed and distance covered was continuously monitored using a software designed in-house to interface with the treadmill. Prior to each study, the treadmill was calibrated by measuring both the treadmill belt length and the time required to complete 50 revolutions at various speeds. The speed shown on the interfaced computer was validated against the actual calculated speeds of the treadmill. In all studies, subjects performed two preliminary tests to determine running economy and \( \dot{V}O_2 \) max (Williams, et al., 1990).

The relationship between oxygen uptake (\( \dot{V}O_2 \)) and running speed was determined by a 16-min continuous submaximal test. The initial speed was set between 2.0 to 3.5 m.s\(^{-1}\) on a level treadmill, depending on the gender and training status of the subjects. The test consisted of 4 stages in which the treadmill speed was increased every 4 minutes by 0.4-0.5 m.s\(^{-1}\). Expired air samples were collected during the last minute of each stage. A regression equation relating \( \dot{V}O_2 \) to running speed was calculated from the result of this 16 min test.

The \( \dot{V}O_2 \) max of each subject was determined during an uphill treadmill running test (Taylor, et al., 1955). Treadmill speed was kept constant throughout the test and the inclination was increased from an initial of 3.5% by 2.5% every 3 min. Expired air samples were collected during the 1:45-2:45 min of each 3 min. A final expired air sample was taken during the last minute of the test immediately after the subject signalled that the running speed could be only be sustained for a final minute. Verbal encouragement was given throughout this test. The highest value for \( \dot{V}O_2 \) during this test was considered to be the \( \dot{V}O_2 \) max of the subject. The criteria used to determine the attainment of \( \dot{V}O_2 \) max were 1) the achievement of a plateau in \( \dot{V}O_2 \) (< 2 ml.kg\(^{-1}\).min\(^{-1}\) increase) with an increase in workrate and 2) a respiratory exchange ratio (RER) >1.10.
In studies 2, 3 and 4 (Chapters 5, 6 and 7 respectively), on a separate occasion at least 3 days before the first main test, subjects completed a one-hour run at ~70 % \( \dot{V}O_2 \max \) to familiarise them with procedures and measurements to be used during the main trials. During this test, subjects followed the drinking pattern, if any, which was used during the main exercise tests (study 2 (Chapter 5) and study 3 (Chapter 6)). The fluid was provided in 30ml plastic syringes during the run to prevent spillage. Expired air samples were collected for 1 minute every 15 minutes.
Fig 3.1 Schematic representation of the experimental procedures

- Meal
- Postprandial Rest
- 70%VO2max

EA: expired air collection (*Study 4 only)
VS: venous sample collection (*Study 4 only)
FI: prescribed fluid intake (Study 2 & 3 only)
MS: muscle biopsy sample (Study 4 only)

Legends:
- 3 hrs rest / digestion
- 5 min warm up at 60 %VO2max
- run at 70 %VO2max
3.4 Main experimental trials

Subjects recorded all their dietary intake and exercise training during the two days prior to the first main trial and replicated these closely before the subsequent trial(s). No exercise training was undertaken in the 24 hour prior to each main trial. These dietary and training control regimen have been shown to result in very similar muscle glycogen concentrations (Tsintzas, et al., 1995). The dietary information obtained was then analysed by a registered dietitian. All energy values are reported as kcal in this thesis, rather than kJ because of the frequent use of the common use of kcal.

Subjects reported to the laboratory at between 0800-1000 hrs after a 12-16 hour overnight fast. This was to ensure an empty stomach and minimise the effect of a previous meal on gastric emptying of the test meals. Venous blood samples were obtained from an ante-cubital vein using an indwelling cannula (Venflon, 16G), which was inserted under local anaesthesia (0.5ml of 1% lignocaine). The cannula was inserted after the subjects had rested for 15 min on an examination couch. The cannula was kept patent by infusion of sterile isotonic saline immediately after cannula insertion and each collection. A fasting blood (10ml) and expired air samples were obtained before each test meal was eaten. The meals were consumed in the laboratory while sitting in a quiet section of the room. The time was noted as the last mouthful of food was swallowed. Expired air and blood samples (10ml) were then collected at intervals as summarised in Fig 3.1. Subjects remained in the laboratory throughout the 3-hour postprandial rest period and maintained a seating (Study 1) or standing (Studies 2, 3 and 4) posture for at least 15 min before and during each blood and expired air collection. This was to minimise the effect of postural change on plasma volume (Harrison, 1985).

In studies 2 (Chapter 5) and 3 (Chapter 6), the final resting (3-hour post-meal) expired air and blood samples were collected with the subject standing on the treadmill. After the collection, the subject immediately drank 350 ml of water. In study 4 (Chapter 7), the 3-hour expired air sample was not collected due to the necessary preparation for the muscle sample to be collected at the same time. Furthermore, as the exercise bout
was not exhaustive, subjects did not receive any fluid just before or during exercise. The subject then proceeded to warm up at \(-60\% \tilde{V}O_2\) max for 5 min before running at \(-70\% \tilde{V}O_2\) max. Each subject ingested a fixed amount (90-150 ml) of fluid every 20 min during the run in studies 2 and 3 (Chapters 5 and 6 respectively) to prevent substantial dehydration. In studies 2 and 3 (Chapters 5 and 6 respectively), exhaustion was defined as the point in time when subjects were no longer able to maintain the treadmill speed. They had the option of slowing the treadmill twice, for two min each time, once to \(-60\% \tilde{V}O_2\) max and again to a walk at \(-5 \text{ km-h}^{-1}\). This was to ensure that subjects were truly fatigued. The run was terminated when subjects could no longer maintain the prescribed running speed i.e. equivalent to 70 \% \tilde{V}O_2\) max. The same procedures of slowing down were repeated during subsequent trials. Subjects were not told their running times until the end of their last trial. In studies 2 and 3 (Chapters 5 and 6 respectively), nude body mass was obtained before and after the run for calculation of sweat loss during the run. Heart rate (HR) was monitored during all exercise tests using short range telemetry (Polar Electro sports tester PE 3000). The runners' rate of perceived exertion (RPE) (Borg, 1973) was recorded during each collection of expired air.
3.5 Carbohydrate feedings

In studies 1 and 3 (Chapters 4 and 6 respectively), two isoenergetic test meals consisting of high and low (LGI) glycaemic index CHO (HGI and low GI respectively) sources of similar volume and macronutrients composition (826 kcal: 67% CHO, 30% protein, 3% fat) were used (Table 3.1). Lentils was chosen for the LGI meal because these have been consistently shown to elicit low glycaemic responses in both normal and diabetic subjects (Foster-Powell and Brand Miller, 1995). Because lentils are high in protein, tuna was added to the HGI meal so that both meals had the same amount of protein. The lentils were boiled in water with a small piece of vegetable tablet (Sainsbury Ltd) at high heat for approximately 10 min and then simmered at low heat for another 10 min. The skinned potatoes were microwaved for 20 min at high heat and consumed together with the tuna and sweetcorn. The crumpets were toasted and served with honey. The amount of CHO ingested was equal to 2 g of CHO kg\(^{-1}\) body mass. In study 2 (Chapters 4 and 5), three test meals were provided (Table 3.2). The high CHO-low fat (CM: 2.5 g CHO kg\(^{-1}\) body mass) and high fat-low CHO (FM: 1 g CHO and 0.7 g fat kg\(^{-1}\) body mass) meals were isoenergetic. The high CHO-high fat meal (HM) contained the same amount of CHO as CM and the same amount of fat as FM. The meals were similar in volume and protein content. In study 4 (Chapter 7), two isocaloric test meals consisting of high (HGI) and low (LGI) GI CHO sources of similar volume and macronutrients composition (817 kcal: 86% CHO, 10% protein, 4% fat) were used (Table 3.3). Within each meal, all subjects consumed the food items in the same order. For each subject, the meals were similar in volume in order to minimise any differences in gastric emptying. All meals were consumed within 15-30 min. Nutritional content of each meal was calculated from information provided by the manufacturer (Table 3.1, 3.2 and 3.3). The incremental areas under the blood and plasma glucose and serum insulin response curves (IAUC) for the initial 1, 2 and 3 hour(s) after ingestion of each meal were calculated using the trapezoidal rule with fasting value taken as the baseline and negative areas ignored (Wolever et al, 1991; Thomas et al, 1994).
Table 3.1 *Study 1 and 3 (Chapters 4 and 6 respectively): Compositions of the HGI and LGI meals per 70 kg body mass*

<table>
<thead>
<tr>
<th>Meal</th>
<th>Description</th>
<th>Nutrient Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGI</td>
<td>230 g dry red lentils + 350 ml flavoured water</td>
<td>826 kcal, 140 g CHO, 63 g protein, 4 g fat, 700 ml water estimated GI = 26*</td>
</tr>
<tr>
<td>HGI</td>
<td>226 g potatoes + 177 g tuna + 113 g crumpets + 120 g sweetcorn + 14 g honey + 350 ml flavoured water</td>
<td>826 kcal, 140 g CHO, 63 g protein, 4 g fat, 700 ml water estimated GI = 74*</td>
</tr>
</tbody>
</table>

* calculated by method described in Wolever (1990) with GI values taken from Foster-Powell and Brand Miller (1995).
<table>
<thead>
<tr>
<th>Meal</th>
<th>Description</th>
<th>Nutrient Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FM</strong></td>
<td>94 g bananas + 20 g Jaffa cakes + 77 g fresh double cream + 120 g ice cream + 403 ml Lucozade placebo</td>
<td>756 kcal, 70 g CHO, 8 g protein, 49 g fat, 715 ml water</td>
</tr>
<tr>
<td><strong>CM</strong></td>
<td>256 g bananas + 43 g raisins + 61 g Jaffa cakes + 213 ml Lucozade original + 140 ml Lucozade placebo</td>
<td>759 kcal, 175 g CHO, 8 g protein, 3 g fat, 715 ml water</td>
</tr>
<tr>
<td><strong>HM</strong></td>
<td>205 g bananas + 17 g raisins + 48 g Jaffa cakes + 68 g fresh double cream + 120 g ice cream + 256 ml Lucozade original</td>
<td>1173 kcal, 175 g CHO, 8 g protein, 49 g fat, 715 ml water</td>
</tr>
</tbody>
</table>
Table 3.3 Study 4 (Chapter 7): Compositions of the HGI and LGI meals per 70 kg body mass

<table>
<thead>
<tr>
<th>Meal</th>
<th>Description</th>
<th>Nutrient Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGI</td>
<td>80 g bran flakes + 200 ml skimmed milk</td>
<td>817 kcal, 175 g CHO,</td>
</tr>
<tr>
<td></td>
<td>+ 360 g canned peach in own juice +</td>
<td>21g protein, 4g fat,</td>
</tr>
<tr>
<td></td>
<td>300 g apples + 500 ml unsweetened apple juice</td>
<td>1100 ml water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>estimated GI = 36*</td>
</tr>
<tr>
<td>HGI</td>
<td>60 g corn flakes + 240 ml skimmed milk</td>
<td>817 kcal, 175 g CHO,</td>
</tr>
<tr>
<td></td>
<td>+ 100 g white bread + 30 g raspberry jam</td>
<td>21g protein, 4g fat,</td>
</tr>
<tr>
<td></td>
<td>+ 265 ml Lucozade original energy drink</td>
<td>1100 ml water</td>
</tr>
<tr>
<td></td>
<td>+ 587 ml water</td>
<td>estimated GI = 80*</td>
</tr>
</tbody>
</table>

* calculated by method described in Wolever (1990) with GI values taken from Foster-Powell and Brand Miller (1995)
3.6 Measurement of gas exchange

The collection of expired air samples was carried out using the Douglas bag method. Expired air were collected through a low resistance respiratory valve and lightweight, wide bore (40mm) tubing (Falconia Ltd) into a 150-litre Douglas bag. Resting and exercising samples were collected for 5 minutes and 1 minute respectively. In studies 1 and 3 (Chapters 4 and 6 respectively), oxygen and carbon dioxide content were analysed using a paramagnetic oxygen analyser (Sybron-Taylor, Servomex model 570A) and an infrared carbon dioxide analyser (Lira, MSA model 303) respectively. In studies 2 (Chapters 4 and 5) and 4 (Chapter 7), another similar analyser was used (Servomax 1440). Instruments were calibrated against nitrogen and known gas mixtures before and between a series of gas analyses. A dry gas meter (Harvard Apparatus) calibrated against a 600-litre Tissot spirometer (Collins Ltd) and a thermometer (Edale Instruments, model C) was used to measure the volume and temperature of the expired air samples. Barometric pressure was obtained from a barometer (Griffen and George Ltd). All gas volumes were corrected to STPD conditions. Using the Haldane transformation formula, minute oxygen uptake ($\dot{V}O_2$), carbon dioxide expired ($\dot{V}CO_2$), ventilation rate (VE) and RER were calculated. Substrate oxidation rates (g/min) were calculated using the $\dot{V}CO_2$ and $\dot{V}O_2$ values and the non-protein RER (Ferrannini, 1988; Frayn, 1983):

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

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

where $\dot{V}CO_2$ and $\dot{V}O_2$ are the gas volumes measured in l.min$^{-1}$. The total CHO and fat oxidised was estimated from the area under the CHO and fat oxidation versus time curve for each subject.
3.7 Treatment and analysis of blood samples

Each blood sample was dispensed into heparin (studies 1 (Chapter 4) and 3 (Chapter 6)) or Na₂EDTA (studies 2 (Chapters 4 and 5) and 4 (Chapter 7)) and serum tubes (Sarstedt). Serum samples were left to clot for 60 min at 3°C and then centrifuged (Burkard) at (3°C) for 15 min at 6000 rpm. The serum obtained was then stored at -70°C and later analysed for insulin (radio-immunoassay, Coat-A-Count Insulin, DPC kit), cortisol (radio-immunoassay, Coat-A-Count Cortisol, DPC kit) (study 3 (Chapter 6)) and prolactin (radio-immunoassay, Coat-A-Count Prolactin, DPC kit) (study 2 (Chapter 5) using a gamma counter (Packard, Cobra 5000). In studies 1 and 3 (Chapters 4 and 6 respectively), the serum was also analysed for free fatty acid (FFA) concentrations (Wako chemicals GmbH kit) using an automatic photometric analyser (Cobas Bio); sodium and potassium concentrations by flame photometry (Ciba Corning 480) and osmolality (Osmotat 030). Duplicate 20μl aliquots of whole blood were used for the determination of haemoglobin concentration by the cyanmethemoglobin method (Boehringer Mannheim, FRG) (studies 2 (Chapter 5) and 3 (Chapter 6)). Triplicate 20μl aliquots of whole blood were also used for the determination of haematocrit values after micro-centrifugation (Hawksley Ltd.) by using a haematocrit reader (Hawksley Ltd.) (studies 2 (Chapter 5) and 3 (Chapter 6)). Changes in plasma volume were estimated from changes in haemoglobin concentrations and haematocrit values (Dill and Costill, 1974). Duplicate 20μl aliquots of blood were also deproteinised in 200 μl of 2.5% perchloric acid. The deproteinised samples were centrifuged for 4 min at 13000 rpm, frozen at -20°C and later analysed for glucose (Boehringer Mannheim Glucose test combination, GOD/Perid method) (studies 1 (Chapter 4) and 3 (Chapter 6)) using a spectrophotometer (Eppendorf 1101M or Cecil 2393) and lactate (Maughan, 1982) (all studies) using a fluorimeter (Locarte, Model 8-9). The remaining volume of blood in the EDTA or heparin tubes was centrifuged for 15 min at 6000 rpm and the plasma obtained, stored at -20°C and later analysed for FFA (Wako chemicals GmbH kit), glucose (Boehringer Mannheim GmbH) using an automatic photometric centrifugal analyzer (Cobas-Mira, Roche) (studies 2 (Chapters 4 and 5) and 4 (Chapter 7)) and glycerol (Laurell and Tibbling, 1966) using a fluorimeter (Locarte,
Model 8-9) (all studies). In studies 2 (Chapter 5) and 4 (Chapter 7), samples for glucagon were also collected in chilled tubes containing Na₂EDTA and 100μl aprotinin (Trasylool), which contained 1000 kallikrein inactivating units (KIU) for 2 ml of whole blood. The blood was centrifuged for 15 min at 6000 rpm and the plasma obtained, stored in glass tubes at -20°C. Plasma glucagon concentrations were determined using double antibody radioimmunoassay procedure (DPC kit) using automatic gamma counter (Packard, Cobra 5000). Analytical procedures for non-automated blood metabolites assays are documented in Appendix B.

The coefficient of variation [(S.D./mean)*100] of the blood, plasma and serum constituents/metabolites assays is shown in Table 3.4.
Table 3.4: Coefficients of variation (CV) of blood, plasma and serum constituents/metabolites assays (n = 10 to 15) (within run assays).

<table>
<thead>
<tr>
<th>Constituents/Metabolite</th>
<th>Concentration</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manual analyses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>11.6 g·dl⁻¹</td>
<td>1.1</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>46 %</td>
<td>0.6</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>6.20 mmol·l⁻¹</td>
<td>2.3</td>
</tr>
<tr>
<td>Blood lactate</td>
<td>2.3 mmol·l⁻¹</td>
<td>2.4</td>
</tr>
<tr>
<td>Plasma glycerol</td>
<td>0.04 mmol·l⁻¹</td>
<td>0.5</td>
</tr>
<tr>
<td>Serum potassium</td>
<td>4.6 mmol·l⁻¹</td>
<td>0.2</td>
</tr>
<tr>
<td>Serum sodium</td>
<td>124 mmol·l⁻¹</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Auto-analyses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>4.2 mmol·l⁻¹</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>12.5 mol·l⁻¹</td>
<td>1.0</td>
</tr>
<tr>
<td>Serum FFA</td>
<td>0.81 mmol·l⁻¹</td>
<td>0.8</td>
</tr>
<tr>
<td>Plasma FFA</td>
<td>0.80 mmol·l⁻¹</td>
<td>0.7</td>
</tr>
<tr>
<td>Serum insulin</td>
<td>11.0 mU·l⁻¹</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>31.1 mU·l⁻¹</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>101.8 mU·l⁻¹</td>
<td>5.5</td>
</tr>
<tr>
<td>Serum cortisol</td>
<td>11.4 µg·dl⁻¹</td>
<td>6.5</td>
</tr>
<tr>
<td>Plasma glucagon</td>
<td>118 ng·ml⁻¹</td>
<td>5.3</td>
</tr>
<tr>
<td>Serum prolactin</td>
<td>8.8 ng·ml⁻¹</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>55 ng·ml⁻¹</td>
<td>1.6</td>
</tr>
</tbody>
</table>
3.8 Collection of muscle samples, extraction and analyses of metabolites.

In study 4 (Chapter 7), muscle samples were obtained from the vastus lateralis using the needle biopsy technique (Bergstrom, 1962) with suction applied (Evans, et al., 1982). The vastus lateralis muscle has been shown to be heavily involved during treadmill running exercise (Tsintzas, et al., 1995; Tsintzas, et al., 1996). Within each trial, samples were obtained from one leg before the test meal, three hour after each test meal and immediately after exercise (Figure 3.1). For the second trial, samples were obtained from the alternate leg. Each sample was taken through a separate skin incision which was made under local anaesthetic (1% lignocaine) using a surgical blade while the subject was lying on an examination couch. After removing the biopsy needle from the leg, the needle was immediately immersed in liquid nitrogen. The muscle sample was then removed from the needle under liquid nitrogen and placed in screw-top plastic tubes (Eppendorf) and stored in liquid nitrogen until it was freeze dried (Edwards RV5, West Sussex, England) at a later date. After samples were freeze dried, they were treated with petroleum ether, dissected free of any visible blood and connective tissues, weighed (Mettler Toledo AG245, Switzerland) and subsequently stored in a dry environment (silica gel) at -70°C.

Muscle metabolites were extracted by adding 1ml of 5 mmol.L⁻¹ perchloric acid (HClO₄) containing 1 mmol.L⁻¹ EDTA.Na (ethylenediamine tetra-acetic sodium salt) for every 10 mg of muscle powder. Each sample was then placed on ice and vortexed intermittently for 10 min, and then centrifuged (3°C) at 14000 rpm for 1 min. The supernatant was then removed using a volumetric pipette, carefully measured, and placed into clean cool plastic tubes. It was then neutralised by the addition of one-fourth volume of 2.2 mol.L⁻¹ potassium bicarbonate (KHCO₃), mixed and left on ice for 5 min for the CO₂ formed to escape, before centrifuging for 5 min at 3°C. One ml of the extract was equivalent to 8 mg of muscle powder and its pH was 7.0 (Harris, et al., 1974).

