THE EFFECTS OF LOW AND HIGH GLYCEMIC INDEX MEALS ON METABOLISM AND PERFORMANCE DURING SOCCER-SPECIFIC INTERMITTENT EXERCISE

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ABSTRACT

The glycemic index (GI) of a pre-exercise meal has been shown to affect substrate oxidation during exercise and may influence exercise performance. Previous research in this area has focused on continuous, moderate intensity exercise. The purpose of this study was to examine the effects of low and high glycemic index (GI) pre-exercise meals on metabolism and performance during soccer-specific intermittent exercise. Thirteen trained male soccer players (22.3 ± 3.3 yrs) participated in four experimental trials in a repeated crossover design. Isocaloric low GI-high protein (lentils), high GI-high protein (potato + egg whites), or high GI-low protein (potato) meals were consumed two hours before a 90-minute treadmill soccer match simulation. A fasted control condition was also employed. Blood and expired gas samples were collected before and during exercise to assess markers of carbohydrate and fat metabolism. The distance covered on five 1-minute sprints (separated by 2.5 minutes of recovery) performed during the last 15 minutes of the match was used to assess performance. Serum insulin concentration at the start of exercise was higher in the high GI-low protein condition compared to all other conditions ($p<0.001$). During exercise, the rate of carbohydrate oxidation was significantly higher and the rate of fat oxidation was significantly lower in the high GI-low protein condition compared to control ($p<0.05$). The distance covered on sprints 1 and 2 was significantly greater in the low GI-high protein condition compared to control ($p<0.05$). The distance covered on sprint 2 was significantly greater in the high GI-high protein condition compared to control ($p<0.05$). There were no significant differences between conditions for sprints 3-5. Ratings of perceived exertion (RPE) throughout exercise were significantly lower in the

low GI-high protein condition compared to both control and high GI-low protein conditions ($p<0.05$). It is concluded that low GI-high protein and high GI-high protein pre-exercise meals result in small improvements in initial repeated sprint performance compared to fasted control. Performance was not improved following a high GI-low protein pre-exercise meal compared to control, indicating that the increased carbohydrate oxidation and decreased fat oxidation in this condition may have negated any potential performance advantage of carbohydrate consumption. The lower RPE throughout exercise in low GI-high protein compared to control and high GI-low protein indicates a possible beneficial effect of consuming a low GI meal prior to soccer-specific intermittent exercise.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ATP – adenosine triphosphate

CV – coefficient of variation

GI – glycemic index

$g.kg^{-1}$ – grams per kilogram

$mmol.l^{-1}$ – millimoles per litre

RPE – rating of perceived exertion

RER – respiratory exchange ratio

VCO_2 – carbon dioxide output

VE – minute ventilation

Vmax – peak treadmill speed

VO_2 – oxygen uptake

VO_2max – maximal oxygen uptake

1. SCIENTIFIC FRAMEWORK

1.1. Introduction

Athletes are constantly searching for ways to maximize their potential and improve performance. Nutrition has long been recognized as a key determinant of athletic performance and is one aspect of competition preparation that an athlete can readily manipulate. For endurance athletes, a pre-competition meal that is rich in carbohydrates increases exercise capacity (Wright et al., 1991) and enhances exercise performance (Sherman et al., 1991). Carbohydrates, in the form of muscle glycogen and blood glucose, are the main fuel source for exercising skeletal muscle during moderate and high intensity exercise (van Loon et al., 2001). Since endogenous carbohydrate stores are limited and depletion of these stores contributes to fatigue (Bergstrom et al., 1967; Hawley et al., 1994) consuming a carbohydrate-rich meal before exercise ensures that exercise commences with optimal carbohydrate stores and can provide additional fuel that becomes available during exercise. Despite substantial evidence demonstrating beneficial effects of high carbohydrate pre-exercise meals, little is known regarding the optimal meal characteristics and type of carbohydrates to consume.

The glycemic index (GI) ranks carbohydrates based on their blood glucose response (Jenkins et al., 1981). High GI foods are absorbed quickly and result in a sharp rise in blood glucose (Jenkins et al., 1981). Low GI foods are digested and absorbed more slowly and result in a more sustained release of glucose into circulation (Jenkins et

al., 1981). The GI of foods consumed before exercise can have profound effects on substrate utilization during exercise. The sharp rise in blood glucose after ingestion of a high GI food is accompanied by a large increase in circulating insulin (Stevenson et al., 2006). The combined effects of insulin and muscle contraction may lead to a fall in blood glucose concentration at the onset of exercise (Jentjens & Jeukendrup, 2002). Insulin also inhibits lipolysis and impairs fat oxidation thereby increasing the reliance on carbohydrate oxidation and decreasing the reliance of fat oxidation during exercise (Horowitz et al., 1997). Increased reliance on carbohydrates may lead to an earlier depletion of carbohydrate stores and result in premature fatigue.

Studies examining the effect of the GI on exercise performance have revealed conflicting results. A few studies show an improvement in endurance capacity when low GI pre-exercise meals are consumed compared to high GI meals (Demarco et al., 1999; Thomas et al., 1991; Wu & Williams, 2006) but most report no difference between low and high GI meals (e.g., Febbraio et al., 2000; Febbraio & Stewart, 1996; Thomas et al., 1994). An important limitation in the literature is that all previous research has focused on continuous exercise. Many team sports (e.g., soccer, hockey, and basketball) involve high-intensity, intermittent exercise. The intermittent activity profile of team sports tends to result in faster depletion of muscle glycogen when compared to continuous exercise (Saltin, 1973) and therefore the results of previous studies may not be applicable to team sport athletes. The aim of this thesis is to examine the performance and metabolic effects of low and high GI pre-exercise meals on soccer-specific intermittent exercise. Soccer was chosen because it involves a large endurance component (matches last 90 minutes) and relevant literature is available on the activity

profile of actual matches. Furthermore, soccer is the most widely played sport in the world yet information regarding the optimal pre-game meal for soccer players is lacking.

1.2 Literature Review

1.2.1 Effects of Pre-Exercise Carbohydrate Ingestion on Endurance Performance

Carbohydrates, in the form of muscle glycogen and blood glucose, are the primary energy sources for exercising skeletal muscle. As a result, depletion of endogenous carbohydrate stores contributes to fatigue during endurance exercise (Bergstrom et al., 1967; Hawley et al., 1994; Nybo, 2003). Reduced muscle glycogen concentration is thought to be a key limiting factor during long duration exercise (Bergstrom et al., 1967; Coyle et al., 1986; Hermansen et al., 1967). Muscular endurance (Jacobs et al., 1981) and the ability to perform high-intensity exercise (Maughan & Poole, 1981) are both impaired when muscle glycogen is depleted. The direct link between muscle glycogen depletion and fatigue has not been fully elucidated, but it appears that when muscle glycogen levels become too low the rapid energy demands of muscular contraction cannot be met (Shulman & Rothman, 2001). A critical reduction in blood glucose, secondary to liver glycogen depletion, also contributes to fatigue. Hypoglycemia may lead to cognitive impairments (Foster et al., 1979; Levine et al., 1924) and/or decreased motor unit recruitment secondary to a reduction in neural drive from the motor cortex (i.e., central fatigue) (Noakes, 2001; Nybo, 2003). Consuming a carbohydrate-rich meal prior to competition can ensure that exercise commences with optimal levels of muscle and liver glycogen. Ongoing digestion and absorption of the carbohydrate meal throughout exercise can also provide additional fuel

(i.e., blood glucose) that can be oxidized during exercise. Accordingly, athletes are generally advised that consuming a carbohydrate-rich meal prior to exercise improves endurance performance (Hargreaves, 2001; Hargreaves et al., 2004; Jeukendrup & Gleeson, 2004; Sherman, 1991; Williams & Serratosa, 2006).

There are several studies that indicate carbohydrate ingestion before exercise enhances performance. Traditionally, the effect of carbohydrate ingestion on exercise performance has been assessed with two approaches; exercise time to exhaustion and time trial performance. Exercise time to exhaustion involves exercising at a constant intensity until volitional exhaustion. If exercise time to exhaustion is prolonged after carbohydrate ingestion compared to a fasted or placebo condition, then endurance capacity can be considered to be greater because of the carbohydrates consumed. Research using this approach has generally supported a performance-enhancing effect of pre-exercise carbohydrate ingestion. Wright et al. (1991) demonstrated that consuming a large quantity of carbohydrate (5.0 grams of available carbohydrate per kilogram body mass) in liquid form four hours before exercise significantly improved cycling time to exhaustion at 70% maximal oxygen uptake (VO_2max) compared to exercise in the fasted state. Solid carbohydrates, a more typical form for a pre-exercise or pre-game meal also enhance cycling time to exhaustion. Breakfast cereals containing primarily carbohydrate (1.0 $g.kg^{-1}$ body mass) consumed 45 minutes before exercise improved cycling time to exhaustion by ~20% (Kirwan et al., 1998; Kirwan et al., 2001). Similarly, Schabort et al. (1999) showed that 100 grams of solid carbohydrate consumed three hours before exercise improved cycling time to exhaustion at 70% VO_2max by ~25%. Studies examining the effect of large carbohydrate meals (2.5 $g.kg^{-1}$ body mass) consumed three