All muscle assays were performed within three days of each other. Concentrations of glucose, glucose-6-phosphate (G-6-P), lactate, ATP, creatine (Cr) and
phosphocreatine (PCr) were determined enzymatically (Lowry and Passonneau, 1972; Harris, et al., 1974). A fraction of the extract as well as the PCA insoluble faction was hydrolysed in 1 mol.L\(^{-1}\) hydrochloric acid (HCl) for the determination of macroglycogen (from the PCA extract) and proglycogen (from the PCA insoluble faction) (Adamo and Graham, 1998; Jansson, 1981). The total mixed muscle glycogen concentration (TG) was calculated by adding the macroglycogen (MG) and proglycogen (PG) concentrations. All the muscle metabolites were assayed spectrophotometrically (DU 650 Beckman, U.S.A.) except for lactate and MG, which were assayed fluorimetrically (F-2000 Fluorescence Spectrophotometer, Hitachi, Tokyo, Japan). Analyses for ATP, PCr, lactate and MG were based on enzyme catalysed reactions in which the coenzymes NAD\(^+\) and NADP\(^+\) were simultaneously reduced to NADH and NADPH, respectively. Analysis for creatine was based on the formation of NAD\(^+\) and acid PG was assayed spectrophotometrically for glucose using a commercially available kit (Boehringer Mannheim Glucose test combination, GOD/Perid method). Procedures of the muscle metabolites assays are documented in Appendix A.

All chemicals (Grade I) were obtained as standard commercial items from Boehringer, Fisons and Sigma Ltd. All reagents were made up with double distilled water on the day(s) prior to analyses. The concentrations of the metabolites were adjusted to the highest content of total creatine (PCr + Cr) in each subjects series (Harris, et al., 1974). The correction for total creatine was performed in order to compensate for any mixture of elements such as connective tissue, fat droplets or blood in the muscle samples. However, muscle glucose and lactate were not adjusted for total creatine content because these two metabolites can occur in significant amounts in both muscle and blood. The coefficients of variation for all the muscle assays are presented in Table 3.5.
**Table 3.5 Coefficients of variation of muscle metabolites assays (n=10 or 12)**

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Concentration (mmol·(kg dw)^{-1})</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proglycogen</td>
<td>342.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Macroglycogen</td>
<td>50.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Adenosine triphosphate</td>
<td>25.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>85.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Creatine</td>
<td>45.3</td>
<td>5.9</td>
</tr>
<tr>
<td>Glucose</td>
<td>50.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>1.17</td>
<td>6.4</td>
</tr>
<tr>
<td>Lactate</td>
<td>13.6</td>
<td>2.6</td>
</tr>
</tbody>
</table>
Chapter 4
Carbohydrate availability controls substrate oxidation in the postprandial state in healthy subjects

4.1 Introduction
It has been well established that an increased carbohydrate (CHO) content in the diet leads to increased CHO oxidation both at rest and during exercise. Some studies have reported that the addition of fat to a CHO meal caused no alteration in the oxidation of individual macronutrients, implying that all the additional fat had been stored (Bennett, et al., 1992; Flatt, et al., 1985; Shutz, et al., 1989). However, these studies compared rates of substrate oxidation after meals with different energy contents, and so energy intake increased according to the amount of fat added to the meal. When short term dietary energy intakes are similar (Roy, et al., 1998; Stubbs, et al., 1993; Thomas, et al., 1992; Verboeket-van de Venne, et al., 1994) or when isoenergetic meals are consumed (Babbioni-Harcsh, et al., 1996; Whithley, et al., 1997), fat oxidation appears to increase in proportion to the fat content of the meal. Nevertheless, shifts in CHO oxidation in response to isoenergetic diets of different CHO and fat composition are greater than changes in fat oxidation (Roy, et al., 1998; Thomas, et al., 1992). Recently, Jebb et al. (1996) demonstrated an oxidative hierarchy from CHO, protein to fat; fat being the most easily stored and least readily oxidised. Most of these studies have not considered the influence of training status of their subjects on the postprandial responses to different meals. Aerobically trained individuals frequently display a diminished early phase insulin secretion (LeBlanc, et al., 1984; Tremblay, et al., 1985), which may reduce the suppression of the activity of hormone sensitive lipase in adipose tissue and increase lipolysis. For example, Tremblay et al. (1983) reported greater fat oxidation in aerobically-trained than untrained men at rest.

Overall substrate metabolism has been postulated to be regulated by the concentrations of blood-borne substrates. Some support for this is provided by studies which utilised lipid or glucose infusions (Boden and Jadali, 1991; Campbell, et al., 1992; Sidossis, et al., 1998; Sidossis and Wolfe, 1996). High plasma FFA concentrations increase hepatic
glucose output and reduce glucose clearance at rest but healthy individuals can 
compensate for these effects with additional secretion of insulin (Boden and Jadali, 
while maintaining a constant rate of FFA re-esterification (Campbell, et al., 1992). 
Furthermore, glucose and insulin may regulate fat metabolism by limiting oxidation of 
long-chain-fatty acid (Sidossis and Wolfe, 1996). These studies infused glucose or 
lipids to increase their circulating concentration. In contrast, there is little information 
on the influence of different types and composition of meals on substrate oxidation. It 
has been well established that large differences exist in acute postprandial glucose and 
insulin responses to different types of CHO (Wolever, 1990). Starchy foods, such as 
bread and potatoes, are digested very rapidly and produce large increases in blood 
glucose concentration compared to foods such as beans and lentils (Foster-Powell and 
Brand Miller, 1995). The glycaemic index (GI) provides a useful method of ranking 
foods according to their glycaemic responses (Wolever, 1990).

Tappy and coworkers (1986) reported that the elevation of glucose oxidation was less 
following the ingestion of beans compared to potatoes. In contrast, Ritz et al. (1991) 
reported that compared to glucose, the dietary use of manioc starch (assumed to be a 
low GI food by the authors) leads to a lower fatty acid concentration, which in turn is 
responsible for higher CHO oxidation and lower body glucose storage. These studies 
provided only a small amount of CHO compared to that consumed during a normal 
meal (Tappy, et al., 1986; Ritz, et al., 1991). It is worth remembering that normal 
meals are made up of a mixture of macronutrients and rarely do they contain CHO 
alone. Fat in a meal delays gastric emptying by increasing the viscosity of the effluent 
through gastrointestinal tract and interferes with the enzyme-CHO interaction and 
protein stimulates insulin secretion (Gulliford, et al., 1989). There may also be a neural 
response to a high fat or high protein meal mediated by cholecystokinin formation, 
slowing gastric motility. Hence both nutrients might be expected to lower the 
glycaemic response of a high GI food such as potatoes.
This chapter describes the result of two studies which examine the influence of different breakfasts on postprandial substrate oxidation in physically active subjects. In the first study of this thesis, we examined the influence of high and low GI breakfasts (HGI and LGI respectively) on postprandial substrate oxidation. The two test meals contained the same amount of CHO, fat and protein. In the second study, we investigated the influence of CHO and fat content of breakfasts on postprandial and exercise metabolism. In order to further elucidate the major influence on postprandial metabolism, the postprandial data of the second study has been included in this chapter. For the sake of clarity, the result of the first study will be presented as Part I and the postprandial data of the second study will be presented as Part II in this chapter.

In Part II, substrate oxidation was compared after three breakfasts containing different amounts of CHO and fat. In addition to a high CHO-low fat (CM) and a high fat-low CHO meal (FM), a third meal which contained the same amount of CHO as in CM and fat as in FM was also provided. This third meal was included as a comparison of the metabolic responses of adding extra CHO to a high-fat meal or conversely, adding extra fat to a high-CHO meal. The postprandial resting metabolic responses in both parts I and II were monitored for 3 hours. A 3-hour observation period was adopted because it is common practice for athletes to eat their pre-exercise meal no later than 3 hours before physical training or competition (Hawley and Burke, 1997).

4.2 Methods
Three women and seven men were recruited for Part I and six men (the six in Chapter 5) were recruited for Part II (Table 4.1). All subjects were healthy and physically active, non-smokers. The subjects in Part I were physically active PE students and subjects in Part II were runners who trained four to seven times a week and averaged a total of 52 ± 3 miles per week.

Part I and II consisted of two and three experimental trials respectively. Trials in part I were between 3 to 7 days apart and in part II they were between 7 to 14 days apart.
All subjects were randomly assigned to all trials. The only factor that distinguished each trial was the type of meal consumed. Subjects were required to record their weighed food intake during the two days prior to the first trial, and to replicate their diet before the subsequent trial(s). The dietary information obtained was then analysed by a registered dietitian. There were no significant differences between trials within each study in the average daily energy intake (overall mean values presented in Table 4.1), or the macronutrient composition (overall mean of 58% CHO, 26% fat and 16% protein) consumed during the two days prior to each trial.

Subjects arrived at the laboratory at 8:00am after a 12 hour overnight fast. After collection of resting venous blood and expired air samples, each subject then consumed the test meal. In Part I, two isoenergetic meals of the same CHO, fat, protein composition (g/70 kg body mass): 140 g, 4 g, 63 g respectively, were given (Table 3.1). In Part II, the three test meals contained CHO, fat, protein in the following amounts respectively (g/70 kg body mass): FM 70 g, 49 g, 8 g; CM 175 g, 3 g, 8 g; HM 175 g, 49 g, 8 g (Table 3.2). The HM provided an additional ~416 kcal of CHO energy when compared to FM. When compared to CM, the HM provided the same amount of additional energy as fat. For each subject, the meals were similar in volume in order to minimise any differences in gastric emptying. The meals were consumed within 15-30 min and subjects remained in the laboratory throughout the 3-hour postprandial rest period during which expired air and venous blood samples were collected (Figure 3.1). During this time, subjects sat quietly reading or watching television.

The incremental area under the blood or plasma glucose and serum insulin response curves (IAUC) for the initial 1, 2 and 3 hour(s) after ingestion of each meal were calculated using the trapezoidal rule with fasting value taken as the baseline and negative areas being ignored (Wolever, et al., 1991). The glucose and insulin IAUC in both parts I and II were not normally distributed and so these were analysed by the Wilcoxon test. Time dependent variables were analysed by using two-way ANOVA with repeated measures (meal x time). Other non-time dependent variables such as
CHO oxidation, fat oxidation and energy expenditure were compared by one way ANOVA (Part I) or paired t-test (Part II). Significant mean differences for the ANOVA were located using the Tukey post hoc test. Statistical significance was set at P < 0.05. All data are reported as mean ± SE.

4.3 Results

Part I

During the 3 hour postprandial period, energy expenditure was 19% (P<0.01) greater after the HGI than the LGI meal (Figure 4.1, upper panel). Furthermore, overall CHO oxidation was 46% higher (P<0.01) and fat oxidation 37% lower (P<0.01) after the HGI than the LGI meal.

Blood glucose concentration increased and reached a peak 15 min after the HGI meal (P<0.01 from LGI and fasting values) but did not change significantly after the LGI meal (Figure 4.2, upper panel). The mean IAUC for blood glucose and serum insulin concentrations are shown in Figure 4.3 (upper and lower panel respectively). The 1, 2 and 3-hour IAUC for blood glucose concentrations were 5.4, 3.2, 1.2-fold greater (P<0.05 in all cases) after the HGI meal than after the LGI meal. According to the method proposed by Wolever for calculating the GI of a mixed meal, the predicted GI was 74 for the HGI meal (Wolever, 1990) and 26 for the LGI meal (Foster-Powell and Brand Miller, 1995). From our results, the calculated GI of the HGI meal in 1, 2 and 3 h was 167, 83, 57 respectively, when the GI of the LGI meal was considered to be 26.

Serum insulin concentration increased by 10-fold from resting value within 15 min after consumption of the HGI meal. The increased in serum insulin concentrations was more pronounced (between 2- to 3-fold) and gradual after the LGI meal (Figure 4.2). Peak serum insulin concentrations ranged from 18 to 71 mIU.l⁻¹ and 59 to 220 mIU.l⁻¹ for the LGI and HGI trials respectively. The 1, 2 and 3-hour IAUC for serum insulin response were 5.1-, 3.8-, 3.0-fold (P<0.05 in all cases) respectively greater after the HGI meal than after the LGI meal.
The plasma glycerol and FFA response curves during the 3-hour postprandial period are shown in Figure 4.4 (upper and lower panel respectively). After ingestion of both the high and low GI meals, plasma glycerol and FFA decreased and remained lower than fasting concentrations. However, plasma glycerol concentration was higher during LGI than HGI trial 1 hour after the meals until the end of the 3 hour postprandial period. Plasma FFA concentrations were also higher during the LGI than the HGI trial from between 15 min to 3 hours after the meals.

**Part II**

During the postprandial period, CHO oxidation and energy expenditure were 40% (P<0.01) and 7% (P<0.05) respectively greater after the CM than after the FM (Figure 4.1, lower panel). When additional fat energy was added to the CM (i.e. CM vs HM), CHO oxidation remained unchanged but fat oxidation increased by 19 kcal (P<0.05). When a similar amount of CHO was added to the FM (i.e. FM vs HM), CHO oxidation increased by 66 kcal (P<0.01) and fat oxidation decreased by 30 kcal (P<0.01).

Plasma glucose concentrations increased and reached a peak in 15 (CM and HM) or 30 (FM) min (P<0.01) after ingestion the respective meals (Figure 4.2, upper panel). One hour after the meals, plasma concentrations returned to that of (HM) or below (CM and FM) fasting values. The 3-hour IAUC for plasma glucose after the FM and HM were 64% lower and 44% higher respectively when compared to that after CM (Figure 4.3, upper panel). However, the difference did not reach statistical significance because of large inter-individual variations.

Serum insulin concentrations increased by 4- to 6-fold from resting values within an hour after consumption of all meals (Figure 4.2, low panel). Peak serum insulin concentrations ranged from 31 to 51 mIU.l⁻¹, 41 to 148 mIU.l⁻¹, 43 to 151 mIU.l⁻¹ for after the FM, CM, HM respectively. Compared to the FM, the 3-hour IAUC for serum insulin concentrations were 1.1- and 1.9-fold larger (P<0.05) after the CM and HM (Figure 4.3). However, serum insulin IAUC was not different between the CM and
HM trials. In contrast, the difference in the serum insulin responses were more exaggerated in part I when meals of different GI were compared (Figure 4.2, lower panel and 4.3, lower panel).

After ingestion of the meals, plasma glycerol decreased and remained lower than fasting concentrations in all cases except at the end of 3 hour following the FM. Plasma glycerol concentrations were higher following the FM than the CM and the HM respectively, from 30 min after the meals until the end of the 3-hour postprandial period (P<0.05). Plasma glycerol concentrations were also higher during HM than CM trial at the same times (P<0.05). After ingestion of the each of the meals, plasma FFA concentrations decreased from fasting values and remained depressed during the 3-hour postprandial period in all cases (P<0.05) with the exception that after the FM, plasma FFA concentration had returned to fasting values at the end of 3 hours postprandial period. During the postprandial period, plasma FFA concentrations were higher following the FM than the CM and the HM (P<0.05 respectively). Plasma FFA concentrations were also higher following the HM than the CM from 30 min after the meal until the end of 3 hours (P<0.05).

4.4 Discussion
In this chapter the influences of I) GI and II) CHO and fat composition of breakfasts on postprandial substrate oxidation were examined. Although the high and low GI meals contained the same amount of CHO and fat, overall substrate oxidation was markedly different (Part I). The exaggerated glycaemic and insulinaemic responses in the 3 hours after ingestion of the HGI compared to the LGI meal suggests that more glucose was available from the gastro-intestinal tract after the HGI than the LGI meal. By increasing the glucose availability in this way, without changing fat and protein content in a single meal, the postprandial substrate oxidation shifted from fat (-37%) to CHO (+46%) and 3-hour postprandial energy expenditure was increased by 19%. This suggests that CHO availability plays a pivotal role in determining postprandial substrate oxidation.
In Part I, the relative importance of CHO and fat content of a meal on postprandial substrate oxidation was examined. Insulin secretion and the postprandial decline in plasma FFA concentrations were greater following the high CHO meal (CM) compared to the high fat meal (FM). This resulted in a lower fat oxidation and a higher CHO oxidation rate in the 3 hour postprandial period. When CHO was added to the FM, plasma glycerol and FFA concentrations decreased, as did fat oxidation, whereas CHO oxidation increased. However, when fat was added to the CM, plasma glycerol, FFA concentrations and fat oxidation increased but CHO oxidation was unchanged. Taken together, these results suggest that both the GI and amount of CHO are more important than fat content in regulating postprandial substrate oxidation.

The finding of the study reported in Part I is in agreement with that of Tappy et al., (1986) who showed that the elevation of glucose oxidation during a 4-hour postprandial period was less following the ingestion of beans (low GI) compared to potatoes (high GI). In contrast, Ritz et al. (1991) reported that 3 hours after ingestion of glucose or manioc starch, glucose oxidation was higher after the starch meal but fat oxidation was not different between the two trials. The authors concluded that the use of low GI CHO (i.e. manioc starch) increases CHO oxidation because of lower plasma FFA concentrations (Ritz, et al., 1991). In that study, however, the postprandial glycaemic and insulinaemic responses of subjects to the starch was only slightly lower than that of glucose. Therefore, manioc starch cannot be considered as a low GI food and there is little basis for the conclusion of Ritz and coworkers (1991). In sharp contrast to the result of Ritz et al. (1991), a previous study (Tappy et al., 1986) and the present study show that a low GI meal increase fat oxidation and reduce CHO oxidation in the postprandial period compared to a high GI meal.

Compared to the HGI meal, ingestion of the LGI meal resulted in a much reduced insulinaemic response. This probably resulted in a greater degree of lipolysis in the LGI than HGI trial. This is supported by the higher plasma glycerol and FFA concentrations in the LGI trial. Because the fat content of the meals was low, it follows that mobilisation and oxidation of fat from endogenous fat stores was greater after a low
than after a high GI meal, at least in physically active subjects. Slabber et al. (1994), using energy restricted diets of similar energy and macronutrient composition, reported that overweight subjects lost more weight after 12 weeks on a low GI compared to a high GI diet. Although, it is not known if the weight lost in that study was the result of a reduction in endogenous fat stores, our result suggests that this may be the case. However, there is also some evidence that glucose and insulin responses to a low GI diet may increase after a period of 30 days, suggesting possible adaptation to such a diet (Kiens and Ritcher, 1996). Nevertheless, even with a slight increase in the glycaemic response to a low GI diet over time, plasma FFA concentrations remained higher in the low GI diet group after 30 days (Kiens and Ritcher, 1996).

The results of Part II do not support the conclusions of previous studies which reported that the addition of fat to a CHO meal caused no alteration in the short term oxidation of fat and CHO (Bennett, et al., 1992; Flatt, et al., 1985; Shutz, et al., 1989). One likely explanation could be that we only monitored postprandial metabolic responses for 3 hours whereas in previous studies the observation lasted for 6 hours (Bennett, et al., 1992; Flatt, et al., 1985). Alternatively, the training status of our subjects may have allowed them to oxidise a proportion of the dietary fat rather than store it. However, Bennett et al (1992) and Roy et al (1998) reported that physical training status does not provide an advantage in avoiding short term storage of excess fat. The differences may be because subjects in both these studies were less well-trained than the subjects in our study. Furthermore, the influence of endurance training on substrate oxidation at rest has not been as well documented as that during exercise.

Part II also confirmed the results of previous studies which reported that, when a range of isoenergetic meals or short term diets are consumed by healthy subjects, there is a reciprocal relationship between fat and CHO oxidation (Babbioni-Haresh, et al., 1996; Roy, et al., 1998; Stubbs, et al., 1993; Verboeket-van de Venne, et al., 1994; Whithley, et al., 1997). By using the HM, we further demonstrated that postprandial substrate oxidation is more sensitive to CHO than fat content in a single meal. Although this oxidative hierarchy has been previously reported in short-term energy balance studies.
(Abbott, et al., 1988; Jebb, et al., 1996), to our knowledge, it has not been reported after the ingestion of a single meal.

The glucose-fatty acid cycle (G-FAC) concept predicts that increased availability of fatty acids for oxidation by muscle will increase fat oxidation and reduce CHO oxidation (Randle, et al., 1963). This concept has been frequently cited as the explanation for the observed inverse relationship between CHO and fat metabolism after ingestion of meals which produced different plasma FFA concentrations (Griffiths, et al., 1994; Whithley, et al., 1997). Plasma FFA and glycerol concentrations were higher following the HM than the CM. This increased FFA availability resulted in an increase in whole body fat oxidation but CHO oxidation remained unchanged during the postprandial period. On the other hand, compared to the FM, plasma FFA and glycerol concentrations decreased when additional CHO was ingested (HM). The increased CHO intake resulted in a large increase in glycaemic and insulinaemic responses. It also resulted in an increase in CHO oxidation and decreased fat oxidation during the postprandial period when compared with the FM. Taken together, these results suggest that CHO availability regulates fat oxidation rather than the converse, which is suggested by the G-FAC concept.

Carbohydrate availability may regulate substrate oxidation by a) decreasing the mobilisation of FFA and b) decreasing the oxidation of the available FFA. The inhibitory effect of insulin on lipolysis is well established (Bonadonna, et al., 1990; Campbell, et al., 1992). Recently, Sidossis and colleagues demonstrated that glucose and insulin directly regulate whole body (Sidossis, et al., 1996; Sidossis and Wolfe, 1996) and hepatic (Sidossis, et al., 1998) oxidation of available fatty acids. Whether the G-FAC cycle operates in resting human skeletal muscle is still a subject of much controversy (Sidossis and Wolfe, 1996; Spriet and Dyck, 1996). Moreover, the exact mechanism by which glucose and insulin regulate fatty acid oxidation in human muscle is still unclear. Nevertheless, our results do not support the operation of a G-FAC during a postprandial resting period in healthy human subjects.
There is a strong association between ageing and the development of insulin resistance (Fink, et al., 1983; Shimokata, et al., 1991). In part II, the age of subjects ranged from 20 to 57. Of the six subjects, 3 were below the age of 35 and the rest were above the age of 50. Therefore, it is possible that age may be a confounding factor in the insulin responses to the meals. However, insulin resistance with ageing can be prevented by regular exercise training (Rogers, et al., 1990). Indeed, the age of subjects in part II and their 3-hour serum insulin IAUC were not correlated ($r = 0.41, P = 0.13$). Therefore, the insulin responses of the older subjects were not different from the younger ones.

One shortcoming of the present studies is that postprandial metabolic responses were only monitored for 3 hours compared to the 6-8 hours in other studies. The resting energy expenditure of our subjects had still not returned to basal values at the end of 3 hours, and so we did not attempt to provide estimates of the total energy cost of CHO or fat utilisation and storage after the meals. However, 3-4 hours after a meal, the thermic effect is likely to be confounded by the influence of a second meal or physical activity in normal active persons.

In summary, the results of these two studies show that the ingestion of CHO in a meal is more important than fat in determining postprandial substrate oxidation. Furthermore, the suppression of fat oxidation following CHO ingestion can be reduced by consumption of low GI CHO, at least in habitually active subjects. In the light of these findings, the value of low GI CHO in helping achieve fat balance may be helpful but require further research.
Table 4.1 *Subjects characteristics* (values are mean ± SE).

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Mass (kg)</th>
<th>BMI (kg.m²)</th>
<th>VO₂max (ml.kg⁻¹.min⁻¹)</th>
<th>Average daily energy intake (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part I</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>n=3, female</td>
<td>23 ± 1</td>
<td>52.7 ± 2.3</td>
<td>20.8 ± 1.2</td>
<td>47.1 ± 1.6</td>
</tr>
<tr>
<td>n=7, male</td>
<td>25 ± 2</td>
<td>69.5 ± 3.9</td>
<td>23.6 ± 1.0</td>
<td>52.7 ± 1.1</td>
</tr>
<tr>
<td>Part II</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>n=6, male</td>
<td>40 ± 6</td>
<td>70.4 ± 1.3</td>
<td>23.5 ± 0.4</td>
<td>59.4 ± 1.7</td>
</tr>
</tbody>
</table>
Figure 4.1 Overall substrate oxidation and energy expenditure for Part I (upper panel) and II (lower panel) during the 3h period after meal ingestion

a:p<0.01 from LGI; b:p<0.01 from CM and HM; c:p<0.05 from CM; d:p<0.05 from HM
Figure 4.2 Plasma or blood glucose (upper panel) and serum insulin (lower panel) responses during the 3h postprandial period.

Dotted lines: a: p<0.01 from LGI; b: p<0.05 from LGI
Solid lines: c: p<0.05 from CM; d: p<0.05 from HM
Figure 4.3 Mean incremental area under blood or plasma glucose (mmol.l⁻¹.min) (upper panel) and serum insulin (mIU.l⁻¹.min) (lower panel) response time curve (IAUC) a:p<0.01 from LGI; b:p<0.05 from CM and HM
Figure 4.4 Plasma glycerol (upper panel) and plasma FFA (lower panel) responses during the 3h postprandial period.

Dotted lines: a: p<0.05 from HGI
Solid lines: b: p<0.05 from CM and HM; c: p<0.05 from CM
Chapter 5
Carbohydrate rather than fat availability before exercise determines endurance running capacity in fasted subjects

5.1 Introduction
Compared to the fasted state, ingestion of a carbohydrate (CHO) meal in the hours before exercise enhances endurance performance (Chryssanthopoulos and Williams, 1997; Neufer, et al., 1987; Sherman, et al., 1989). However, utilisation of plasma free fatty acids (FFA) decreases after a CHO meal because of CHO-induced rise in circulating insulin concentration inhibits the mobilisation of fatty acids, and hence supply of this substrate. Montain et al. (1991) demonstrate that the suppression of fat oxidation during exercise at 70 % \( \dot{V}O_2 \text{ max} \) persists for at least 4 hours following a CHO meal. This is accompanied by increased muscle glycogenolysis (Coyle, et al., 1985), muscle glucose uptake and reduced hepatic glucose production (Conus, et al., 1996).

The concentration of plasma FFA influences the rate of fat oxidation during exercise. Therefore, it is not surprising that several nutritional strategies have been proposed to increase the availability of FFA for muscle metabolism. Pre-exercise fat ingestion with heparin administration can increase plasma FFA concentrations during exercise of moderate to high intensity (\(-70 \% \dot{V}O_2 \text{ max}\)) (Costill, et al., 1973; Vukovich, et al., 1993). However, the strong anticoagulant properties of heparin makes it unacceptable for use in human other than in clinical situation. Most (Costill, et al., 1973; Dyck, et al., 1993; Odland, et al., 1998; Romijn, et al., 1995; Vukovich, et al., 1993) but not all (Hargreaves, et al., 1991) of the studies on this topic reported that increased circulating FFA reduces muscle glycogen degradation during exercise. If and when this occurs, it is reasonable to predict an increase in endurance capacity as a consequence of the muscle glycogen being used more economically throughout exercise.
Okano et. al. and Whitley et. al. compared the influence of isoenergetic high- (Okano, et al., 1996; Whitley, et al., 1998) or moderate- (Okano, et al., 1998) CHO meals with low CHO-high fat pre-exercise meals on metabolism and endurance cycling performance. Substrate oxidation also shifted from CHO towards greater metabolism of fat when the low CHO-high fat meal was used (Okano, et al., 1996), at least during the early stages of exercise (Okano, et al., 1998; Whitley, et al., 1998). However, performances were similar regardless of the CHO composition of the meals (Okano, et al., 1998; Okano, et al., 1996; Whitley, et al., 1998). Nevertheless, the addition of fat to a high GI CHO meal may optimise the availability of CHO to muscle and liver before exercise and yet maintain higher concentrations of FFA during exercise. Any glycogen sparing following the ingestion of additional fat may then enable subjects to extend their endurance times beyond that achieved after a high CHO meal.

The lack of substrate availability may not be the only cause of fatigue and other possibilities need exploring. The central fatigue hypothesis suggests that high concentrations of plasma free-tryptophan is related to fatigue during exercise (Davis and Bailey, 1997). There is a strong correlation between concentrations of plasma free-tryptophan and FFA (Blomstrand, et al., 1988; Davis, et al., 1992; Zanker, et al., 1997). It is conceivable that fat ingestion may be detrimental to exercise performance by accentuating the rise in FFA during exercise. This possible confounding effect of elevated FFA on fatigue has not been considered in previous studies which measured endurance performance after acute dietary manipulations. There is evidence for serotonergic control of exercise-induced prolactin secretion in men (DeMeirleir, et al., 1985; Fischer, et al., 1991), and so serum prolactin concentrations may reflect the serotonergic activity beyond the blood brain barrier. Therefore, the purpose of the present study (Study 2) was to examine the influence of CHO and fat content in three different meals, taken 3 hours before exercise, on metabolic responses, serum prolactin concentrations and endurance running capacity during subsequent exercise. A high fat-
low CHO meal (FM) and a high CHO-high fat meal (HM) were compared with a high CHO-low fat meal (CM).

5.2 Methods

Six trained male runners volunteered to serve as subjects for the experiment (Table 5.1). Preliminary and main tests were as described in Chapter 3. All subjects in this study were highly motivated and experienced volunteers who had participated in similar running studies in the past and were, therefore, familiar with the sensation and symptoms of fatigue during prolonged running to exhaustion.

Table 5.1. Descriptive characteristic of subjects (mean ± SE).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>40 ± 6</td>
</tr>
<tr>
<td>Running mileage, miles per week</td>
<td>52 ± 3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174 ± 1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.4 ± 1.3</td>
</tr>
<tr>
<td>maximal oxygen uptake, ml·kg⁻¹·min⁻¹</td>
<td>59.4 ± 1.7</td>
</tr>
<tr>
<td>maximum heart rate, beat·min⁻¹</td>
<td>179 ± 5</td>
</tr>
<tr>
<td>treadmill running speed at 70%VO₂max, m·s⁻¹</td>
<td>3.6 ± 0.3</td>
</tr>
</tbody>
</table>

The experimental trials were conducted on three occasions separated by at least 7 days as described in Chapter 3. Briefly, subjects arrived at the laboratory at 8:00am after a 12 hour overnight fast on test days. After collection of resting blood and expired air samples, the subjects were given the test meals in a random order. The test meals they received contained energy, CHO, fat, protein in the following amounts respectively (per 70 kg bodyweight): FM 756 kcal, 70 g, 49 g, 8 g; CM 759 kcal, 175 g, 3 g, 8 g; HM 1173 kcal, 175 g, 49 g, 8 g (Table 3.2). Only one investigator was aware of the content of the test meal for each trial. The meals were consumed within
15-20 min. During the 3 h postprandial period, the subjects sat quietly reading or watching a video in a quiet corner of the laboratory. Just before exercise, each subject ingested 350 ml of water. After a standard warm-up subjects ran at \(~70\ \%\ \dot{V}O_2\max\) until exhaustion (EXH). Subjects were not told their running times until the end of their last trial.

Heart rate (HR) was monitored continuously during exercise (Polar Electro sports tester PE 3000). The runners' perceived rate of exertion (RPE) (Borg, 1973) were recorded during each expired air collection. During exercise, a fixed volume of water was ingested after each expired air collection. The drinking pattern was replicated during subsequent trials for each subject. All trials were conducted under similar conditions of temperature and relative humidity (FM: 19.6 ± 0.8 °C; 52 ± 4%, CM: 19.9 ± 0.4°C; 49 ± 5% and HM: 19.4 ± 0.6°C; 52 ± 5%). An electrical fan in front of the runners circulated air and wet sponges were also available for the subjects to use ad libitum throughout each run in order to minimise thermal stress. Nude body weight was obtained before and after the run following the removal of any sweat from the skin, for calculation of overall sweat loss.

The changes in physiological and biochemical variables were analysed by a two-way ANOVA for repeated measures (meal x time). The glucose and insulin IAUC as well as run times were not normally distributed and were analysed by the Wilcoxon test. The dietary data, total substrate oxidation and energy expenditure were analysed by a one-way repeated measures ANOVA. Statistical significance was set at \(P < 0.05\). The Tukey post hoc test was used to locate any significant difference(s) as revealed by the ANOVA. All data are reported as mean ± SE.

5.3 Results

Nutritional analysis. During the two days before each trial there were no significant differences between the three trials in the average daily energy intake, and composition
in terms of CHO, fat or protein consumed. The mean daily energy intake and macronutrient composition during the two days prior to each trial were FM: 3242 ± 279 kcal (57% CHO, 28% fat and 16% protein), CM: 3247 ± 221 kcal (55% CHO, 28% fat and 17% protein) and HM: 3284 ± 339 kcal (57% CHO, 27% fat and 16% protein).

Exercise performance. Mean relative exercise intensities were similar in all three trials: 71.1 ± 0.6, 71.1 ± 0.5 and 70.8 ± 0.5 % \( \dot{V}O_2 \) max for FM, CM and HM trials, respectively. Compared to the CM (111 ± 4 min; range 90-120 min) and HM (111 ± 3 min; range 98-115 min) trials, running time to exhaustion was 14% less during the FM trial (96 ± 3 min; range 86-103; P<0.05). There were no difference between the running times following CM and HM. Furthermore, no difference was found when time to exhaustion for the treatments were analysed by order (T1: 108 ± 5 min; T2:105 ± 4 min; T3:106 ± 5 min).

Heart rates increased with the duration of exercise but there were no differences between trials (Table 5.2). The RPE was higher during the FM trial (P<0.05) at 60 min into exercise than at the same time during the CM and HM trials (Table 5.2). Sweat loss (FM: 1.9 ± 0.2 kg, CM: 2.1 ± 0.2 kg; HM: 2.1 ± 0.2 kg, ns) and changes in plasma volume (FM: -3.7 ± 0.7%, CM: -4.0 ± 0.7%, HM: -4.0 ± 0.7%, ns) were slightly lower during FM than CM and HM trial. The total amount of water consumed just before and during exercise in the three trials were similar (FM: 886 ± 77, CM: 921 ± 83, HM: 916 ± 89 ml).

Metabolic and hormonal measures. Plasma glucose, glucagon and serum insulin response curves during the 3 h postprandial period and exercise are shown in Figure 5.1. The 3 h IAUC for plasma glucose was greatest following HM and smallest following FM but the difference did not reach statistical significance. At the onset of and 20 min into exercise, plasma glucose concentrations in the HM (4.1 ± 0.1
mmol·L⁻¹) trial were lower than in the FM (4.5 ± 0.2 mmol·L⁻¹; P < 0.05) trial (Figure 5.1A). Thereafter, plasma glucose concentrations remained stable during exercise in all trials and did not fall below 3.4 mmol·L⁻¹ in any of the subjects. Serum insulin concentrations increased by 5- to 10-fold from resting values within an hour after consumption of all the meals. Peak serum insulin concentrations ranged from 31 to 51 mIU·L⁻¹, 41 to 148 mIU·L⁻¹, 43 to 151 mIU·L⁻¹ for FM, CM and HM trials respectively. Compared to the FM trial, the 3h IAUC for serum insulin concentrations were 1.1- and 1.9-fold larger (P < 0.05) after the CM and HM trials (Figure 5.1B). However, this did not differ between the CM and HM trials. At the onset of exercise, serum insulin concentration during the FM (10.9 ± 0.9 mIU·L⁻¹) trial was lower compared to CM (17.7 ± 3.7 mIU·L⁻¹; P < 0.1) and HM (19.0 ± 2.6 mIU·L⁻¹; P < 0.05) trials. At this time, serum insulin concentrations during CM and HM were still ~2-fold greater than fasting (P < 0.05) values but not for FM. Serum insulin concentrations decreased to similar values during exercise in all trials. Plasma glucagon concentrations were not different between the trials during the 3 h postprandial period, and increased with the duration of exercise, reaching the highest values at the end of exercise. After 60 min of exercise and at EXH, plasma glucagon concentrations were higher in the FM trial compared to CM and HM (P < 0.05, Figure 5.1C).

The plasma FFA, glycerol and serum prolactin response curves are shown in Figure 5.2. The suppression of plasma FFA and glycerol concentrations during the 3 h postprandial period were least after FM (P < 0.05 from CM and HM) and greatest after CM (P < 0.05 from HM) (Figure 5.2A). At the onset of exercise, plasma FFA and glycerol in the HM and CM trials were lower than (P < 0.05) fasting values but this was not the case in the FM trial. Plasma FFA and glycerol concentrations increased with duration of exercise but were highest during the FM trial (P < 0.05 from HM and CM) and lowest during the CM trial (P < 0.05 from HM). Serum prolactin concentrations increased during exercise in all trials and reached the highest concentration at EXH (P < 0.01 from onset of exercise). After 60 min of exercise and
at EXH, serum prolactin concentrations were higher during the FM trial than CM and HM trials (P<0.05). There was a modest correlation between the concentrations of plasma FFA and serum prolactin (r = 0.61; p < 0.001). Plasma FFA concentrations of two subjects exceeded 1.00 mmol·L⁻¹ at EXH during the FM trial. These two subjects also reported sensation of sleepiness and had the highest serum prolactin concentrations at the same time. This was not observed in other trials nor with other subjects.

Respiratory exchange ratio during exercise and blood lactate concentrations during the 3 h postprandial period and exercise are shown in Figure 5.3. Oxygen uptake (Table 5.2) and RER remained constant throughout each exercise bout until the point of exhaustion Oxygen uptake was not different between the trials. Throughout exercise, RER was lower during FM (mean of 0.88 ± 0.01) compared to CM (mean of 0.94 ± 0.01; P < 0.05) and HM trials (mean of 0.92 ± 0.02; P < 0.05) but RER values were not different during CM and HM trials. The 3 h IAUC for blood lactate following the CM and HM were 111% (P < 0.01) and 88% (P < 0.05) respectively greater than following FM. During exercise, blood lactate concentrations were between 2.0 and 3.6 mmol·L⁻¹ in the trials but were lower during the FM trial at 20, 60 min and at EXH than during CM and HM trials (P < 0.05). Overall substrate oxidation and energy expenditure during 80 min of exercise is shown in Figure 5.4. During the 3 h postprandial period, total CHO oxidation was 45 ± 3, 61 ± 2 and 60 ± 1 g in the FM (P < 0.05 from CM and HM), CM and HM trials respectively. During the first 80 min of exercise in the FM trial, total CHO oxidation was 19% and 14% lower compared to CM and HM (P < 0.01) respectively (FM: 189 ± 10 g; CM: 233 ± 9 g; HM: 220 ± 10 g) with a compensatory increase in fat oxidation, such that the total energy expended in the trials were similar (Figure 5.4).
5.4 Discussion

In agreement with previous studies (Okano, et al., 1996; Whitley, et al., 1998), we demonstrate that, in comparison with an isoenergetic CM, pre-exercise FM ingestion results in elevated concentration of plasma FFA, increased fat oxidation and decreased CHO oxidation during exercise. However, in contrast to these studies (Okano, et al., 1996; Whitley, et al., 1998), endurance capacity is poorer after the FM than after the CM. Moreover, running time to exhaustion was longer in the HM trial compared to FM but not different from the CM trial. Therefore, dietary CHO rather than fat availability before exercise determines endurance capacity in fasted subjects.

The relationship between dietary CHO availability and endurance exercise capacity is well documented. Compared to the fasted condition, ingestion of a high CHO meal 3-4 hours before exercise maintains CHO oxidation rates during exercise (Chryssanthopoulos and Williams, 1997; Coyle, et al., 1985; Neufer, et al., 1987; Sherman, et al., 1989), and enhances endurance capacity and performance (Chryssanthopoulos and Williams, 1997; Neufer, et al., 1987; Sherman, et al., 1989). Liver glycogen concentration is significantly reduced after an overnight fast (Nilsson and Hultman, 1973). Previous investigators in the same laboratory have consistently demonstrated that the same training and dietary control regimen adopted in the present study results in very similar muscle glycogen concentrations (Tsintzas, et al., 1995; 1996). Therefore, it is reasonable to assume that CHO stores of the subjects were similar before the meals. The mean amounts of CHO provided during the FM, CM and HM trials were 70g, 175g and 175g respectively. The estimated CHO used following the postprandial period and 80 min of exercise were 234g, 294g and 280g in the FM, CM and HM trials respectively. Therefore, compared to the FM trial, CHO availability at the final stages of exercise would be 45g and 59g higher in CM and HM trials respectively.
The difference in the amount of CHO provided between the isoenergetic high CHO and high fat meal in studies by Okano et al. (1996) and Whitely et al. (1998) were greater than the present study - 136g and 165g respectively. In one study, subjects cycled for 2 h at 65% $VO_2$ max before pedalling to exhaustion at 80% $VO_2$ max (approximately 5 min) (Okano, et al., 1996). In the other study, subjects cycled at 70% $VO_2$ max for 90 min and then performed a 10 km time trial (approximately 14 min) (Whitley, et al., 1998). At the commencement of the respective performance tests, the difference in subjects' CHO availability between the high-CHO and high-fat trials in these studies must be greater than ours because the reported CHO sparing in the respective high-fat trials were less (Okano, et al., 1996; Whitley, et al., 1998). Therefore, it is likely that exercise times will be longer in their respective high CHO trials than the high fat trials if their subjects had continued to exercise at the same intensities (Okano, et al., 1996; Whitley, et al., 1998).