hours before running at an intensity of 70% VO_2max also report increased time to exhaustion compared to placebo (Chryssanthopoulos & Williams, 1997; Cryssanthopoulos et al., 2002).

Not all studies using exercise time to exhaustion have supported an ergogenic effect of pre-exercise carbohydrate ingestion. For example, Hargreaves et al. (1987) noted no difference in cycling time to exhaustion when glucose (75 g), fructose (75 g) or sweetened placebo beverages were consumed 45 minutes before exercise. Gleeson et al. (1986) reported that five of six subjects cycled for longer when they consumed glucose (1 $g.kg^{-1}$ body mass) 45 minutes before exercise compared to placebo, but the results were not statistically significant. Similar studies comparing consumption of small amounts of carbohydrate (43 grams, from candy bars) 30 minutes before exercise also report no significant differences between carbohydrate and placebo trials (Alberici et al., 1993; Devlin et al., 1986; Maliszewski et al., 1995). Foster et al. (1979) reported a decrease in cycling time to exhaustion at 80% VO_2max when a glucose drink was consumed 30 minutes before exercise compared to a fasting condition. This is the only study to show a detrimental effect of pre-exercise carbohydrate ingestion. The results of this study have been questioned because it appears that the researchers stopped the exercise tests using subjective criteria instead of the subjects exercising to volitional exhaustion (Sherman, 1991). In addition, exercise time to exhaustion at 100% VO_2max was unaffected by pre-exercise glucose ingestion in the same study (Foster et al., 1979). The majority of studies reporting no benefit of pre-exercise carbohydrate ingestion involve study designs where small amounts of carbohydrate are consumed 30 to 45 minutes before the start of exercise after an overnight fast. It is highly unlikely that an

athlete would prepare for competition in this way. It is more likely that an athlete would consume a large carbohydrate-rich meal 2-4 hours before exercise. Although there are fewer studies that follow this more practical feeding schedule, beneficial effects of carbohydrate-rich meals on exercise time to exhaustion have been reported (Cryssanthopoulos & Williams, 1997; Cryssanthopoulos et al., 2002; Schabort et al., 1999; see discussion above). Overall, the majority of the research demonstrates increased exercise time to exhaustion when both moderate and large amounts of carbohydrate are ingested before moderate intensity (~70% VO_2max) cycling and running exercise.

The applicability and reliability of exercise time to exhaustion as a performance measure have been questioned. Motivation, boredom, and familiarity may profoundly influence the amount of time an individual is able to continue exercising (Jeukendrup et al., 1996). In most endurance activities (e.g., road cycling and distance running races) athletes are required to cover a set distance in the shortest time possible rather than exercise for the longest time possible. A more valid indicator of performance is a time trial, where participants must complete a set amount of work (e.g., distance) as fast as possible or cover the most distance in a set amount of time. This latter measure is likely more applicable to team sport athletes, where the ability to cover more distance during the game, especially in the later stages, is a characteristic of superior players (Stolen et al., 2005). Jeukendrup et al. (1996) reported a coefficient of variation (CV) of ~27% based on six repeats of a cycling time to exhaustion test at 75% of maximal workload. The CV for six repeats of a time trial test was ~3.5%, indicating that a time trial is a considerably more reliable measure of performance than exercise time to exhaustion

(Juekendrup et al., 1996). As such, drawing scientifically sound as well as practical conclusions from studies that examine the effect of pre-exercise carbohydrate ingestion on exercise time to exhaustion is difficult.

In light of this, using time trials to measure performance is a more valid and reliable way to assess the effects of pre-exercise carbohydrate ingestion. The common way to do this is with a preloaded time trial approach. In these study designs, subjects exercise at a constant intensity for a set amount of time followed by the time trial. Often, the trials involve 90-120 minutes of steady-state exercise followed by high-intensity performance tests (e.g., Febbraio & Stewart, 1996; Febbraio et al., 2000; Sherman et al., 1991). This allows researchers to monitor exercise metabolism under the same conditions across experimental trials (during the steady-state period) and assess performance within the same trial. Using this approach, Sherman et al. (1991) reported that consuming 1.1 or 2.2 grams of carbohydrate per kilogram body mass (in liquid form) 60 minutes before exercise improved cycling performance over placebo in a time trial completed after 90 minutes of steady state cycling. These authors also showed that ingesting a liquid meal containing a large amount of carbohydrate (312 grams) four hours before exercise improved cycling performance over placebo in a time trial performed after 95 minutes of moderate-intensity intermittent exercise (Sherman et al., 1989). Furthermore, Neufer et al. (1987) demonstrated improved time trial performance after 90 minutes of steady state cycling when a meal containing 200 grams of carbohydrate was consumed 4 hours prior to exercise when compared to placebo and two conditions where small amounts of carbohydrate were consumed immediately before exercise. The findings of improved time trial performance following pre-exercise

carbohydrate ingestion are not universal. Some studies have reported no difference in preloaded time trial performance in the carbohydrate and placebo condition when carbohydrates were consumed 30 to 60 minutes before (Febbraio & Stewart, 1996; Febbraio et al., 2000; Sparks et al., 1998) and 3.5 to 4 hours before (Paul et al., 2003; Whitley et al., 1998) cycling exercise. Once again, the applicability of the studies that employ designs where subjects consume small amounts of carbohydrate 30 to 60 minutes before exercise after an overnight fast can be questioned (Febbraio & Stewart, 1996; Febbraio et al., 2000; Sparks et al., 1998). It is important to note that carbohydrate consumption prior to exercise has never been reported to be detrimental to time trial performance. The balance of the evidence, especially in studies that use feeding schedules that are typical of endurance athletes (i.e., large carbohydrate feedings 2-4 hours before exercise,) suggests that pre-exercise carbohydrate ingestion is beneficial (Neufer et al., 1987; Sherman et al., 1989; Sherman et al., 1991).

1.2.2 Pre-exercise Carbohydrate Ingestion: Possible Mechanisms of Performance Enhancement

The performance-enhancing effect of pre-exercise carbohydrate ingestion is thought to be related to improved maintenance of blood glucose during the later stages of exercise (Kirwan et al., 2001; Sherman et al., 1989; Wright et al., 1991). During prolonged exercise, hepatic glucose output decreases as liver glycogen stores become exhausted yet muscle glucose uptake remains elevated (Ahlborg & Felig, 1982). As a result of this imbalance, hypoglycemia may develop. Hepatic gluconeogenesis is increased during exercise but cannot totally compensate for the decline in liver glycogen availability (Trimmer et al., 2002). Therefore, it appears that consuming carbohydrates

before long duration exercise acts to increase or replenish hepatic glycogen stores thereby allowing exercise to continue for longer before critical hypoglycemia develops. Delaying hypoglycemia is important because blood glucose availability plays key roles in cognitive functioning (Levine et al., 1924), motor unit recruitment (Nybo, 2003) and skeletal muscle carbohydrate oxidation (Coggan and Coyle, 1991). However, not all studies demonstrate that pre-exercise carbohydrate ingestion preserves blood glucose concentration compared to placebo (Alberici et al., 1993; Sherman et al., 1989) and hypoglycemia is not the only cause of fatigue during endurance exercise (Hawley et al., 1994). Ingestion of carbohydrates before exercise may also act to replenish or "top-up" muscle glycogen stores so that more carbohydrate fuel is immediately available for skeletal muscle (Chryssanthopoulos et al., 2004; Wee et al., 2005). Alternatively, ongoing absorption of a pre-exercise carbohydrate meal could help to maintain blood glucose oxidation, reducing the reliance on muscle glycogen as a fuel source (Tsintzas et al., 1995; Tsintzas et al., 1996). This latter mechanism has not been directly supported by studies that examine muscle glycogen utilization during exercise following pre-exercise carbohydrate ingestion (Devlin et al., 1986; Hargreaves et al., 1987; Neufer et al., 1987). However, these studies have used cycling as the mode of exercise and have measured muscle glycogen in mixed muscle samples. Tsintzas et al. (1995 & 1996) have demonstrated that carbohydrate ingestion during running exercise decreases reliance on glycogen in type I muscle fibres only. This same effect is not supported in studies that use cycling as the mode of exercise (Bosch et al., 1994; Coyle et al., 1986; Hargreaves & Briggs, 1988; Jeukendrup et al., 2005). Recent evidence actually suggests that carbohydrate consumption before and during cycling exercise decreases the reliance on