It is possible that substrate availability is not a limiting factor during high intensity exercise employed in these studies (Okano, et al., 1996; Whitley, et al., 1998). Although our result suggests that fat supplementation in the fasted state does not improve endurance performance, there may be some benefit when FFA availability is increased in glycogen supercompensated subjects (Pitsiladis, et al., 1998).

One interesting observation was that oxygen consumption remained relatively constant throughout exercise in all three trials. This is similar to that observed in other studies on the metabolic effects of pre-exercise CHO feeding (Coyle, et al., 1985; Sherman, et al., 1989; Okano, et al., 1996). In the present study, this may be explained by the relatively constancy of RER with the duration of exercise in all three trials. Indeed CHO oxidation may not decrease during constant pace running even when muscle glycogen stores are reduced to low levels (Tsintzas, et al., 1996).
High CHO meals containing foods of similar GI to those in the present study have been reported to increase muscle glycogen content of between 10% to 40% over a 4 h postprandial period (Coyle, et al., 1985; Neufer, et al., 1987; Taylor, et al., 1993), resulting in greater muscle glycogen degradation during subsequent exercise (Coyle, et al., 1985; Neufer, et al., 1987). In these studies, the modest increase in muscle glycogen concentration suggests that some of the ingested CHO goes to the liver (Coyle, et al., 1985; Neufer, et al., 1987; Taylor, et al., 1993). The prevailing insulinaemia in the postprandial period after the CM and HM suggests significant muscle and liver glycogen storage (Young, et al., 1988) compared to FM before commencement of exercise. Serum insulin concentrations were 2-fold higher than fasted values at the onset of exercise during the CM and HM trials. This suggests that gastric emptying of some additional CHO from these two meals also continued into the exercise period. Therefore, the enhanced CHO oxidation during the CM and HM trials compared to FM is probably the consequence both greater muscle glycogenolysis (Coyle, et al., 1985; Hargreaves, et al., 1995) and greater glucose uptake and oxidation (Conus, et al., 1996). The lower plasma glucose concentrations at the onset of and during the first 20 min of exercise during the HM than FM trial (Figure 5.1A) suggests a greater glucose uptake and oxidation in the former due to the persistent effect of hyperinsulinaemia before exercise. Nevertheless, this did not limit running performance as it did not last beyond 20 min and none of the subjects experienced any symptom of hypoglycaemia.

During prolonged exercise, the rise in glucagon is essential for the increase in hepatic glucose production and gluconeogenesis (Lavoie, et al., 1997). Galbo et. al. (1979) reported that the increase in plasma glucagon during exhaustive running at 70 % VO2max is greater following a high fat-low muscle glycogen than a high CHO-high muscle glycogen diet. Furthermore, this increase is suppressed with glucose infusion at the end of exercise when muscle glycogen is depleted (Galbo, et al., 1979). The observation that plasma glucagon concentrations were higher after 60 min of exercise
in the FM than the CM and HM trials (Figure 5.1C) is likely to result in a higher hepatic glucose output in the FM trial in order to make up for the lack of CHO availability from other sources.

Our results and others (Okano, et al., 1996; 1998; Whitley, et al., 1998), demonstrate that acute fat meal (FM) ingestion can result in elevated levels of plasma FFA during exercise. Although the observed plasma FFA concentrations was somewhat lower than that achieved after fat ingestion or infusion in combination with heparin administration (Costill, et al., 1973; Dyck, et al., 1993; Odland, et al., 1998; Romijn, et al., 1995; Vukovich, et al., 1993), CHO oxidation during exercise was nevertheless reduced compared to the CM and HM trials. The magnitude of CHO sparing is not related to the increase of plasma FFA concentration above control values but is dependent of saturation of the fatty acids’ transport system via albumin (Vukovich, et al., 1993). The observation that during exercise, plasma glycerol and FFA concentrations were higher in the HM than CM trial suggests that the dietary fat provided in the HM may be immediately available for muscle metabolism (Griffiths, et al., 1994). The large insulin response after the HM meal would have activated adipose tissue lipoprotein lipase (LPL) (Sadur and Eckel, 1982), and the added fat in the HM may then directly enter into the plasma FFA pool via the action of LPL on chylomicron-triglyceride. On the other hand, the additional plasma FFA available for oxidation in the muscle during exercise in the FM trial was likely to have originated from the adipose tissue because insulin inhibition of hormone sensitive lipase would have ceased when insulin concentrations normalised at the onset of exercise.

The HM contained 414 ± 14 kcal of additional fat compared to the CM, and 417 ± 14 kcal of additional CHO compared to the FM. Plasma FFA was higher during the HM trial than the CM trial, but this did not result in any significant shifts in substrate oxidation. This observation is not consistent with the glucose-fatty acid cycle which predicts that increased availability of fatty acids for oxidation by muscle will increase
fat oxidation and reduce CHO oxidation (Randle, et al., 1963). The observation that substrate oxidative pattern during exercise in the HM resembles CM rather than FM (Figure 5.4) provides evidence that it is CHO rather than fat availability that exerts predominant control over substrate oxidative pattern during exercise. In the fasted state, circulating FFA does not limit fat oxidation at exercise intensities of up to 65 %VO₂ max (Romijn, et al., 1993). However, even small elevation in circulating insulin before exercise can suppress lipolysis during exercise at low intensity and limit fat oxidation (Horowitz, et al., 1997). Pre-exercise insulin concentrations were higher but plasma FFA and glycerol concentrations lower, before and during exercise in the CM and HM trials than FM. This suggests that the suppression in fat oxidation during exercise in the CM and HM trials compared to FM is at least partly related to insulin inhibition of lipolysis. There is also evidence that increases in glycolytic flux and CHO oxidation following CHO ingestion limits the oxidation of long chain fatty acids in skeletal muscle during exercise (Coyle, et al., 1997). The preferential oxidation of CHO rather than fat when both substrates are made available to the muscle may be related to the inhibition of long chain fatty acid transport into the mitochondria via carnitine palmitoyltransferase (Coyle, et al., 1997), but the exact mechanism remains unclear.

Our observations of serum prolactin responses are similar to that of Johannessen et al. (1981) who reported that concentrations are higher at the end of exhaustive running exercise following a high-fat than a high-CHO diet. However, their suggestion that prolactin responses to exercise are enhanced by low glucose concentrations (Johannessen, et al., 1981) is not supported by our result. Plasma glucose concentrations were not different between the three trials after 20 min of exercise. There is some evidence for serotonergic control of exercise-induced prolactin secretion in men (DeMeirleir, et al., 1985; Fischer, et al., 1991). In the present study, there was a modest correlation between the concentrations of plasma FFA and serum prolactin during exercise. The observation that at EXH in the FM
trial, the two subjects who reported sleepiness, had plasma FFA concentrations > 1 mmol·l⁻¹ also had the highest serum prolactin concentrations (> 100 ng·ml⁻¹) provides circumstantial evidence that impairment in endurance performance during the FM trial, at least for these two subjects, may be partly mediated by central factors.

The central fatigue hypothesis suggests that an increase in the concentration of the neurotransmitter serotonin in certain areas of the brain might ensure a high rate of neuronal firing in a specific part of the brain, which then increases the sensitivity to fatigue (Davis and Bailey, 1997; Newsholme, et al., 1993). Tryptophan, a precursor of serotonin, exists in bound and free forms, which are in equilibrium. It binds to the same sites on plasma albumin as fatty acids. This equilibrium is changed in favour of free tryptophan when plasma FFA concentrations are raised above 1 mmol·l⁻¹, probably by the favourable binding of FFA to albumin (Newsholme, et al., 1993). Therefore, when the concentration of plasma FFA exceeds 1 mmol·l⁻¹, tryptophan is displaced and the amount of free-tryptophan available for transport across the blood brain barrier increases, leading to a rise in the concentration of serotonin and possibly resulting in the sensation of fatigue.

In summary, substrate utilisation during CM and HM were similar but there was an decreased reliance on CHO during FM trial. These results suggest that it is the CHO rather than fat availability before exercise that exerts predominant control over substrate selection during exercise and endurance running capacity. The observed metabolic responses may partly be attributed to the inhibitory effect of insulin on lipolysis. Additional research is needed to delineate the mechanism by which the earlier onset of fatigue occurs during the FM trial.
Table 5.2. Effects of exercise to exhaustion after ingestion of FM, CM and HM on physiological parameters. Values are mean ± SE.

<table>
<thead>
<tr>
<th>Time</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
<th>80 min</th>
<th>100 min*</th>
<th>Exhaustion</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \dot{V}O_2 ) (l·min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>FM</td>
<td>2.90±0.04</td>
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<td>2.92±0.06</td>
<td>2.91±0.06</td>
<td>2.90±0.07</td>
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<td>CM</td>
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<td>2.92±0.08</td>
<td>2.89±0.06</td>
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<td>2.87±0.07</td>
<td>2.89±0.07</td>
<td>2.90±0.07</td>
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</tr>
<tr>
<td>RPE</td>
<td>FM</td>
<td>11±1</td>
<td>12±1</td>
<td>*14±1</td>
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<td>12±1</td>
<td>13±1</td>
<td>14±1</td>
<td>16±1</td>
<td>18±1</td>
</tr>
<tr>
<td>HM</td>
<td>11±1</td>
<td>12±1</td>
<td>13±0</td>
<td>15±0</td>
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<td>Heart rate</td>
<td></td>
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<td>(beats·min(^{-1}))</td>
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* n=3 for FM, n=5 for CM and HM

* P<0.05 from CM and HM
Figure 5.1. Plasma glucose, serum insulin and plasma glucagon concentrations during the 3h postprandial period and exercise. Values are means ± SE.

*P<0.05 from CM; †P<0.05 from HM.
Figure 5.2. Plasma FFA, glycerol concentrations during the 3h postprandial period and exercise and serum prolactin concentrations during exercise. Values are means ± SE. \(^{a}P<0.05\) from CM; \(^{b}P<0.05\) from HM.
Figure 5.3. Respiratory exchange ratio during exercise and blood lactate concentrations during the 3h postprandial period and exercise. Values are means ± SE. *p<0.05 from CM and HM.
Figure 5.4. Substrate oxidation and energy expenditure during 80 min of exercise. Values are means ± SE. $^a$P<0.01 from CM. $^b$P<0.01 from HM. $^c$P<0.05 from HM.
Chapter 6
Influence of high and low glycaemic index meals on endurance running capacity

6.1 Introduction

A carbohydrate (CHO) meal ingested 3-4 hours before exercise can increase liver (Nilsson and Hultman, 1973) and muscle glycogen concentrations (Coyle, et al., 1985). It may also continue to provide an absorbable source of CHO as it empties from the stomach during exercise (Wright, et al., 1991). Pre-exercise CHO meals also affect the metabolic response and substrate utilisation during exercise. Chapter 5 described a study which showed that the composition of CHO and fat determines pattern of substrate utilisation. To study the influence of pre-exercise CHO on energy metabolism during exercise, other investigators have used different monosaccharides, whole foods with different GI values, foods that are processed differently, and the addition of other macro-nutrients to a CHO source.

A few studies have considered the GI of foods when examining the effects of pre-exercise ingestion of CHO meals (Febbraio and Stewart, 1996; Guezennec, et al., 1993; Thomas, et al., 1991; Thomas, et al., 1994). Despite differences in the metabolism of fast and slowly digested starches, the benefits from eating starch on endurance performance remain unclear. In order to provide high and low GI CHO meals, Thomas et al. (1991, 1994) and Febbraio et al. (1996) used lentils and potatoes in their studies. The amount of potatoes and lentils consumed was calculated to provide 1g CHO·kg⁻¹ body mass. However, in lentils there is significantly more protein than in potatoes. From values provided by Thomas et al. (1994), the protein and energy content of the lentils meal was 208% and 36% higher respectively than in the potatoes meal. Therefore, their pre-exercise meals were not iso-energetic nor of the same macronutrient composition. Because of the observed energy differences between the test meals, the results of these studies should be interpreted with caution. Furthermore, it is not known how much of the enhanced insulinaemic, depressed
glycaemic and other metabolic response can be attributed to the increased protein content of the lentils meal.

Therefore, the aim of the present study (study 3) was to examine the influence of high and low GI CHO meals, containing 2g CHO·kg⁻¹ body mass, ingested 3 hours before exercise on subsequent endurance running capacity. The amount of food and 3-hour postprandial rest period before exercise was chosen to closely resemble the practice which athletes adopt before training or competition.

6.2 Methods

Five males and three females recreational runners volunteered to participate in this study. The age, bodyweight, \( \dot{V}O_2 \) max, and maximum heart rate were 33.2 ± 3.8 yr, 74.7 ± 1.7 kg, 51.9 ± 1.5 ml·kg⁻¹·min⁻¹ and 184 ± 5 beat·min⁻¹ for the male subjects and 27.0 ± 1.6 yr, 68.0 ± 0.4 kg, 44.7 ± 1.0 ml·kg⁻¹·min⁻¹ and 197 ± 1 beat·min⁻¹ for the female subjects respectively. At the time of the study, all subjects were involved in endurance running at a frequency of at least four times per week. None of the subjects had a history of diabetes mellitus. The female subjects were pre-menopausal, non-oral contraceptive users and were not on any estrogen replacement therapy. Preliminary and main tests were as described in Chapter 3. Briefly, the subjects were required to run to exhaustion at 70% \( \dot{V}O_2 \) max on a motorised treadmill on two different occasions separated by one week. Furthermore, the female subjects undertook the main trials during the last half of their monthly menstrual cycle so as to avoid major fluctuations in estrogen level that may influence substrate utilisation during exercise. Two isocaloric test meals consisting of high (HGI) and low (LGI) GI CHO sources of similar volume and macronutrients composition (826 kcal: 67% CHO, 30% protein, 3% fat) were ingested three hours before exercise (Chapter 3; Table 3.1). Each subject consumed a fixed amount (90-150 ml) of water just before and every 20 minutes during the run to prevent substantial dehydration.

Wet sponges were available for the subjects to use ad libitum throughout each run. Nude body weight was obtained before and after the run following the removal of any
sweat from the skin, for calculation of overall sweat loss. The runners’ perceived rate of exertion (RPE) (Borg, 1973) was recorded during each collection of expired air. All trials were conducted under similar conditions of temperature and relative humidity (LGI: 21.1±0.8 °C; 57±3% and HGI: 21.1±0.8 °C; 56±3%).

The changes in physiological and biochemical variables were analysed by a two-way ANOVA for repeated measures (meal x time). For values attaining this criterion, the Tukey post hoc test was used to locate the difference(s). The dietary data, total substrate oxidation and energy expenditure were analysed by Student paired t-tests. The glucose and insulin IAUC were not normally distributed and so were analysed by Wilcoxon test. Statistical significance was set at P < 0.05. All data are reported as mean ± SE.

6.3 Results

Dietary analysis. There were no differences in the daily energy intakes nor macronutrient composition of the subjects during the three days before each main trial (LGI: 2246±247 kcal, CHO: 323±17g, fat: 54±5g, protein: 91±6g; HGI: 2185±212 kcal, CHO: 324±13g, fat: 51±5g, protein: 87±6g).

Postprandial responses. Blood glucose peaked at 15 min (p<0.01) after consumption of the HGI meal and after 3 hours it had returned to fasting values (Fig 6.1). In contrast, blood glucose did not change significantly after the LGI meal. The incremental area under the blood glucose curve for the 1, 2 and 3 hour(s) after the HGI meal were 14-, 7- and 5-fold greater compared that after the LGI meal respectively (P<0.05, Fig 6.2).

After the LGI meal, serum insulin concentrations increased gradually and remained at between 80-160% higher than fasting values (Fig 6.1). At the beginning of exercise, serum insulin concentrations were 96% higher than fasting values (p<0.01). Serum insulin concentrations after consumption of HGI meal increased by 10-fold and peaked at 15 min. At the beginning of exercise, they were still 210% higher than fasting values.
(p<0.01). Serum insulin concentrations throughout the postprandial period were higher during HGI than LGI trial (p<0.01), but the difference did not reach statistical significance at the onset of exercise (p=0.08). Overall, the 3-hour incremental area under the serum insulin curve for HGI exceed that of LGI by 336% (p<0.05, Fig 6.2).

Blood lactate concentrations increased by more than 100% during the 30 min (p<0.01) after the HGI meal and remained higher than fasting values for 2 h (Fig 6.1). Blood lactate concentrations during the HGI trial were higher than following the LGI meal during the first hour (p<0.01). Serum FFA and plasma glycerol (Fig 6.3) concentrations decreased after both meals and remained lower than fasting values during the 3-hour postprandial period. However, serum FFA and plasma glycerol concentrations were higher during the postprandial period following the LGI meal than following the HGI meal (p<0.05).

Table 6.1 shows the respiratory exchange ratio, CHO and fat oxidation rates during the postprandial period. Postabsorptive resting metabolic rate was the same before both trials. Metabolic rate increased following both meals but was higher after the HGI trial than after the LGI meal throughout the 3-hour postprandial period (p<0.05). The RER increased and remained above the pre-meal values after both meals, reflecting an increase in the proportion of CHO oxidised. Rates of CHO oxidation were higher after the HGI meal at all time points. In the 3-hour postprandial period, 49% more CHO was oxidised during HGI (HGI: 52.0±3.9g vs LGI: 35.0±3.8g, p<0.01) and 69% more fat was oxidised during LGI (HGI: 3.9±1.3g vs LGI: 6.6±1.7g; n.s.).

Serum cortisol concentrations decreased after both meals and were different from fasting values after 1 h in both treatments, but there were no difference between trials (Fig. 6.3). Serum sodium, potassium and osmolality did not change with ingestion of either meals before exercise and were not different between treatments.

*Responses during exercise.* Exercise time to exhaustion was similar for both treatments (LGI: 111±6 min; range: 93-139 min and HGI: 113±4 min; range: 98-136 min; n.s.).
No difference was found when time to exhaustion for the treatments were analysed by order (T1: 111±6 min vs T2: 113±5 min).

The mean % $\dot{V}O_2\text{max}$ sustained during exercise for the treatments were LGI: 69±1% and HGI: 67±2% (n.s.). Heart rates and $\dot{V}O_2$ during exercise were similar during both trials (Table 6.2). The rate of perceived exertion was lower during the LGI trial at 60 min into exercise than at the same time during the HGI trial (Table 6.2).

Blood glucose concentrations throughout exercise were similar to pre-exercise levels during the LGI trial (Fig 6.1). However, at 20 min into exercise during the HGI trial, blood glucose concentration declined sharply to values (3.6±0.3 mmol·L$^{-1}$) lower than that at the start of exercise (4.3±0.2 mmol·L$^{-1}$). Three subjects had blood glucose concentration which were less than 3.5 mmol·L$^{-1}$ but none of the subjects reported any symptoms of hypoglycaemia. Their low blood glucose concentration increased after 20 min of exercise. Blood glucose concentrations were not different between the two trials afterwards. During the early phases of exercise, serum insulin concentrations declined during both trials and there were no difference between trials throughout exercise (Fig 6.1).

Blood lactate concentrations were also similar throughout exercise during both trials (Fig 6.1). Serum FFA and plasma glycerol (Fig 6.3) concentrations rose with exercise duration during both trials and were higher during LGI than during the HGI trial. Apart from plasma glycerol concentrations at 80 min (p=0.07) and point of fatigue (p=0.06), serum FFA and plasma glycerol were all significantly different between the two trials. Serum cortisol concentrations increased with the duration of exercise during both trials but were not different between trials (Fig 6.3).