muscle glycogen in type II muscle fibres but not type I (De Bock et al., 2007). Research regarding fibre type specific muscle glycogen content is somewhat difficult to interpret because different semi-quantitative techniques have been employed in different studies. Nonetheless, carbohydrate ingestion appears to have fibre type specific effects on muscle glycogen utilization which are different during running as compared to cycling. There are no studies that examine the effect of pre-exercise carbohydrate ingestion on muscle fibre type specific glycogen utilization during running. Therefore, ongoing absorption of glucose from a pre-exercise carbohydrate meal could serve to spare muscle glycogen in type I fibres during running exercise.

It is possible that all three mechanisms (i.e., increased hepatic glycogen, increased muscle glycogen, decreased reliance on muscle glycogen in type I fibres) play a role in the performance-enhancing effect of pre-exercise carbohydrate ingestion. All three of these mechanisms help to ensure that skeletal muscle carbohydrate oxidation is maintained during the later stages of exercise. The adenosine triphosphate (ATP) yield per unit of oxygen consumed is greater when carbohydrates are oxidized in mitochondria compared to fatty acids (Krogh & Lindhard, 1920). Therefore, carbohydrate is a more efficient fuel and becomes increasingly important in high-intensity exercise when oxygen may be limiting. The ability to maintain carbohydrate oxidation, whether from muscle glycogen or blood glucose, is therefore a crucial factor in preventing fatigue.

Consuming a pre-exercise meal may also help to prevent feelings of hunger during the later stages of exercise, thereby increasing mental alertness and focus (Welsh et al., 2002). Regardless of the mechanism, the general consensus is that athletes should

consume a large meal containing 140 to 300 grams of carbohydrate 3-4 hours before exercise (Hargreaves, 2001; Hargreaves et al., 2004; Jeukendrup & Gleeson, 2004; Williams & Serratosa, 2006).

1.2.3. Type and Amount of Carbohydrate

Despite the evidence that carbohydrate ingestion before exercise is beneficial, the characteristics of the ideal pre-exercise meal remain largely unknown. It is quite clear that consuming carbohydrates in the hours leading up to exercise affects substrate utilization during the exercise session (Coyle et al., 1985; Horowitz and Coyle, 1993; Montain et al., 1991). Specifically, pre-exercise carbohydrate ingestion increases carbohydrate oxidation and decreases fat oxidation compared to exercising in a fasted state (Coyle et al., 1985; Horowitz and Coyle, 1993; Montain et al., 1991). The primary mechanism for this reduction in fat oxidation is thought to be a decrease in adipose tissue lipolysis and skeletal muscle fatty acid oxidation due to the rise in circulating insulin after carbohydrates are consumed. This insulin-mediated reduction in free fatty acid mobilization and oxidation persists for up to four hours after a carbohydrate meal (Montain et al., 1991). If the quantity of carbohydrates ingested before exercise does not offset the insulin induced increased rate of carbohydrate oxidation, depletion of endogenous carbohydrate stores could occur earlier and exercise performance could be impaired. This may be the reason why Foster et al. (1979) reported a 19% decrease in exercise time to exhaustion at 80% VO_2max when subjects consumed a glucose drink (75 grams) compared to water 30 minutes prior to exercise. Studies that use similar designs where small amounts of carbohydrates are ingested within the hour before exercise may also fall victim to this phenomenon; the increased rate of carbohydrate

oxidation following ingestion of the small amount of carbohydrate offsets any potential performance-enhancing effect of increased carbohydrate availability (Alberici et al., 1993; Devlin et al., 1986; Febbraio & Stewart, 1996; Febbraio et al., 2000; Hargreaves et al.,. 1987; Maliszewski et al., 1995; Sparks et al., 1998). For example, Febbraio et al. (2000) found that the rate of carbohydrate oxidation during 120 minutes of moderate intensity cycling was approximately one gram per minute higher following consumption of mashed potatoes (containing 1.0 $g.kg^{-1}$ carbohydrate) compared to a fasted condition. The mean body mass of the subjects in this study was ~73 kilograms. Therefore, the small quantity of carbohydrate consumed (~73 grams) would not have offset the increased rate of carbohydrate oxidation, possibly negating any potential performance-enhancing effect of the pre-exercise carbohydrate ingestion. Indeed, performance was not different between conditions during a 15-minute time trial performed after the 120 minutes of moderate intensity cycling (Febbraio et al., 2000). Since consuming carbohydrates leads to an increase in circulating insulin and an accompanying increase in the rate of carbohydrate oxidation, a carbohydrate-rich food that is digested and absorbed more slowly, resulting in a lower insulin response may be the ideal pre-exercise carbohydrate source.

1.2.4. The Glycemic Index

The glycemic index (GI) classifies carbohydrate sources according to their postprandial blood glucose response (Jenkins et al., 1981). The GI of a food is determined using a standardized laboratory method which compares the two-hour blood glucose response after ingestion of 50 grams of available carbohydrate from that food with the blood glucose response after ingestion of 50 grams of a reference food (Jenkins

et al., 1981). The standard reference food is a glucose drink, however some researchers use white bread (Foster-Powell et al., 2002). The GI of a test food is calculated by dividing the area under the two-hour blood glucose curve for the test food by the area under the 2-hour blood glucose curve for the reference food and multiplying the result by 100 (equation 1.1).

$$(1.1) \quad GI = \frac{\text{area under the blood glucose curve for test food}}{\text{area under blood glucose curve for reference food}} \times 100$$

With glucose as the reference, a carbohydrate source can be classified as low GI (<55), moderate GI (55-70), or high GI (>70) (Brand-Miller et al., 1996). Table 1.1 lists the GI of some common foods. There are many factors that influence the GI, including the fibre content, fat and protein content, amylose:amylopectin ratio, method of preparation, and degree of processing (Foster-Powell et al., 2002; Walton & Rhodes, 1997).

Table 1.1. Glycemic index (GI) values of some common foods categorized as low, medium and high GI (using glucose as the reference food). GI values are from Foster-Powell et al., 2002.

Food	**GI**
Low GI (<55)	
Apple juice	40
Lentils	30
Chocolate Milk	43
Spaghetti, boiled	38
Banana	52
Moderate GI (55-70)	
Blueberry Muffin	60
Power Bar, chocolate	56
Rice, white, boiled	64
Honey	55
High GI (>70)	
Mashed potato	85
Gatorade	78
Bagel	72
Corn Flakes	81
White bread	73

The magnitude of the insulin response to foods with differing GIs generally follows the blood glucose response (Febbraio & Stewart, 1996; Febbraio et al., 2000; Stevenson et al., 2006; Wee et al., 1999, Wee et al., 2005). Hence, ingestion of a high GI food leads to a marked increase in circulating insulin whereas ingestion of a low GI food leads to a slight rise in circulating insulin. It is the insulin response that is primarily responsible for the contrasting metabolic effects when comparing high and low GI pre-exercise meals. Carbohydrate oxidation is augmented and fat oxidation suppressed when high GI compared to low GI foods are consumed before exercise (Febbraio et al., 2000; Stevenson et al., 2006; Wee et al., 1999; Wee et al., 2005; Wu et al., 2003; Wu & Williams, 2006). If the increased rate of carbohydrate oxidation following a high GI meal leads to an earlier depletion of endogenous carbohydrate stores, then low GI pre-exercise meals could provide a performance advantage.

1.2.5 The Effect of the Glycemic Index on Exercise Metabolism

High GI foods are digested and absorbed quickly, resulting in a rapid increase in blood glucose and insulin (Febbraio & Stewart, 1996; Febbraio et al., 2000; Stevenson et al., 2006; Wee et al., 1999, Wee et al., 2005). This type of food is thought to be most effective for increasing muscle and liver glycogen stores (Burke et al., 1993; Walton & Rhodes, 1997). However, ingestion of a high GI meal before exercise may produce a metabolic environment that is not conducive to improving endurance performance because elevated insulin levels can be counter-productive during exercise. Both insulin and muscle contraction independently signal glucose transporter protein (GLUT4) translocation to the muscle membrane (Lund et al., 1995). As a result, during exercise after a high GI meal the combined effects of insulin and muscle contraction can result in