Carbohydrate oxidation rate was lower at the end of exercise compared to the first hour of exercise during both trials (Table 6.2). With the exception at 20 min (p=0.06) and 40 min (p=0.06), there were differences in the CHO oxidation rates. Overall, the total amount of CHO utilised during exercise was higher during the HGI trial.
than the LGI trial (269±16g; p<0.01). During the first 80 min of exercise in the LGI trial, CHO oxidation was 12% lower (HGI: 232±18g vs LGI:205±20g; p<0.01) and fat oxidation 118% higher than the HGI trials (HGI: 9±2g vs LGI:19±3g; p<0.01). The overall metabolic rate and substrate oxidation during the postprandial period and first 80 min of exercise is summarised in Fig 4.4.

Sweat loss (HGI: 1.5±0.3 kg vs LGI:1.6±0.3 kg; n.s.) and changes in plasma volume (HGI: -3.6±0.8% vs LGI: -3.7±0.6%; n.s.) during exercise were similar for both trials. The amount of water each subject consumed just before and during both exercise was the same in both trials (1250±100ml). Serum potassium concentrations (0 min: 4.3±0.2 mmol·l⁻¹ (LGI), 4.3±0.2 mmol·l⁻¹ (HGI); 60 min: 4.8±0.2 mmol·l⁻¹ (LGI), 4.7±0.1 mmol·l⁻¹ (HGI)) and osmolality (0 min: 295±3 mOsm.kg⁻¹ (LGI), 294±3 mOsm.kg⁻¹ (HGI); 60 min: 298±3 mOsm.kg⁻¹ (LGI), 298±3 mOsm.kg⁻¹ (HGI)) were significantly elevated after 60 min of exercise during both trials but were not different between trials. Serum sodium concentrations (0 min: 138±1 mmol·l⁻¹ (LGI), 138±1 mmol·l⁻¹ (HGI); 60 min: 139±1 mmol·l⁻¹ (LGI), 139±1 mmol·l⁻¹ (HGI)) did not change with exercise duration during both trials and were not different between trials.

6.4 Discussion

The present study demonstrates that the GI of a CHO meal ingested 3 hours prior to exercise does not influence subsequent endurance running capacity. However, compared to a high GI meal, ingestion of a low GI meal before exercise resulted in a 12% reduction in CHO oxidation and 118% increase in fat oxidation during 80 min of exercise. The persistent effect of pre-exercise insulin resulted in a transient fall in blood glucose below pre-exercise values and a trend towards decreased FFA concentrations during exercise in the HGI trial.

A number of studies have examined the effect of the GI of CHO meal consumed in the hour before prolonged exercise on metabolism and endurance performance. Compared to a high GI CHO meal, a low GI CHO pre-exercise meal results in a lower CHO oxidation rate during the first 90-120 min of exercise and higher plasma FFA
concentrations during the latter stages of exercise (Guezennec, et al., 1993; Thomas, et al., 1991; 1994). Apart from the study by Thomas and co-workers (1991), the contention that such metabolic effects may benefit endurance performance has not been supported by previous (Febbraio and Stewart, 1996; Goodpaster, et al., 1995; Thomas, et al., 1994) nor the present studies. Hence, apart from individuals who may be sensitive to hypoglycaemia, there is little evidence to substantiate the advice given to athletes to avoid high GI CHO foods and drinks before exercise in favour of their low GI CHO (Brand-Miller, et al., 1996).

Sportsmen and women normally eat a light meal a few hours before, rather than eating just before, undertaking endurance exercise. The present study is the first to examine the influence of iso-energetic high and low GI CHO meals eaten three hours before exercise on exercise metabolism and capacity. Although the shift in substrate utilisation observed in this study is consistent with earlier studies (Guezennec, et al., 1993; Thomas, et al., 1991; Thomas, et al., 1994), the underlying mechanism is likely to be different because of the greater amount of CHO ingested and longer time period before exercise.

The two meals had similar fat and protein contents, therefore, the 336% greater incremental area under the postprandial serum insulin curve after the high GI meal, is not related to the presence of fat or protein. In the present study, both the high and low GI CHO meals provided an average of 144g of CHO. Apart from the estimated -30g of CHO oxidised, the prevailing insulinaemia observed during the HGI trials suggests that most of the remaining -114g of high GI CHO may have been stored as muscle and liver glycogen before exercise began (Nilsson and Hultman, 1973; Nilsson and Hultman, 1974; Young, et al., 1988). Previous studies using foods of similar GI to the HGI meal, providing ~2-2.5g CHO·kg\(^{-1}\) body mass, reported increased muscle glycogen content of between 10% to 40% over a 3 to 4 h postprandial period (Chryssanthopoulos, 1995; Coyle, et al., 1985; Neufer, et al., 1987). The increased CHO oxidation in the HGI trial in the present study provides some support for this speculation. Coyle et al. (1985) reported that compared to the fasted state, increased
CHO oxidation during exercise paralleled the elevated pre-exercise muscle glycogen concentration after a high GI pre-exercise CHO meal. This increased muscle glycogen degradation (Hargreaves, et al., 1995; Ritcher and Galbo, 1986) is probably the result of enhanced glycogen phosphorylase activity (Hespel and Ritcher, 1992). In contrast, a greater portion of the ~125g of unoxidised CHO from the low GI meal may have remained in the gut at the onset of exercise (Thorne, et al., 1983).

With the onset of exercise, blood glucose concentrations during LGI were maintained while during the HGI trial, subjects experienced a transient fall in blood glucose below basal levels. This is consistent with studies in which CHO was provided in the hour (Thomas, et al., 1991; 1994) or 4 hours before exercise (Coyle, et al., 1985; Sherman, et al., 1989). The effect of high pre-exercise serum insulin concentration on hepatic glucose production and muscle glucose uptake provides some explanation for this observation. Pre-exercise glucose ingestion is accompanied by increased muscle glucose uptake and reduced hepatic glucose production during exercise (Conus, et al., 1996). The influence of insulin on hepatic glucose metabolism is exaggerated due to the exposure of the liver to a higher insulin concentration compared to other tissues. Hence, hepatic glucose output during the early stages of exercise is likely to be lower during the HGI than during the LGI trial. The persistent effect of insulin (Montain, et al., 1991) probably increased muscle glucose uptake during early stages of exercise in the HGI to a greater extent compared to the LGI trial. The exercise induced rise in hepatic glucose output matches the increased glucose uptake by contracting skeletal muscles providing that liver glycogen stores are sufficient. Beyond 30 min of exercise, blood glucose concentrations were maintained during the HGI trial but decreased during the LGI trial after 60 min. These are likely to be the combined consequences of lower liver glycogen storage during the postprandial period and higher liver glycogenolysis during the LGI compared to the HGI trial.

Continued gastric emptying and intestinal absorption of CHO from a pre-exercise meal may account for the enhanced CHO oxidation during exercise (Chryssanthopoulos, 1995; Wright, et al., 1991). A significant amount of the CHO from the LGI meal may
have been available for continued absorption into the blood during exercise as more than three hours is required for the complete digestion and absorption of low GI CHO (Thorne, et al., 1983). However, increased activity of the sympathetic nervous system during exercise which causes splanchnic vasoconstriction probably further reduced the rate of absorption of monosaccharides from the low GI CHO. Furthermore, more than 30% of high amylose starches could escape digestion (Berry, 1986; Granfeldt, et al., 1993). Hence, the higher amylose content of lentils (~40%) compared to other starches (~25%) (Thorne, et al., 1983) may mean that less CHO was available to the contracting muscle during exercise.

The effect of insulin on lipolysis (Wolfe, et al., 1986) is responsible for the trend towards lower plasma glycerol and serum FFA concentrations in the HGI compared to the LGI trial. During the postprandial period and first 80 min of running, the amount of CHO oxidised during the LGI trial was 19% (or 44g) lower than in the HGI trial. Total energy expenditure was similar during exercise in both trials. It is also reasonable to assume similar contribution of protein to energy metabolism during both trials because the protein content of, and the cortisol responses to both meals were similar. This suggests that the 13% (or 27g) lower CHO oxidation during the first 80 min of exercise for the LGI trial was compensated by the 125% (or 10g) increase in fat oxidation. The amount of FFA employed as an energy substrate during exercise intensity of ~65% \( \dot{V}O_2 \text{max} \) is closely related to its availability (Coyle, 1995).

Two possible-limitations of the present study are the lack of a control condition (ie fasted) and the use of both male and female subjects. The benefits of CHO ingestion 3-4 hours compared to overnight fasting before exercise on endurance performance is well documented (Sherman, et al., 1989; Wright, et al., 1991; Chryssanthopoulos, 1995). Compared to a fasted condition, the increased CHO supply during both HGI and LGI trials will most likely result in higher RER during exercise. The observation at the onset of exercise, that serum insulin remained 96% (LGI) and 210% (HGI) higher than before meal ingestion provides some support for this.
If there are gender differences in metabolism during exercise, using 5 men and 3 women as a group may introduce a confounding factor to the data. A recent review of 10 studies on this topic by Tate and Holtz (1998) showed that 5 of the studies reported that women had a lower mean RER than men during exercise while the other 5 did not find any difference. There is evidence that circulating estrogen may be critical to whether there is a gender difference in exercise metabolism (Kendrick and Ellis, 1991; Rooney, et al., 1993). In the present study, the 3 female subjects were non-contraceptive users and not on estrogen replacement therapy. They were also tested in the phase of their menstrual cycle when estrogen fluctuation was minimal so as to avoid any gender effect on exercise metabolism.

In summary, the results of the present study show that ingestion of a high or low GI CHO 3 hours before exercise results in similar endurance running capacity. Compared to a low GI meal, ingestion of a high GI meal results in a shift in substrate utilisation from fat to CHO during exercise. In terms of CHO nutrition, the metabolic challenge is to maintain CHO supply to the muscles but to slow its depletion by relying optimally on fat as a fuel. However, it is difficult to optimise the availability of glucose to muscle and liver before exercise and still maintain high concentrations of FFA during exercise.
Fig 6.1 Blood glucose, serum insulin and blood lactate concentrations during the 3-h postprandial period and exercise (mean ± SE)

a: P<0.05 from 0:00; b: P<0.05 from 3:00; c: P<0.05 from LGI;
d: P<0.01 from 3:20, 3:40, 4:00 and Exh.
Fig 6.2 Mean incremental area under blood glucose (mmol.L⁻¹.min) and serum insulin (mIU.L⁻¹.min) response time curve (IAUC)
Fig 6.3 Serum FFA, plasma glycerol and serum cortisol concentrations during the 3-h postprandial period and exercise (mean ± SE)

a: P<0.05 from 0:00; b: P<0.05 from 3:00; c: P<0.05 from HGI.
Fig 6.4 Overall metabolic rate during the 3 h postprandial period and first 80 min run (mean ± SE).
* P<0.01 from HGI; # P=0.06 from HGI
Table 6.1 Respiratory exchange ratio, CHO and fat oxidation rate during the postprandial period. Values are mean ± SE.

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
</tr>
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<tr>
<td>RER</td>
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<td>0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
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<td>Fat oxidation</td>
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</tbody>
</table>

a: P<0.01 from 0 min; b: P<0.01 from L; c: P<0.05 from LGI
Table 6.2 *Oxygen uptake (VO₂), heart rate (HR), perceived rate of exertion (RPE), respiratory exchange ration (RER) and CHO oxidation during exercise. Values are mean ± SE.*

<table>
<thead>
<tr>
<th>Variable</th>
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<th>60min</th>
<th>80min</th>
<th>EXH</th>
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<td>173±8b</td>
<td>175±8</td>
<td>179±10</td>
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<tr>
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<td>169±9b</td>
<td>172±11b</td>
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<td>12±1a</td>
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<td>60min HGI</td>
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<td>12±1a</td>
<td>14±1ac</td>
<td>15±2a</td>
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<td>CHO oxd (g.min⁻¹)</td>
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<td>20min LGI</td>
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<td>2.3±0.2</td>
<td>2.3±0.2</td>
<td>2.3±0.1</td>
</tr>
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<td>40min HGI</td>
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<td>2.3±0.2</td>
<td>2.3±0.2</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>60min LGI</td>
<td>162±8b</td>
<td>169±9b</td>
<td>173±8b</td>
<td>175±8</td>
<td>179±10</td>
</tr>
<tr>
<td>60min HGI</td>
<td>161±9b</td>
<td>169±9b</td>
<td>172±11b</td>
<td>174±10</td>
<td>180±11</td>
</tr>
<tr>
<td>80min LGI</td>
<td>10±1a</td>
<td>12±2a</td>
<td>12±1a</td>
<td>13±2a</td>
<td>18±0</td>
</tr>
<tr>
<td>80min HGI</td>
<td>11±1a</td>
<td>12±1a</td>
<td>14±1ac</td>
<td>15±2a</td>
<td>18±0</td>
</tr>
</tbody>
</table>
| a: P<0.01 from EXH; b: P<0.05 from EXH, c: P<0.05 from LGI, d: P<0.01 from LGI
Chapter 7
Effect of high and low glycaemic index meals on muscle glycogen and exercise metabolism

7.1 Introduction
Compared to exercise after an overnight fast, the benefits of a carbohydrate (CHO) meal (2.5 - 4g CHO·kg\(^{-1}\) body mass) 3-4 hours before exercise on endurance performance are well documented (Chryssanthopoulos and Williams, 1997; Neufer, et al., 1987; Schabort, et al., 1999; Sherman, et al., 1989; Wright, et al., 1991). In accordance with these findings, it is commonly recommended that athletes undertaking strenuous daily training or competition should eat a moderate-sized meal about 3 hours before exercise for optimal restoration of liver and muscle glycogen (Brooks, et al., 1996; Burke, et al., 1999). However, any large glycaemic and insulinaemic perturbation accompanying such pre-exercise CHO ingestion may result in greater muscle glycogenolysis (Coyle, et al., 1985), and/or muscle glucose uptake and oxidation (Conus, et al., 1996) as well as reduced plasma free fatty acids availability and oxidation (Coyle, et al., 1997; Horowitz, et al., 1997) during subsequent exercise. In the study reported in Chapter 6, the low GI meal induced smaller metabolic changes during the postprandial period and exercise, which included a lower CHO oxidation during exercise (Wee, et al., 1999).

Among the studies that have examined the influence of GI of pre-exercise CHO foods on exercise metabolism and/or performance (Burke, et al., 1998; DeMarco, et al., 1999; Febbraio and Stewart, 1996; Kirwan, et al., 1997; Sparks, et al., 1998; Thomas, et al., 1991; Thomas, et al., 1994), only one has directly compared muscle glycogen utilisation (Febbraio and Stewart, 1996). Febbraio and Stewart (1996) reported that irrespective of glycemic and insulineemic responses of the ingested meals, net muscle glycogen utilisation were not different during 2-hour of cycling exercise at 70% \(\dot{V}O_{2\text{max}}\). In this latter study, a small quantity of CHO was provided less than an
hour before exercise. Therefore, it is possible that this may be insufficient to elicit large enough metabolic perturbation in their subjects. Furthermore, their subjects also appeared have very high pre-exercise glycogen concentrations (>600 mmol·kg\(^{-1}\) dw) (Febbraio and Stewart, 1996). Therefore, the primary purpose of the present study (Study 4) was to determine whether the GI of high CHO pre-exercise breakfast affects muscle glycogen storage during a 3-hour postprandial period, and muscle glycogen utilisation early during running exercise. The amount of CHO (2.5 CHO·kg\(^{-1}\) body mass) and timing for ingestion of the pre-exercise meals are in line with current recommendation for athletes (Brooks, et al., 1996; Burke, et al., 1999).

7.2 Methods

Subjects. Seven male recreational male runners [age 31 ± 4; body height 174 ± 2 cm; body mass 71.7 ± 3.1 kg; maximal oxygen uptake (\(\dot{V}O_2\) max) = 55.1 ± 2.3 ml·kg\(^{-1}\)·min\(^{-1}\)] volunteered as subjects for this study. Preliminary and main tests were as described in Chapter 3. The experimental trials were conducted on two occasions separated by 14 days (Figure 3.1). On test day, subjects arrived at the laboratory between 8:00-10:30am after a 12 hour overnight fast. After collection of baseline data, they received isoenergetic high and low GI tests meals of identical macronutrient content and volume (Table 3.3). During a 3-h postprandial period, the subjects relaxed in a quiet corner of the laboratory. Each subject then warmed up by running at ~60 % \(\dot{V}O_2\) max for 5 min before running at ~70 % \(\dot{V}O_2\) max for 30 min. Heart rate (HR) was monitored continuously during exercise (Polar Electro sports tester PE 3000). All trials were conducted under conditions of similar temperature and relative humidity (HGI: 19.4 ± 0.1 °C; 52 ± 2%, LGI: 19.3 ± 0.2°C; 52 ± 1%).

The changes in physiological and biochemical variables were analysed by a two-way ANOVA for repeated measures (meal x time). For values attaining this criterion, the
Tukey post hoc test was used to locate the difference(s). The glucose and insulin IAUC were not normally distributed and were analysed by the Wilcoxon test. The dietary data, total substrate oxidation and energy expenditure were analysed by Student paired t-tests. Statistical significance was set at \( P < 0.05 \). All data are reported as mean ± SE.

7.3 Results

During the two days before each trial there were no significant differences between the two trials in the recorded average daily energy intake, or amounts of CHO, fat or protein consumed. The mean daily energy intake and macronutrient composition were LGI: 3017 ± 269 kcal, 422 ± 8g CHO, 97 ± 3g fat, 121 ± 7g protein and HGI: 2909 ± 288 kcal, 414 ± 15g CHO, 84 ± 3g fat and 124± 14g protein. All subjects replicated their training schedule on the second day before the second trial and also refrained from exercise, caffeine and alcohol 24 h before each trial.

Plasma glucose, glucagon, serum insulin concentrations and ratio of glucagon-to-insulin concentrations during the 3-h postprandial period and exercise are shown in Figure 7.1. Plasma glucose concentrations peaked at 15 min after ingestion of both meals and returned to basal levels by the end of the 3-h postprandial period. The incremental area under the plasma glucose response curve for 1, 2 and 3-hour after the HGI meal were 1.9- 3.2- and 3.9-fold greater, respectively, than those after the LGI meal (P<0.05 in all cases). At 10 min into exercise during the HGI trial, plasma glucose concentrations declined sharply to values (4.05 ± 0.18 mmol·l⁻¹; \( P<0.05 \) from LGI) lower than that at the onset of exercise (4.75 ± 0.29 mmol·l⁻¹; \( P<0.05 \)). However, by the end of exercise, plasma glucose concentrations in the HGI trial (5.16 ± 0.20 mmol·l⁻¹) had increased to values higher than those in the LGI trial (4.79 ± 0.24 mmol·l⁻¹; \( P<0.05 \)).
Serum insulin concentrations peaked at 15-30 min after ingestion of both meals. The incremental area under the serum insulin response curve for 1, 2 and 3 hour after the HGI meal were 1.0-1.2- and 1.4-fold greater, respectively, than those after the LGI meal (P<0.05 in all cases). Just prior to exercise, serum insulin concentrations during the HGI trial (24.0 ± 3.6 mIU·l⁻¹) was twice that at the same time during LGI trial (12.1 ± 0.6 mIU·l⁻¹; P<0.05). At this time, serum insulin concentrations during both trials (P<0.01) were still elevated when compared to fasting values (7.6 ± 0.2 mIU·l⁻¹). After 10 min of exercise, serum insulin concentrations during the HGI trial (10.9 ± 1.4 mIU·l⁻¹) were still slightly higher compared to the LGI trial (8.0 ± 0.3 mIU·l⁻¹; P=0.07) but decreased to similar values thereafter.

After the LGI meal, plasma glucagon concentrations increased above fasting values and remained elevated for 2-h (P<0.05). Plasma glucagon concentrations were higher in the LGI than the HGI trial throughout the 3-h postprandial period (P<0.05). The differences in pancreatic hormonal responses after the high and low GI meal became more apparent when the glucagon-insulin ratios were compared. These ratios were higher throughout the postprandial and exercise period following the LGI than the HGI meal (P<0.05).

The plasma FFA and glycerol response curves are shown in Figure 7.2. Plasma FFA and glycerol concentrations were lower than fasting values after both meals during the 3-h postprandial period (P<0.05). However, this suppression is less after the LGI meal than after the HGI meal. Plasma FFA and glycerol concentrations were higher at the end of the postprandial period and during exercise in the LGI than the HGI trial (P<0.05).