a sharp decline in blood glucose (Febbraio et al., 2000; Foster et al., 1979; Guezennec et al., 1993; Jentjens & Jeukendrup, 2002; Sparks et al., 1998; Wee et al., 1999; Wee et al., 2005; Wu et al., 2003). This phenomenon may lead to critically low blood glucose levels and has been termed "rebound hypoglycemia" (Jentjens & Jeukendrup, 2002). Increased insulin may further influence blood glucose levels by inhibiting glucose output from the liver (Febbraio et al., 2000). Moreover, an increase in circulating insulin inhibits adipose tissue lipolysis (Horowitz et al., 1997), thus limiting the availability of free fatty acids for oxidation during exercise (Febbraio & Stewart, 1996; Sparks et al., 1998; Wee et al., 1999). Inhibition of adipose tissue lipolysis is not the only mechanism by which insulin may impair fat oxidation. Horowitz et al. (1997) have shown that elevating plasma free fatty acid levels by infusing a lipid preparation does not fully restore fat oxidation when insulin levels are high. This indicates that insulin impairs fatty acid oxidation at a step distal to free fatty acid availability. Possible sites of limitation are sarcolemmal or mitochondrial transport (Coyle et al., 1997; Siddosis et al., 1996) or intramuscular triglyceride hydrolysis. The overall effect of ingesting a high GI meal before exercise is increased whole body carbohydrate oxidation and suppressed fat oxidation (Febbraio et al., 2000; Horowitz et al, 1997; Jose-Cunilleras et al., 2002; Sparks et al., 1998; Wee et al., 1999; Wee et al., 2005; Wu et al., 2003; Wu & Williams, 2006). If blood glucose drops at the onset of exercise, yet carbohydrate oxidation is increased, it seems plausible that muscle glycogen depletion could be augmented during exercise following a high GI meal. In fact, early research demonstrated that ingestion of a glucose drink (GI = ~100) prior to exercise resulted in a rapid decline in blood glucose, a decreased availability of free fatty acids, and increased reliance on muscle glycogen (Costill et al., 1977). Since

low levels of muscle glycogen are a key factor that contributes to fatigue (Hermansen et al., 1967; Shulman & Rothman, 2001), high GI foods may not represent the ideal pre-exercise meal.

A low GI carbohydrate source is digested and absorbed more slowly and results in a delayed release of glucose into the blood (Jenkins et al., 1981). As a result, the insulin response is much lower when a low GI meal is compared to a high GI meal (Burke et al., 1998b; Febbraio et al., 2000; Guezennec et al., 1993; Stevenson et al., 2006; Wee et al., 1999; Wee et al., 2005). Thus, following a low GI meal, a rapid decline in blood glucose at the onset of exercise (i.e., rebound hypoglycemia) does not occur (Febbraio & Stewart, 1996; Wu et al., 2003). The blunted insulin response results in an increased availability of free fatty acids and increases the reliance on lipids as a fuel source (Febbraio et al., 2000; Stevenson et al., 2006; Wee et al., 1999; Wee et al., 2005; Wu et al., 2003; Wu & Williams, 2006). Studies using expired gas analysis to estimate substrate oxidation have reported increased fat oxidation and decreased carbohydrate oxidation during steady-state exercise following a low GI compared to a high GI meal (Thomas et al., 1991; Stevenson et al., 2006; Wee et al., 1999; Wee et al., 2005; Wu et al., 2003; Wu & Williams, 2006). Increased reliance on fat and decreased reliance on carbohydrate in the low GI condition has also been reported using stable isotope glucose tracers to more precisely measure blood glucose and carbohydrate oxidation (Febbraio et al., 2000). Another beneficial effect of a low GI pre-exercise meal may be the delayed release of glucose into the blood, contributing to improved maintenance of blood glucose levels during exercise (DeMarco et al., 1999; Thomas et al., 1991; Thomas et al., 1994; Wu & Williams, 2006). As a result, the rate of muscle

glycogenolysis during exercise may be lower, preserving muscle glycogen for later stages of exercise (Wee et al., 2005). Ongoing intestinal absorption of a low GI meal during exercise may also help maintain blood glucose oxidation late in exercise once muscle glycogen levels have declined. This has led some researchers to recommend that athletes consume a low GI meal prior to competition (Brand-Miller et al., 1996; Burke et al., 1998a; DeMarco et al., 1999; Kirwan et al., 2001; Siu & Wong, 2004; Thomas et al., 1991; Walton & Rhodes, 1997).