Mean oxygen uptake, RER, blood lactate concentrations and HR during both trials are shown in Table 7.1. Oxygen uptake and RER were higher during the postprandial period compared to the fasted state during both trials but were not different between
each trial. Blood lactate concentrations were higher during the 3-h postprandial period after the LGI (P<0.05) than the HGI meal. During exercise, oxygen uptake and heart rates were not different between the trials. The average intensities sustained during exercise were 71.0 ± 1.2 % $\dot{V}O_2\text{max}$ and 71.0 ± 1.3 % $\dot{V}O_2\text{max}$ during the LGI and HGI trials respectively. The mean RER values and blood lactate concentrations during exercise were lower in the LGI than the HGI trial (P<0.05; Table 7.1). Overall, substrate oxidation during the postprandial period did not differ between trials (Figure 7.3). During exercise, total CHO oxidation was 12% lower (P<0.05) during the LGI trial with a compensatory increase in fat oxidation (P<0.05) compared to the HGI trial, such that the overall energy expenditure were similar (Figure 7.3).

Pre-meal, pre-exercise and post-exercise mixed muscle glycogen (TG) concentrations are shown in Figure 7.4. The exercise and dietary control before each trial resulted in similar mixed muscle glycogen concentration before the respective meals (LGI: 380 ± 26 mmol·kg$^{-1}$ dw; HGI: 407 ± 45 mmol·kg$^{-1}$ dw). Three hours following the HGI meal, muscle glycogen concentration was elevated by 15% (P<0.05) but remained unaltered after the LGI meal. At the end of 30 min exercise, glycogen concentrations were reduced in both trials (P<0.05) and values did not differ between trials (LGI: 315 ± 16 mmol·kg$^{-1}$ dw; HGI: 340 ± 46 mmol·kg$^{-1}$ dw). However, net muscle glycogen utilisation was 46% greater (P<0.01) during the HGI (129 ± 16 mmol·kg$^{-1}$ dw) compared to the LGI (88 ± 15 mmol·kg$^{-1}$ dw) trial.

Concentrations of the macro- (MG) and pro-glycogen (PG) pools are shown in Figure 7.5. The PG fraction was always in excess of MG, which varies from 2 to 21% of TG. Similar to the changes in TG, MG (+32%; P<0.05) and PG (+15%; P<0.05) concentrations increased 3-h following the HGI meal but remained unaltered following the LGI meal. Following exercise in both trials, the respective pools were reduced to similar values. In the LGI trial, MG decreased by 59% (P<0.05) while PG decreased by 23% (P<0.05). In the HGI trial, MG decreased by 41% (P<0.01) while
PG decreased by 20% (P<0.01). However, where changes were statistical significant, these were always greater in the MG pool compared to the PG pool. Following the HGI meal, MG increased from being 12 to 14% (P<0.05) of TG. Following exercise, MG decreased from being 11 to 8% (P=0.07) and 14 to 7% (P=0.07) of TG in the LGI and HGI trial respectively.

Changes in concentrations of other muscle metabolites are presented in Table 7.2. Changes in adenosine triphosphate, phosphocreatine and glucose-6-phosphate, if any, were similar between both trials. In agreement with the blood lactate data during exercise, muscle lactate concentration were higher in the HGI than the LGI trial following exercise (P<0.05).

7.4 Discussion
The major finding of this study was that when different pre-exercise breakfasts conforming to current nutritional recommendation for athletes were consumed, a low GI meal contributed less CHO to the muscle during a 3-h postprandial period compared to a high GI meal. However, plasma FFA availability and fat oxidation were better maintained during the LGI trial, together with a more economical utilisation of muscle glycogen and lower muscle lactate accumulation during 30 min of running exercise. Furthermore, our result also suggests that when muscle glycogen concentration is normal, that is, neither supercompensated nor depleted, changes in the MG faction appears to occur more readily than the PG faction in response to diet and exercise.

A number of studies have quantified muscle glycogen storage 3 or 4-hour following breakfast consisting of high or moderate GI CHO foods and reported glycogen accumulation ranging from 10-42% (Chryssanthopoulos, et al., 1998; Coyle, et al., 1985; Neufer, et al., 1987; Taylor, et al., 1993). The 15% increase in TG concentration 3-hour following the HGI meal is in close agreement with these studies. However, TG
concentration did not alter following the LGI meal of identical macronutrient content. In another study, muscle glycogen storage after 24-hour recovery of following glycogen depleting exercise was greater with a high than a low GI diet (Burke, et al., 1993). The subjects in the present study were on a normal mixed diet and agreed not to exercise the day before the experiment. Their pre-meal TG concentration were normal and not supercompensated (Figure 7.4). 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-hour postprandial period. This is supported by the greater incremental area under the plasma glucose (3.9-fold) and serum insulin (1.4-fold) response curves 3-h following the HGI compared to the LGI meal (Figure 7.1A and B). This result demonstrate that even without stimulation of prior muscle contraction, high GI foods confers advantage in term of muscle glycogen storage compared to low GI foods.

Utilisation of plasma FFA decreases after a CHO meal partly because the CHO-induced rise in insulin inhibits the mobilisation and hence availability of circulating FFA. One hour following glucose ingestion, lipolysis is suppressed to a point which it limits fat oxidation during low intensity exercise (Horowitz, et al., 1997). Pre-exercise ingestion of glucose either increase (Costill, et al., 1977; Hargreaves, et al., 1985) or does not influence (Fielding, et al., 1987) muscle glycogen utilisation during exercise at 70 % VO2 max. A number of investigators have applied the GI concept to address the still controversial question of CHO intake within the hour before exercise (DeMarco, et al., 1999; Febbraio and Stewart, 1996; Kirwan, et al., 1997; Sparks, et al., 1998; Thomas, et al., 1991; 1994). However, sportsmen and women normally eat a light meal a few hours before, rather than eating shortly before undertaking endurance exercise. Nevertheless, the suppression of fat oxidation during exercise persists for at least 4 hours following a high CHO (2g CHO·kg-1 body mass) meal, even when insulin had returned to pre-exercise concentrations (Montain, et al., 1991). Apart from Study 3 of this thesis (Chapter 6) (Wee, et al., 1999), another study also examined the effect
of GI of pre-exercise meals consumed in the hours before exercise on metabolism and performance (Burke, et al., 1998). Burke et al. (1998) reported that GI of pre-exercise meals does not influence exercise metabolism and performance when large amounts of CHO are ingested during cycling exercise. As it is difficult to deliver as much fluid and CHO during running compared to cycling, the pre-exercise meal is likely to play a more important role in preparation for running. In the present study and Chapter 6 (Wee, et al., 1999), eating a low GI meal (2-2.5g CHO·kg⁻¹ body mass) 3-hour before running at ~70 % \( \dot{V}O_2 \text{max} \) resulted in reduced glycaemic and insulinaemic responses, increased plasma FFA availability and a shift in substrate utilisation from CHO to fat during exercise compared to a high GI meal. The greater CHO metabolism during the HGI trial is accompanied by a greater degradation of muscle glycogen (Figure 7.4) and accumulation of muscle (Table 7.2) and blood lactate (Table 7.1) in 30 min of exercise.

The present study is first report of reduced muscle glycogen utilisation during exercise after a LGI was compared to a HGI meal. The present finding is in contrast with that of Febbraio and Stewart (1996), who reported that irrespective of the GI of pre-exercise meals, pre-exercise CHO ingestion has no effect on muscle glycogen utilisation. A number of factors could account for the discrepancy. Firstly, in Febbraio's study, subjects ingested only 1g CHO·kg⁻¹ body mass of high and low GI foods 45 min before 2-hour of cycling exercise at 70 % \( \dot{V}O_2 \text{max} \) (Febbraio and Stewart, 1996). In contrast to the present study, such a CHO load may not be sufficient to elicit large enough metabolic perturbation especially in well-trained and insulin sensitive subjects (Montain, et al., 1991). Secondly, any increase in muscle glycogenolysis following pre-exercise ingestion of high GI CHO is likely to be more pronounced at the early stages of exercise (Costill, et al., 1977), especially when muscle glucose uptake is not matched by glucose output from the liver (Conus, et al., 1996). In Febbraio's study, plasma FFA concentrations were not different between high and low GI trials beyond the first hour of exercise. Consequently, it may be more difficult to detect any differences in rate of muscle glycogen utilisation over 2-hour of
exercise (Febbraio and Stewart, 1996) compared to the 30-min in the present and other studies (Costill, et al., 1977; Hargreaves, et al., 1985). Lastly, the very high pre-exercise muscle glycogen concentrations (>600 mmol·kg\(^{-1}\) dw) of their subjects (Febbraio and Stewart, 1996) may also have reduced any possible difference in muscle glycogen utilisation. In the present study, the glucagon-to-insulin ratio during exercise in the HGI trial is lower than that in the LGI trial (Figure 7.1D). This suggests hepatic glucose output is likely to be lower in the former. After 10 min of exercise, plasma glucose concentration in the HGI trial declined sharply to values lower than that at the onset of exercise or the LGI trial (Figure 7.1A). The effect of insulin and contraction on muscle glucose uptake is addictive. This sharp decline is probably a reflection of insufficient glucose provision in the face of increased muscle uptake in the HGI trial.

During 30 min of exercise, total CHO oxidation during the two trials were LGI: 99 ± 7 g and HGI: 112 ± 9 g; and muscle glycogen utilisation were LGI: 88 ± 15 mmol·kg\(^{-1}\) dw and HGI: 129 ± 16 mmol·kg\(^{-1}\) dw. Assuming that the dry mass of two legs is equal to ~5% of total body weight (Katz, 1986) and all this muscle is involved in contraction during running, then the estimated amount of dry muscle mass engaged in exercise is about 3.7 kg. Compared to the HGI trial, 41 mmol·kg\(^{-1}\) dw less muscle glycogen was used during the LGI trial which translates to 152 mmol or 27 g of CHO. Therefore, the lower CHO oxidation (13 g) during the LGI trial is entirely explained by the lower rate of muscle glycogen utilisation. Furthermore, it can be deduced that total glucose oxidation in the LGI trial exceeded that in the HGI trial by 14g.

As distinct from cycling, CHO ingestion just before and during running (~70 % \(\dot{V}_O_{2,max}\)) delays the onset of fatigue by reducing the rate of muscle glycogen utilisation (Tsintzas and Williams, 1998). All being equal, it is reasonable to predict a greater endurance running capacity following the LGI than the HGI meal due to a more economical utilisation of TG during the earlier stages of exercise. However, glycogen storage after the HGI meal within the 3-hour postprandial period was 40
mmol·kg\(^{-1}\) dw higher than after the LGI meal. This is similar to the difference in muscle glycogen utilisation between the HGI and LGI trial (41 mmol·kg\(^{-1}\) dw). Consequently, TG concentration was similar in both trials at the end of 30 min of exercise (Figure 6.4). Therefore, the greater fat utilisation and lower glycogen utilisation following the LGI meal may not translate to a greater endurance capacity than the HGI meal. Indeed, this was the finding in Chapter 6 (Wee, et al., 1999).

Glycogen exists in two forms in human muscle: one form is acid soluble termed MG and the other is acid insoluble termed PG (Adamo and Graham, 1998; Jansson, 1981). Recent studies suggest that rates of accumulation and utilisation of both forms are different, depending on total glycogen (TG) concentration and other metabolic factors (Adamo, et al., 1998; Asp, et al., 1999). In the glycogen depleted state, the PG pool is more sensitive to CHO intake (Adamo, et al., 1998). In contrast, glycogen supercompensation is associated with a greater synthesis in the MG pool (Adamo, et al., 1998). Furthermore, Asp et al. reported that a greater fraction of MG than PG is utilised during a marathon (Asp, et al., 1999). In the present study, the PG and MG faction of muscle glycogen was measured following perchloric acid extraction of muscle samples (Adamo and Graham, 1998; Jansson, 1981). The storage and depletion patterns of both pools are similar to that of TG following the meals and exercise. However, the fractional increase following the HGI meal was greater in the MG (32%) than PG (15%) pool such that MG fraction of TG increased with the meal. Similarly the fractional decrease during exercise in both trials was greater in the MG (LGI: 59%; HGI: 41%) than the PG (LGI: 23%; HGI 20%) pool. The fractional decrease in glycogen factions observed in the present study is in agreement with that reported after a marathon (MG: 72%; PG: 34%) (Asp, et al., 1999). This suggests that the preferential utilisation of the MG faction may be independent of TG concentrations. Taken together, the results of the present and other studies (Adamo, et al., 1998; Asp, et al., 1999) suggest that compared to MG, PG may be more tightly regulated within critical limits in response to diet and exercise.
In summary, these findings indicate that 1) a high GI meal results in greater muscle glycogen storage than a low GI meal in overnight fasted subjects with normal glycogen concentration; 2) the relative shift in substrate metabolism during 30 min exercise from CHO to fat 3-hour following a LGI compared to a high GI meal is due to reduced rate of muscle glycogenolysis and not glucose oxidation; and 3) compared to MG, concentrations of PG appears to be more closely regulated when TG is not supercompensated nor depleted. These findings support that of Chapter 6 that the GI of CHO ingested 3-hour before exercise does not influence endurance running capacity (Wee, et al., 1999).
Figure 7.1 A) Plasma glucose, B) serum insulin, C) plasma glucagon and D) ratio of glucagon-insulin concentrations during the 3h postprandial period and 30 min exercise. Values are mean ± SE. a: P<0.05 from LGI; b: P<0.05 from 0:00, c: P<0.05 from 3:00.
Figure 7.2 A) Plasma FFA and B) glycerol concentrations during the 3h postprandial period and exercise. Values are means ± SE. *P<0.05 from HGI.
Figure 7.3  Estimated substrate oxidation during 3 h postprandial rest period and 30 min exercise. Values are means ± SE. a: P<0.05 from HGI.
Figure 7.4 Muscle glycogen concentrations pre-meal, pre-exercise and post-exercise. Values are means ± SE. a: P<0.05 from pre-meal, b: P<0.05 from pre-exercise.
Figure 7.5 Muscle pro- and macro-glycogen concentrations pre-meal, pre-exercise and post-exercise. Values are means ± SE. a: P<0.05 from pre-meal, b: P<0.05 from pre-exercise.
Table 7.1. Mean oxygen uptake, RER, blood lactate concentrations and heart rates during LGI and HGI trials (values are mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>LGI</th>
<th>HGI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxygen uptake (l·min⁻¹)</strong></td>
<td></td>
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</tr>
<tr>
<td>Basal</td>
<td>0.29 ± 0.02</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>Postprandial</td>
<td>0.37 ± 0.02</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td>Exercise</td>
<td>2.85 ± 0.18</td>
<td>2.82 ± 0.21</td>
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<tr>
<td><strong>RER values</strong></td>
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<td>0.82 ± 0.03</td>
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<tr>
<td>Postprandial</td>
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<td>0.94 ± 0.02</td>
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<td>Exercise</td>
<td>0.96 ± 0.01*</td>
<td>0.99 ± 0.01</td>
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<tr>
<td><strong>Blood lactate concentrations (mmol·l⁻¹)</strong></td>
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<tr>
<td>Basal</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
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<tr>
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<tr>
<td>Exercise</td>
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</tr>
<tr>
<td><strong>Heart rate (beats·min⁻¹)</strong></td>
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<tr>
<td>Exercise</td>
<td>161 ± 6</td>
<td>162 ± 5</td>
</tr>
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</table>

* P<0.05 from HGI
Table 7.2. Other muscle metabolites concentrations (mmol·kg $dw^{-1}$) during HGI and LGI trials (values are mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>LGI</th>
<th>HGI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre-meal</td>
<td>pre-exercise</td>
</tr>
<tr>
<td>ATP</td>
<td>25.7 ± 0.6</td>
<td>25.9 ± 0.8</td>
</tr>
<tr>
<td>PCr</td>
<td>83.2 ± 2.1</td>
<td>83.5 ± 2.1</td>
</tr>
<tr>
<td>G-6-P</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Lactate</td>
<td>4.7 ± 0.2</td>
<td>5.8 ± 0.3</td>
</tr>
</tbody>
</table>

*P < 0.05 from HGI
Chapter 8
General Discussion

Postprandial blood glucose and insulin responses play a key role in subsequent exercise metabolism. Many studies have investigated the effects of different types of CHO meals before exercise (Chapter 2, Sections 2.5.1, 2.5.2). In these studies, the meals were consumed 30-60 min before exercise. However, the benefits of eating CHO meals within the hour before exercise are not entirely clear. In contrast, the benefits of a high CHO meal 3-4 hours before exercise on endurance performance are well documented (Chapter 2, Section 2.5.3). Therefore, it is commonly recommended that athletes undertaking strenuous daily training or competition eat a moderate-sized meal about 3 hours before exercise (Brooks et al., 1996; Burke et al., 1999). However, the optimal type and amount of CHO to be consumed is unclear. Only a few studies have investigated the effect of composition (Okano et al., 1996, 1998; Whitley et al., 1998) or glycaemic index (Burke et al., 1998) of a CHO meal, in the hours before exercise on metabolism and endurance cycling performance. Therefore, this thesis examined the effect of the glycaemic index and composition of CHO breakfasts on postprandial and exercise metabolism and also on endurance running capacity.

In each of the studies described in this thesis, the subjects were either physically active students or endurance trained runners of similar training status. The meals were designed to produce markedly and contrasting glycaemic and insulinaemic responses during the postprandial period. Similar experimental procedures were used in all the studies reported in this thesis and the metabolic and physiological responses of the subjects followed similar patterns after the same type of meal.

The aim of this final chapter is to reflect on and integrate the main observations made during each study so as to facilitate a greater understanding of the influence of the type of CHO meals on postprandial and subsequent exercise metabolism.
8.1 Carbohydrate availability controls substrate oxidation in the postprandial state

Postprandial substrate metabolism may be controlled by the CHO composition or glycaemic and insulinaemic responses to a meal. Some support for this statement is provided by studies which utilised glucose and insulin infusions (Campbell et al., 1992; Sidossis et al., 1996, 1998) rather than real foods. In the study reported in Chapter 4, a high GI meal increased glucose delivery to the bloodstream and resulted in a shift in postprandial substrate oxidation from fat to CHO when compared to the responses to a low GI meal. This observation provides evidence that CHO availability plays an important role in determining postprandial substrate oxidation. Furthermore, a high CHO-low fat meal (CM) resulted in a lower fat oxidation and higher CHO oxidation rate in the 3 hours following the meal compared with the responses to an iso-energetic low CHO-high fat meal (FM). When CHO was added to the FM (HM vs FM), plasma glycerol and FFA concentrations decreased, whereas CHO oxidation increased and fat oxidation decreased. However, when fat was added to the CM (HM vs VM), plasma glycerol, FFA concentrations and fat oxidation increased but CHO oxidation was unchanged. This provides evidence that role of CHO availability is more important than that of fat availability in regulating postprandial substrate oxidation. When exercise was undertaken 3 hours after the high and low GI meals, the higher fat oxidation in the low GI trial continued for a further 90 min until the end of exercise (Chapter 6). Apart from limiting dietary fat and energy intake, these findings suggest a possible role for low GI CHO as part of an effective dietary strategy towards fat loss. Further research is needed to provide evidence, if any, of the potential benefits of a low GI diet in helping to achieve and maintain fat balance.

In the studies reported in Chapters 4, 6 and 7 the incremental area under the serum insulin response curves during the 3-hour postprandial period after the high GI meal was 1.4 to 3.3 fold greater than after the low GI meal. During the same period following the low GI meal, the suppression of plasma glycerol and fatty acid concentrations were not as great as after a high GI meal. Some studies reported that a prolonged low GI diet resulted in lower fasting insulin concentration in
hyperinsulinaemic subjects (Slabber et al., 1994) or elite athletes (Tegelman et al., 1996) compared to a high GI diet. However, fasting insulin concentration is only a crude reflection of whole body insulin sensitivity. Studies which examined the effect of high and low GI diets on insulin sensitivity reported contrasting results. Frost and co-workers (1996; 1998) reported that a low GI diet reduced insulin response to a glucose load and improved adipocyte insulin sensitivity in women with, or at risk of, CHD and in healthy controls. Behall et al. (1989) did not find any difference in insulin response in healthy men to a standard glucose load 4 weeks after a diet containing 34% of energy as 70% amylose or amylopectin starch. In a study involving healthy lean young men, Kiens & Ritcher (1996) reported that switching CHO from high to low GI sources for 4 weeks decreased whole body glucose disposal at a high but not physiologic plasma insulin concentration. This result is unexpected and difficult to explain. It is suggested that the higher plasma fatty acid concentration after low GI than high GI meals may impair insulin-stimulated glucose uptake when on the low GI diet (Kiens & Ritcher, 1996). This seems unlikely because studies supporting the inhibitory effect of fatty acid on glucose uptake have reported this effect to occur at plasma FFA concentrations well above normal fasting values (Boden et al., 1991, 1994; Walker et al., 1991). However, in the studies reported in Chapters 4, 6, 7 and in the study of Kiens and Ritcher (1996), plasma FFA concentrations did not exceed those of fasting values when subjects were at rest.

On the other hand, a recent study showed that 15 hours of moderate hyperglycaemia (~8 mmol·L⁻¹) reduced fatty acid oxidation in the splanchnic region even when fatty acid availability was maintained (Sidossis et al., 1998). A similar result was reported for the fasted state after two weeks on a high CHO compared to a high fat diet (Sidossis & Mittendorfer, 1999). As a result of hyperglycaemia (Sidossis et al., 1998) or high CHO diet (Sidossis & Mittendorfer, 1999) there was a significant increase in the hepatic triglyceride secretion and plasma VLDL-triglyceride concentration. In the studies reported in Chapters 4, 6 and 7 the incremental area under the blood glucose response curve was 1.2 to 5 fold greater during the 3-hour postprandial period after the high GI compared to the low GI meal. Many people spend a significant amount of
their time in the postprandial state, and so a low GI diet which avoids postprandial hyperglycaemia and hyperinsulinaemia compared to a high GI diet, may result in a reduction in hepatic triglyceride secretion and plasma VLDL-triglyceride concentration.

In summary, postprandial substrate metabolism is controlled by the amount and type of CHO rather than fat in a meal. Although there are studies which point to the potential health benefits of low GI diets (Chapter 2, Section 2.3.6), there is, as yet, no conclusive evidence to show that a habitual low GI diet may improve fat balance, increase whole body insulin sensitivity or reduce hypertriglyceridaemia.

8.2 Influence of glycaemic index of pre-exercise meals on exercise metabolism

Chapter 6 reported a study in which the ingestion of a low GI meal before exercise resulted in a reduction in CHO oxidation and increase in fat oxidation during 80 min of exercise when compared to the responses to a high GI meal. The higher pre-exercise insulin concentration following the high GI meal resulted in a transient fall in blood glucose concentration early during exercise and a trend towards decreased plasma FFA concentrations (Table 8.1).

Using other high and low GI foods, this result was reproduced during 30 min of exercise in the study described in Chapter 7. In addition, muscle glycogen concentration was shown to increase by 15% 3 hours after the high GI meal but it did not change after the low GI meal. However, muscle glycogen utilisation and lactate accumulation were lower during 30 min of exercise in the low GI than the high GI trial. This was the first study to confirm the hypothesis of Thomas and colleagues (1991) and Guezenne (1995) that a low GI pre-exercise meal results in reduced muscle glycogen utilisation during exercise when compared to a high GI pre-exercise meal. It is also estimated that overall glucose oxidation in the LGI trial during the 30 min of exercise exceeded that in the HGI trial. Hence the lower CHO oxidation during the LGI trial can be entirely explained by the lower rate of muscle glycogen utilisation and higher rate of glucose oxidation. The higher rate of muscle glycogen utilisation in
the HGI trial is likely to be related to the sharp decline in plasma glucose concentration during the first 10-20 min of exercise, in order to make up for the lack of hepatic glucose output in the face of increased CHO requirement. It is difficult to attribute the increased muscle glycogenolysis entirely to either reduced blood glucose or FFA availability. There are mechanisms other than feedback regulation of hepatic glucose output to prevent hypoglycaemia during exercise when exercise intensity exceeds 60% \( \dot{V}O_2\text{max} \) (Kjaer, 1995). Therefore, increased muscle glycogenolysis may be another necessary means of preventing excessive decline in blood glucose concentration.

Table 8.1 Serum insulin concentration just before exercise and plasma FFA concentrations just before and at the end of exhaustive exercise in LGI and HGI trials (mean ± SE (range))

<table>
<thead>
<tr>
<th></th>
<th>Serum insulin concentration at the onset of exercise (mU/ml)</th>
<th>Plasma FFA concentration at the onset of exercise (mmol/l)</th>
<th>Plasma FFA concentration at exhaustion (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGI</td>
<td>20 ± 2 (13 to 29)</td>
<td>0.25 ± 0.08 (0.04 to 0.68)</td>
<td>1.08 ± 0.30 (0.41 to 2.90)</td>
</tr>
<tr>
<td>HGI</td>
<td>30 ± 7 (9 to 52)</td>
<td>0.07 ± 0.02 (0.01 to 0.16)</td>
<td>0.92 ± 0.30 (0.38 to 2.89)</td>
</tr>
</tbody>
</table>

8.3 Influence of CHO and fat availability on substrate oxidation during exercise

It is well known that the content of CHO and fat in the diet before exercise influences the reciprocal relationship between CHO and fat metabolism during exercise. In the studies reported in Chapters 6 and 7, the high and low GI meals contained the same amount of CHO, fat and protein. Yet different postprandial glycaemic and insulinaemic responses resulted in markedly different muscle glycogen and plasma FFA concentrations before exercise. Therefore, it is not surprising that substrate metabolism was also different during exercise. However, the relative importance of CHO and fat availability on substrate oxidation during exercise was not known. This question was addressed in Chapter 5.

In the study reported in Chapter 5, pre-exercise FM ingestion resulted in elevated concentration of plasma FFA, greater fat oxidation and lower CHO oxidation during exercise when compared to the iso-energetic CM. Overall substrate oxidation during exercise in the HM trial resembled the CM rather than the FM trial. This provides
evidence that it is CHO rather than fat availability that exerts predominant control over substrate utilisation during exercise.

Pre-exercise CHO ingestion may exert a dual effect on fat oxidation during exercise. The first is the effect of insulin on lipolysis. During exercise at 70 % $\dot{V}O_2_{max}$, fat oxidation is limited by the concentration of circulating FFA. Table 8.2 shows the serum insulin concentration just before exercise and plasma FFA concentrations just before and at the end of exercise. The differences in circulating FFA were maintained throughout exercise. Therefore, the suppression of fat oxidation during exercise in the CM compared to the FM trial is at least partly related to lack of plasma FFA availability. It is suggested that the lower concentration of plasma FFA in the CM compared to the FM trial is due to insulin inhibition of lipolysis (Horowitz et al., 1997), at least during the initial stages of exercise.

The second is the effect of increased CHO flux on fat oxidation independent of the effect on lipolysis. The HM contained 414 ± 14 kcal of additional fat compared to the CM, and 417 ± 14 kcal of additional CHO compared to the FM. Although plasma FFA concentration was higher during the HM trial than the CM trial, this did not result in any significant shift in substrate oxidation. This suggests a preferential oxidation of CHO rather than fat when both substrates are made available to the muscle. This observation provides some support for the results of a recent study which demonstrated that increases in glycolytic flux and CHO oxidation following pre-exercise CHO ingestion may limit the oxidation of long chain fatty acids in skeletal muscle during exercise (Coyle et al., 1997). The proposed mechanism is that during increased glycolytic flux, carnitine mediated entry of long-chain fatty acid (LCFA) into the mitochondria is limited (Sidossis, 1998; Coyle, 1999). Furthermore, it is speculated that this discrimination against LCFA transport is due to the inhibition of the carnitine acyl-transferase enzyme by malonyl-CoA (Sidossis, 1998; Coyle, 1999).

Table 8.2 Serum insulin concentration just before exercise and plasma FFA concentrations just before and at the end of exhaustive exercise in high fat-low CHO
(FM), high CHO-low fat (CM) and high CHO-high fat (HM) trials (mean ± SE (range))

<table>
<thead>
<tr>
<th></th>
<th>Serum insulin concentration at the onset of exercise (mU·mL⁻¹)</th>
<th>Plasma FFA concentration at the onset of exercise (mmol·L⁻¹)</th>
<th>Plasma FFA concentration at exhaustion (mmol·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM</td>
<td>11 ± 1 (7 to 14)</td>
<td>0.29 ± 0.03 (0.20 to 0.37)</td>
<td>0.88 ± 0.13 (0.42 to 1.20)</td>
</tr>
<tr>
<td>CM</td>
<td>18 ± 4 (10 to 35)</td>
<td>0.06 ± 0.02 (0.01 to 0.11)</td>
<td>0.46 ± 0.06 (0.36 to 0.66)</td>
</tr>
<tr>
<td>HM</td>
<td>19 ± 3 (13 to 29)</td>
<td>0.14 ± 0.03 (0.05 to 0.23)</td>
<td>0.60 ± 0.07 (0.40 to 0.85)</td>
</tr>
</tbody>
</table>

8.4 Effect of type of pre-exercise CHO on endurance running capacity

In the study reported in Chapter 6, the GI of the CHO in the pre-exercise meal did not influence endurance running capacity. It is reasonable to predict a greater endurance running capacity following the LGI than the HGI meal due to a more economical utilisation of muscle glycogen during the earlier stages of exercise (Chapter 7). However, glycogen storage after the HGI meal within the 3-hour postprandial period was 40 mmol·kg⁻¹ dw higher than after the LGI meal. This is similar to the difference in muscle glycogen utilisation between the HGI and LGI trial (41 mmol·kg⁻¹ dw). Consequently, muscle glycogen concentration was similar in both trials at the end of 30 min of exercise. Therefore, the greater fat utilisation and lower glycogen utilisation following the LGI meal does not eventually translate to a greater endurance capacity than the HGI meal (Chapter 6).

There may be a trade-off in terms of any benefit conferred by additional CHO before exercise because of increased CHO utilisation during exercise. On the other hand, high fat diets or pre-exercise fat ingestion which increases fat oxidation and causes a more more economical rate of glycogen degradation throughout exercise may enhance endurance capacity. However, there is no convincing evidence that adaptation to a high fat diet may enhance endurance performance (Chapter 2, section 2.5.4). Chapter 5 described the results of a study which examined the influence of CHO and fat content of pre-exercise meals on endurance capacity. Running time to exhaustion was longer after the CM than the FM. Moreover, running time to exhaustion was longer in the HM trial compared to FM but not different from the CM trial. It seems that the amount of CHO provided in the CM was enough to cover any deficit in terms of increased
CHO oxidation and endurance capacity was not decreased but increased compared to the FM trial. Furthermore, additional fat in the HM did not further increase endurance capacity compared to the CM trial. Therefore, dietary CHO rather than fat availability before exercise determines endurance capacity in overnight fasted subjects.

In order to delay the onset of fatigue during exercise, this thesis had explored two different dietary strategies to increase CHO supply to the muscles and also to delay its depletion by using as much fat as possible for energy production. The study reported in Chapter 7 showed that a high CHO and high GI meal increased muscle glycogen storage before exercise. Compared to such a meal, carbohydrate metabolism during exercise could be reduced either by eating a high fat (Chapter 5) or a low GI meal (Chapters 6 and 7) before exercise. However, the effects of these two dietary strategies on endurance run times to exhaustion were quite different. Despite lowering fat oxidation and increasing CHO oxidation, a high CHO-low fat pre-exercise meal enhanced endurance running capacity compared to a low CHO-high fat meal (Chapter 5). When the amount of CHO was the same, the GI of a pre-exercise meal did not influence endurance running capacity even though CHO oxidation was lower after a low than a high GI meal (Chapter 6). Taken together, these results suggest that endurance running capacity is determined by the difference between the amount of CHO supplied to and that utilised by the muscles during exercise.

In the study reported in Chapter 5, the CM and HM contained additional CHO compared to the FM. The estimated whole body CHO availability after 80 min of exercise in the CM and HM trials were 45g and 59g higher respectively, than the FM trial. Furthermore, the higher glucagon concentration in the FM compared to the CM and HM trials after 60 min of exercise suggests increased hepatic glucose output compensates for the apparent lack of CHO for muscle metabolism. A feed-forward mechanism is likely to account for the increased hepatic glucose output (Kjaer, 1995) because plasma glucose concentration did not decline during exercise. Previous studies conducted in our laboratory suggest that fatigue during prolonged running is clearly linked to glycogen depletion in type I muscle fibres (Tsintzas et al., 1995, 1996).
Therefore, further studies would be needed to confirm if the earlier onset of fatigue in the FM trial is due to depletion of glycogen in the type-I fibres of the active muscle.

In the same study (Chapter 5), there was also a modest correlation between the concentrations of plasma FFA and serum prolactin during exercise. At exhaustion in the FM trial, two subjects who reported sleepiness also had plasma FFA concentrations greater than 1 mmol·l⁻¹ as well as the highest serum prolactin concentrations. These observations provide circumstantial evidence that impairment in endurance performance during the FM trial, at least for these two subjects, may be partly mediated by central factors. It is not known if fatigue during exercise in the study reported in Chapter 6 is mediated by central factors. The higher plasma FFA concentration at the end of exercise in this study (Table 8.1) than that reported in Chapter 5 (Table 8.2) can be attributed to a female subject who had equally high FFA concentration at exhaustion in both trials (~2.9 mmol·l⁻¹). When these value are excluded, the mean values at the end of exercise were LGI: 0.8 ± 0.1 mmol·l⁻¹ and HGI: 0.6 ± 0.1 mmol·l⁻¹. No other subject had plasma FFA concentration greater than 1 mmol·l⁻¹ at exhaustion. However, as there were no markers of central fatigue, it is not possible to speculate on the role of the central component of fatigue in this case.

In the studies reported in Chapters 6, and 7, ingestion of 2 to 2.5 g kg⁻¹ body mass of high GI CHO was associated with a transient reduction in blood glucose concentration during the first 20 min of exercise. Glucose concentrations were measured in blood (Chapter 6) and in plasma (Chapter 7). In Chapter 6, three subjects had blood glucose concentration which were less than 3.5 mmol·l⁻¹ after 20 min of exercise during the HGI trial. In Chapter 7, one subject had a plasma glucose concentration of less than 3.5 mmol·l⁻¹ after 10 min of exercise in the HGI trial. However, none of the subjects reported any symptoms of hypoglycaemia. In contrast, none of the subjects in the LGI trial in either study recorded blood or plasma glucose concentration of less than 3.5 mmol·l⁻¹. The results of Chapters 6 and 7 showed that the transient hypoglycaemia can be avoided by consuming low instead of high GI CHO before exercise. Nevertheless, the early temporary reduction in blood glucose concentration do not appear to
influence the rate of perceived exertion nor exercise performance in the present and earlier studies (Chapter 2, Section 2.5.3).

Therefore, in selecting the appropriate foods for breakfast before participating in endurance sports, one with a high CHO content should be favoured over one with a low CHO content, especially if athletes have not followed a CHO loading diet. The choice of high or low GI CHO may be less important for endurance performance than had been indicated elsewhere (Brand-Miller et al., 1996) and perhaps should be dictated by individual preferences. For athletes who have followed a glycogen supercompensation regimen or who are sensitive to hypoglycaemia, a low GI pre-exercise breakfast may be more beneficial than a high GI breakfast.

8.5 Conclusions

The rate of CHO and fat oxidation in the postprandial state is controlled by the type and amount of CHO rather than the fat content of a meal. After an overnight fast, increasing the CHO content of a pre-exercise meal from 1.0 to 2.5 g.kg\(^{-1}\) body mass enhances subsequent endurance running capacity. On the other hand, consuming additional fat does not enhance endurance running capacity. In addition, despite difference in muscle metabolism, the glycaemic index of the CHO in a meal which is consumed 3 hours before exercise does not influence endurance running capacity.
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Appendix A

Mixed muscle metabolites assays

Appendix A1
1. Adenosine triphosphate, Phosphocreatine and Glucose-6-phosphate

Principle
PCr + NADP → G6P-DH → P-Gluconolactate + NADPH

ATP + Glucose → HK → ADP + G6P

PCr + ADP → CPK → Cr + ATP

Standards
D-glucose-6-phosphate (Sigma G-7250, 2 mol/mol H2O) 1.1mM or 0.38 mg/ml
ATP (Sigma A5394, 2.5 mol/mol H2O) 1.1 mM or 0.67 mg/ml
PCr (Sigma P6915, 5 mol/mol H2O) 1.1 mM or 0.39 mg/ml

Reagents (in 339 μL)
37.5 μL D1 (18.6g/100ml Triethanolamine pH 7.5-7.6, 2.2g/100ml Mg(Ac)2·4H2O,
0.4g/100ml EDTA·Na2·2H2O)
7.5 μL Dithiothreitol (DTT, Boeringher 197 777) (7.8mg/ml)
15 μL NADP (Boeringher 128 031) (20.9 mg/ml)
1.5 μL ADP (Boeringher 236 675, MW=501.3) (5.1 mg/ml)
15 μL Glucose (22.5 mg/ml)
262.5 μL Water

Enzymes
G6P-DH (Boeringher 127 035). Dilute 1 part enzyme to 2 parts water. Use 3 μL per sample.
HK (Boeringher 1426 362). Dilute 1 part enzyme to 1 part water. Use 5 μL per sample.
CPK (Sigma C-3755) (15mg/ml in 0.5%NaHCO3+ 0.05% BSA (D5)). Dilute 2 parts enzyme to 1 part water. Use 5 μL per sample.

Procedure
1. Pipette 37.5 μl of water blank or extract into each cuvette (or 12.5 μl each of G-6-P,
ATP and PCr standards).
2. Pipette 350 μl of reaction mixture into each cuvette.
3. Set spectrophotometer (DU650, Beckman, U.S.A.) to read samples at 340 nm for 4000 secs.
4. Start plotting the reaction. Read absorbance (A1) for ~2 mins.
5. Add 3 μl of G6P-DH to each cuvette, agitate to mix and continue plotting until a plateau (A2) is reached (about 5 mins).
6. Add 5 μl of HK to each cuvette, agitate to mix and continue plotting until a plateau (A3) is reached (about 10 mins).
7. Add 5 μl of CPK to each cuvette, agitate to mix and continue plotting until a plateau (A4) is reached (about 25 mins).
\[
\text{[G6P]} = \frac{390.5 \times (A_2 - \text{Blank}_2) - 387.5 \times (A_1 - \text{Blank}_1) \times \text{Extraction factor} \times 1.25}{37.5 \times \text{extinction coefficient}}
\]

\[
\text{[ATP]} = \frac{395.5 \times (A_3 - \text{Blank}_3) - 390.5 \times (A_2 - \text{Blank}_2) \times \text{Extraction factor} \times 1.25}{37.5 \times \text{extinction coefficient}}
\]

\[
\text{[PCr]} = \frac{400.5 \times (A_4 - \text{Blank}_4) - 395.5 \times (A_3 - \text{Blank}_3) \times \text{Extraction factor} \times 1.25}{37.5 \times \text{extinction coefficient}}
\]
Appendix A2
2. Creatine

**Principle**

Pyruvate + NADH $\xrightarrow{\text{LDH}}$ Lactate + NAD$^+$

ADP + PEP $\xrightarrow{\text{PK}}$ ATP + Pyruvate

Creatine + ATP $\xrightarrow{\text{CK}}$ PCr + ADP

**Standard**

Creatine 1.5 mM or 0.1965 mg/ml

**Reagents (in 450 μL)**

- 150 μL Glycine buffer (D4, 2.4g glycine (aminoacetic acid) and 0.4g Mg(Ac)$_2$·4H$_2$O per 100 ml water, pH 9.0-9.1)
- 7.5 μL KCl (D3, 15g/100ml)
- 30 μL ATP·Na$_2$·5H$_2$O (15.4 mg/ml)
- 22.5 μL PEP (11.6mg/ml)
- 7.5 μL NADH (9 mg/ml)
- 0.75 μL LDH (Boehringer 107034 or 107042)
- 0.75 μL PK (Boehringer 128155)
- 231 μL Water

Prepare CPK (Sigma C-3755) (15mg/ml in 0.5% NaHCO$_3$ + 0.05% BSA (D5)). Use 10 μL per sample.

**Procedure**

1. Pipette 30 μl of water blank, standard or extract into each cuvette
2. Pipette 450 μl of reaction mixture into each cuvette.
3. Set spectrophotometer (DU650, Beckman, U.S.A.) to read samples at 340 nm for 2000 secs.
4. Start plotting the reaction. Read absorbance (A1) for ~4 mins.
5. Add 10 μl of CPK to each cuvette, agitate to mix and continue plotting until a plateau (A2) is reached (about 20 mins).

\[
[\text{Cr}] = \frac{490 \times (A1 - \text{Blank1}) - 480 \times (A2 - \text{Blank 2}) \times \text{Extraction factor} \times 1.25}{30 \times \text{extinction coefficient}}
\]
Appendix A3

3. Lactate

Principle
Lactate + NADH $\xrightarrow{\text{LDH}}$ Pyruvate + NADH + H^+

Standard
Lactate 0.5 mmol·l⁻¹ (stock solution) (L-Lactic acid MW=112.06, Aldrich)

Reagents (in 203.6 µl)
200 µl Hydrazine buffer 1.1 mmol·l⁻¹, pH 9.0 with 1 mmol·l⁻¹ EDTA
2 µl NAD Cofactor (50 mmol·l⁻¹ or 33.17 mg/ml, Boeringher 127 965, MW=663.4)
1.6 µl LDH (undiluted, 5500 U·ml⁻¹, Boeringher 107 085)

Additional reagents: Carbonate buffer 20 mmol·l⁻¹, pH 10.0

Working reagents were prepared daily from the stock solutions as follows:

<table>
<thead>
<tr>
<th>Stock solution (µl)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (µl)</td>
<td>100</td>
<td>90</td>
<td>800</td>
<td>70</td>
<td>40</td>
</tr>
<tr>
<td>Lactate concentration (µmol·l⁻¹)</td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>150</td>
<td>300</td>
</tr>
</tbody>
</table>

Immediately prior to the analysis, add 50 µl of top standard to 1 ml reagent and follow the reaction on Fluorescence Spectrophotometer (F-2000, Hitachi, Tokyo, Japan)

Procedure
1. 10µl of undiluted extract was added to 1 set of duplicate water blanks, standards and samples.
2. 0.2 ml of reaction mixture was added to each cuvette
3. A further 1 ml of reaction mixture was added to 50 µl of 300 µmol·l⁻¹ lactate standard and the reaction followed on the Fluorescence Spectrophotometer (F-2000, Hitachi, Tokyo, Japan) at (EX 340, EM 460).
4. After incubating for 30 mins at room temperature, 1 ml of carbonate buffer was added to each cuvette, and after thorough mixing, fluorescence (EX 340, EM 460) was measured.
Lactate concentration (µmol·l⁻¹) was calculated using a linear regression model.

\[
\text{[La]} \text{ (mmol·kg}^{-1}\text{·dm)} = \text{[La]} \text{ (µmol·l}^{-1}\text{)} \times 100 \times \frac{1.25}{1000}
\]
Appendix A4

4. Total glycogen (TG)

The assay for glycogen was carried out on both the acid precipitated muscle pellet (proglycogen) and the neutralised extract (macroglycogen) by analysis of glucosyl units obtained after acid hydrolysis of glycogen by the method of Jansson (1981).

Acid hydrolysis

Reagents
HCl 1 mol·l⁻¹; NaOH 6 mol·l⁻¹

Procedure
For each muscle sample, 0.1 ml of 1 mol·l⁻¹ HCl per 1 mg of muscle powder was added to the precipitated muscle pellet. 0.1 ml of 1 mol·l⁻¹ HCl was also added to 20 μl of undiluted neutralised extract. Both samples were gently mixed and incubated from 2 hours in tightly sealed screw-top micro-reaction eppendorf tubes in a boiling water bath. The tubes were then centrifuged for 1 min and left at room temperature to cool down. The acid hydrolysed extract only was neutralised with 15 μl of 6 mol·l⁻¹ NaOH. Macroglycogen was assayed spectrophotometrically using a Glucose Test combination, GOD/Perid method. Proglycogen was assayed fluorimetrically for glucose.

4a. Proglycogen (acid insoluble glycogen)

Principle

\[
glucose + O_2 + H_2O \xrightarrow{GOD} gluconate + H_2O_2 \\
H_2O_2 + ABTS \xrightarrow{POD} coloured complex + H_2O
\]

where:
GOD is glucose oxidase
POD is horseradish peroxidase
ABTS is di-ammonium 2,2'-azino-bis (3 ethylbenzothiazoline-6-sulphonate)

The intensity of the colour change is directly proportional to the concentration of glucose. Therefore, the glucose concentration of a sample can be calculated by using a standard of known concentration.

Reagents
Buffer/enzymes/chromogen: made up as directed in kit (phosphate buffer 0.1 mol·l⁻¹, pH 7.0; POD 0.8 U·ml⁻¹; GOD 10 U·ml⁻¹; ABTS 1.0 mg·ml⁻¹). This was found to remain active for several months when stored protected from sunlight at -4-8°C.

Standard
Glucose 0.505 mmol·l⁻¹ (included in kit)

Procedure
1000 μl of the GOD/Perid reagent was added to 5 μl of duplicate blanks, standards and samples which were consisted of double-distilled water, standard and the unneutralised
hydrolysed extract of the precipitated muscle pellet, respectively. The samples were incubated at room temperature for 30 mins and their absorbance (A) determined on a spectrophotometer (DU650, Beckman, U.S.A.) at 420 nm.

Proglycogen concentration (glucosyl units mmol·kg⁻¹ dm) was calculated using the following equation:

\[
[PG] = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 0.505 \times 100
\]
4b. Macroglucogen (+ G6P + glucose)

**Principle**

Glucose + ATP $\xrightarrow{HK} G6P + ADP$

$G6P + NADP^+ \xrightarrow{G6P-DH} G-P-Gluconolactone + NADPH + H^+$

**Standard**

Glucose 0.505 mmol·l$^{-1}$ (stock solution from GOD-Perid kit)

**Reagents per ml of buffer**

Buffer: TRIS-HCl 0.1 mol·l$^{-1}$, BSA 0.02%, pH 8.1

6 μl of 3.937 mg/ml NADP (Boeringher 128 031)

6 μl of 121.04 mg/ml ATP (Boeringher 519 979)

10 μl of 7.715 mg/ml Dithiothreitol (Boeringher 197 777)

10 μl of 0.1 mol·l$^{-1}$ MgCl$_2$

5 μl of 0.1 mol·l$^{-1}$ EDTA and 1 mol·l$^{-1}$ HCl

Enzymes to be added to reaction mixture just before analyses:

G6P-DH (Boeringher 127035). Dilute 5 μl with 245 μL water. Use 3 μL per ml buffer.

HK (Boeringher 1426362). Dilute 5 μL with 245 μL water. Use 5 μL per ml buffer.

Additional reagents: Carbonate buffer 20 mmol·l$^{-1}$, pH 10.0

**Working standards were prepared daily from the glucose standard as follows:**

<table>
<thead>
<tr>
<th>Stock solution (μl)</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (μl)</td>
<td>505</td>
<td>485</td>
<td>455</td>
<td>405</td>
<td>305</td>
</tr>
<tr>
<td>Glucose concentration (μmol·l$^{-1}$)</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>

**Procedure**

1. Three sets of 20 μl water blanks and working standard and two sets of samples (neutralised hydrolysed extract) were pipetted into fluorimetric cuvettes.

2. 200 μl of reaction mixture was added to each cuvette.

3. A further 1 ml of the reagent was added to 50 μl of the 200 μmol·l$^{-1}$ glucose standard and the reaction in this tube followed on the on the Fluorescence Spectrophotometer (F-2000, Hitachi, Tokyo, Japan) at (EX 340, EM 460).

4. After incubating for 30 mins at room temperature, 1 ml of carbonate buffer was added to each cuvette, and after thorough mixing, fluorescence was measured. Macroglucogen concentration (as glucosyl units (μmol·l$^{-1}$)) was calculated from the standard linear regression line. The resulting value was multiplied by (0.125*6.75) to correct for the dilution involved in the extraction and acid hydrolysis. Muscle free glucose and G6P was subtracted from the result to give the true macroglucogen concentration.
4c. Free glucose (+ G6P)
(This assay is performed together with 4. Macroglycogen (+ G6P + glucose))

**Principle**

\[
\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G6P} + \text{ADP}
\]

\[
\text{G6P} + \text{NADP}^+ \xrightarrow{\text{G6P-DH}} \text{6-P-Gluconolactone} + \text{NADPH} + \text{H}^+
\]

**Standard**

Glucose 0.505 mmol·l⁻¹ (stock solution from GOD-Perid kit)

**Reagents per ml of buffer**

Buffer: TRIS-HCl 0.1 mol·l⁻¹, BSA 0.02%, pH 8.1
6 μl of 3.937 mg/ml NADP (Boeringher 128 031)
6 μl of 121.04 mg/ml ATP (Boeringher 519 979)
10 μl of 7.715 mg/ml Dithiothreitol (Boeringher 197 777)
10 μl of 0.1 mol·l⁻¹ MgCl₂
5 μl of 0.1 mol·l⁻¹ EDTA and 1 mol·l⁻¹ HCl

Enzymes to be added to reaction mixture just before analyses:
G6P-DH (Boeringher 127035). Dilute 5 μL with 245 μL water. Use 3 μL per ml buffer.
HK (Boeringher 1426362). Dilute 5 μL with 245 μL water. Use 5 μL per ml buffer.

Additional reagents: Carbonate buffer 20 mmol·l⁻¹, pH 10.0

Working standards were prepared daily from the stock solutions as follows

<table>
<thead>
<tr>
<th>Stock solution (μl)</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (μl)</td>
<td>505</td>
<td>485</td>
<td>455</td>
<td>405</td>
</tr>
<tr>
<td>glucose concentration (μmol·l⁻¹)</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

**Procedure**

1. Three sets of 20μl water blanks and working standard and one set of sample (undiluted extract) were pipetted into fluorimetric cuvettes.
2. 200 μl of reaction mixture was added to each cuvette.
3. A further 1 ml of the reagent was added to 50 μl of the 200 μmol·l⁻¹ glucose standard and the reaction in this tube followed on the on the Fluorescence Spectrophotometer (F-2000, Hitachi, Tokyo, Japan) at (EX 340, EM 460).
4. After incubating for 30 mins at room temperature, 1 ml of carbonate buffer was added to each cuvette, and after thorough mixing, fluorescence was measured. Muscle free glucose + G6P concentration (as glucosyl units (μmol·l⁻¹)) was calculated from the standard linear regression line. The resulting value was multiplied by 0.125 to correct for the dilution involved in the extraction. The G6P concentration was subtracted from this value to obtain the muscle free glucose concentration.
Appendix B

Blood metabolites assays

Appendix B1

1. Blood lactate
Fluorometric method based upon Maughan, 1982.

Principle:

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

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

Reagent solutions:
1. 1.1M Hydrazine buffer, pH=9
One litre of buffer contains:
46.167 ml of hydrazine hydrate
20.77 g of hydrazinium sulphate
0.37224 g of EDTA
Stable at room temperature.

2. Lactate diluent
0.07 M HCl solution.

3. Reaction mixture (make up at the start of assay)
2.0 mg NAD\(^+\) and 10 μl of LDH for each ml of hydrazine buffer (200 μl of reaction mixture per test tube).
NAD\(^+\), free acid, grade II, ~98%, Boehringer Mannheim, No. 127 990
LDH - 5500 U/ml, Boehringer Mannheim, No. 107 085.

4. Standards
Made from 1.0 M L-Lactate stock solution (Boehringer Mannheim, No. 125 440). A 10 mM standard is made and diluted with 2.5% perchloric acid (PCA) to make 1, 1, 2, 4, 6 and 8 mM standards.

A 20 μl of whole blood sample by deprotenised by adding it to 200 μl 2.5% PCA already dispensed in an eppendorf tube. The sample is thoroughly mixed and then centrifuged for 3 min before stored at -20°C.

Procedures
1. The samples, standard and reagent solution are allowed to warm to room temperature.
2. The samples are then mixed using a whirlmixer and centrifuged for 3 mins.
3. Twenty μl of supernatant and standard is transferred to a glass fluorimeter test tube using an air-displacement piston pipette.
4. Two hundred μl of reaction mixture is then added. (All measurements are made in duplicate. Water and reagent solution are used as blank).
5. Mix thoroughly and allow to incubate for 30-40 mins.

6. One ml of the Lactate diluent (0.07 M HCl) is then added to each test tube using either an air-displacement piston pipette or an automatic dispenser (Hamilton). Mix well.

7. The fluorescence of the samples is measured against the blanks and standards using a fluorimeter (Locarte Model 8-9). The blanks are used to zero the fluorimeter and the top standard to set the upper limit of the range of measurement (~180 units).

8. Lactate concentrations of the samples are calculated using a linear regression model.
Appendix B2
2. Blood glucose (GOD Perid colourimetric method)

Principle
Glucose + O$_2$ + H$_2$O $\xrightarrow{\text{GOD}}$ Gluconate + H$_2$O$_2$
H$_2$O$_2$ + ABTS $\xrightarrow{\text{POD}}$ Coloured complex + H$_2$O

A Boehringer Mannheim Diagnostica kit (Cat. No. MPR3 124 036) is used to produce the reaction mixture and standard for this assay.

Reagent solution
100 mmol·l$^{-1}$ phosphate buffer, pH=7.0
8 U·ml$^{-1}$ GOD
0.35 U·ml$^{-1}$ POD
1.0 mg·ml$^{-1}$ ABTS (di-ammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)

Standard
A 0.505 M standard is also provided and used undiluted

A 20 µl of whole blood sample by deprotenised by adding it to 200 µl 2.5% PCA already dispensed in an eppendorf tube. The sample is thoroughly mixed and then centrifuged for 3 min before stored at -20°C.

Procedures
1. The samples, standard and reagent solution are allowed to warm to room temperature.
2. The samples are then mixed using a whirlmixer and centrifuged for 3 mins.
3. Twenty µl of supernatant and standard is transferred to a plastic or glass test tube using an air-displacement piston pipette.
4. One ml of reagent solution is also added using either an air-displacement piston pipette or an automatic dispenser (Hamilton). Use 1 ml of reagent solution as a blank. (All measurements are made in duplicate). Mix well.
5. The test solutions are allowed to incubate for at least 30 mins.
6. The absorbance (A) of the blanks, standards and samples are measured using a photometer (Eppendorf 1101M, at Hg 436 nm) or spectrophotometer (CECIL 2393 at 610 nm), in a plastic, glass or quatz cuvette with a 1.0 cm light path. The blanks are used to zero the spectrophotometer or photometer.
7. Glucose concentrations (C) of the samples are calculated as

\[ C = 5.55 \times \frac{A_{\text{sample}}}{A_{\text{standard}}} \]

The reaction mixture is then added. (All measurements are made in duplicate. Water and reagent solution are used as blank).
Appendix B3
3. Plasma glycerol
Fluorometric method modified from that of Laurell and Tibbling, 1966

Principle

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

GK = Glycerol kinase
GDH = Glycerol-3-phosphate dehydrogenase
NAD\(^+\) is converted to NADH in amount proportional to the amount of glycerol

Reagent solutions
1. 0.087 M Zinc sulphate (ZnSO\(_4\)-7H\(_2\)O)
   6.25 g of ZnSO\(_4\)-7H\(_2\)O (MW=287.54) dissolved in 250 ml distilled water. Kept at room temperature.

2. 0.083 M Barium hydroxide (Ba(OH)\(_2\)-8H\(_2\)O)
   6.55 g of Ba(OH)\(_2\)-8H\(_2\)O (MW=315.4) dissolved in 250 ml distilled water. Kept at room temperature.

3. 0.2 M Cysteine
   24.3 mg Cysteine dissolved in 1.0 ml of 0.4 M NaOH. To be prepared daily.
   (0.4 M NaOH: 400 mg of NaOH dissolved in 25 ml of distilled water).

4. 1.0 M Hydrazine-HCl buffer with 1.5 M MgCl\(_2\):
   12.15 ml of hydrazine-hydrate (99% solution) and 76.2 mg of MgCl\(_2\) dissolved in 250 ml of distilled water. Adjust pH with HCl to 9.4. To be kept at \(-3^\circ\text{C}\).

5. Diluent: 0.01 M NaOH with 1.0 mM EDTA:
   400 mg of NaOH and 372.24 mg of EDTA dissolved in 1000 ml of distilled water. To be kept at room temperature.

6. Reaction mixture (make up at the start of assay):
   (100 \(\mu\)l of reaction mixture is required per test tube)

Each ml of reaction mixture contains:
- 700 \(\mu\)l of 1.0 M Hydrazine-HCl buffer
- 200 \(\mu\)l of distilled water containing 12 mg of ATP and 20 mg of NAD (0.1 M ATP, 0.15 M NAD)
- 100 \(\mu\)l of 0.2 M cysteine
- 1.0 \(\mu\)l glycerol kinase
- 5.0 \(\mu\)l glycerol-3-phosphate dehydrogenase

NAD\(^+\), free acid, grade II, \(-98\%\), MW=663.4, Boehringer Mannheim, No. 127 990.
ATP, MW=605.2, Boehringer Mannheim, No. 519 979 (1g) or 127 531 (10g).
GK - 1.0mg/ml, Boehringer Mannheim, No. 127 159.
GDH - 2.0 mg/ml, Boehringer Mannheim, No. 127 124.
7. Standards
These are made from a Glycerol AR stock solution (99% solution, wt/ml: 1.259, MW=92.10, FISONS, No. G/0650). A 4.0 mM standard solution is prepared (36.8 mg in 100 ml distilled water). The 4.0 mM standard is then diluted with distilled water to make up a 2.0 mM solution (2.5 ml of 4.0 mM standard in 7.5 ml distilled water). Take 2.0 mM as 100% and then prepare the following working standards:

<table>
<thead>
<tr>
<th>Working standards</th>
<th>1.0 mM (ml)</th>
<th>Distilled water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>2% (0.02 mM)</td>
<td>0.05</td>
<td>2.45</td>
</tr>
<tr>
<td>4% (0.04 mM)</td>
<td>0.10</td>
<td>2.40</td>
</tr>
<tr>
<td>20% (0.2 mM)</td>
<td>0.5</td>
<td>2.0</td>
</tr>
<tr>
<td>40% (0.4 mM)</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>80% (0.8 mM)</td>
<td>2.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Procedures
1. The samples, standard and reagent solution are allowed to warm to room temperature.
2. The samples are then mixed using a whirlmixer and centrifuged for 3 mins.
3. 0.25 ml of zinc sulphate is transferred into Eppendorf tubes using an air-displacement pipette.
4. 50 μl of sample or standard is added.
5. 0.25 ml of Barium hydroxide is then added. Mix well immediately.
6. 200 μl of the supernatant is transferred to glass fluorimeter test tubes.
7. One hundred μl of reaction mixture is then added. (All measurements are made in duplicate. Water and reagent solution are used as blank).
8. Mix thoroughly and allow to incubate for 60 mins.
9. One ml of the diluent is then added to each test tube using either an air-displacement piston pipette or an automatic dispenser (Hamilton). Mix well.
10. The fluorescence of the samples is measured against the blanks and standards using a fluorimeter (Locarte Model 8-9). The blanks are used to zero the fluorimeter and the top standard to set the upper limit of the range of measurement (~180 units).
11. Glycerol concentrations of the samples are calculated using a linear regression model.
Appendix B4

4. Electrolytes (Na⁺, K⁺)
Performed on plasma using flame photometry (Ciba Corning, Model M435)

Solutions

\( R_B \)
3 M Li⁺ diluted 1:200 to give 15 mmol·l⁻¹
(5.0 ml of 3 M Li⁺ in 1 litre distilled water)

Standard
140 mmol·l⁻¹ Na⁺; 5 mmol·l⁻¹ K⁺ diluted 1:200
(0.5 ml in 100 ml of 15 mmol·l⁻¹ lithium working solution)

Calibration
A zero base-line was achieved against \( R_B \), whereas the one point standard solution (140 mmol·l⁻¹ Na⁺; 5 mmol·l⁻¹ K⁺) established the working range. Repeat until readings are stable.

NB Recalibrate every 20 samples

Procedures
1. Pipette 30 μl of sample or standard into bijou bottles.
2. Add 5 ml of 15 mmol·l⁻¹ lithium solution, mix and read.