Despite the theoretical benefits of a low GI meal, research that directly compares low to high GI pre-exercise meals has revealed somewhat conflicting results. Ingestion of a high GI compared to a low GI meal has resulted in increased carbohydrate oxidation and decreased fat oxidation in most (DeMarco et al., 1999; Febbraio et al., 2000; Guezennec et al., 1993; Sparks et al., 1998; Thomas et al., 1991; Wee et al., 1999; Wee et al., 2005; Wu et al., 2003; Wu & Williams, 2006) but not all (Febbraio & Stewart, 1996) studies. Evidence of a rapid decline in blood glucose at the onset of exercise in the high GI condition is also evident in most studies (Febbraio & Stewart, 1996; Febbraio et al., 2000; Stannard et al., 2000; Thomas et al., 1991; Wee et al., 2005; Wu et al., 2003). Traditionally, a blood glucose concentration of less than ~3.3 $mmol.l^{-1}$ has been considered hypoglycemic (Sherman, 1991). At these extremely low levels of blood glucose, cognitive performance may be impaired and symptoms of nausea, dizziness, and confusion can arise (Levine et al., 1924). Recently it has been shown that when blood glucose drops below ~3.5 $mmol.l^{-1}$ cerebral blood glucose uptake is impaired and motor output from the central nervous system to exercising muscles decreases (Nybo, 2003). Therefore, it seems likely that maintaining blood glucose concentration above 3.5

mmol.l^{-1} is optimal for exercise performance. It is unclear whether the blood glucose nadir during exercise following a high GI meal reaches critically low values (i.e., < 3.5 mmol.l^{-1}), a phenomenon that has been termed rebound hypoglycemia (Jentjens & Jeukendrup, 2002; Kuipers et al., 1999). It appears that some individuals are more susceptible to rebound hypoglycemia than others, although the reasons for this are unknown (Jentjens & Jeukendrup, 2002; Kuipers et al., 1999). The prevalence of rebound hypoglycemia does not seem to be related to muscle mass, insulin sensitivity, or training status (Jentjens & Jeukendrup, 2002). When mean results are presented for a group of study participants it is difficult to ascertain whether some individuals experienced rebound hypoglycemia with a blood glucose nadir of less than 3.5 mmol.l^{-1}. Only one study has ever reported decreased performance due to rebound hypoglycemia (Foster et al., 1979), perhaps because rebound hypoglycemia only appears to be a transient event occurring approximately 15 minutes into exercise (Foster et al., 1979; Jentjens & Jeukendrup, 2002). However, considering that some individuals are more susceptible than others and that rebound hypoglycemia may have severe adverse effects on certain athletes (Noakes, 2001 pp. 131-132), low GI foods may confer a substantial advantage for some endurance athletes if rebound hypoglycemia is prevented. Indeed, Jentjens and Jeukendrup (2003) demonstrated that consuming low GI carbohydrate drinks 45 minutes before exercise decreased the incidence of rebound hypoglycemia in those that experienced this response when consuming high GI drinks, although exercise performance was unaffected in this study.

Regardless of whether blood glucose concentration declines to critically low values, if a high GI meal increases the reliance on carbohydrate yet blood glucose levels

are reduced during the first 15 minutes of exercise, reliance on muscle glycogen may be increased (Costill et al., 1977; Hargreaves et al., 1985). In turn, exercising after a high GI meal may lead to premature fatigue. Only three studies, producing contradictory results, have directly examined muscle glycogen utilization after low and high GI meals with the needle biopsy technique. Two studies from the same laboratory (Febbraio & Stewart, 1996; Febbraio et al., 2000) reported no difference in muscle glycogen utilization during 120 minutes of cycling exercise at ~70% VO_2max in highly trained cyclists when low GI and high GI pre-exercise meals containing 1.0 grams of carbohydrate per kilogram body mass were consumed 60 minutes before exercise. In contrast, Wee et al. (2005) reported a significantly greater rate of muscle glycogen degradation during 30 minutes of moderate intensity running (~70% VO_2max) in recreational athletes after a high GI versus a low GI meal. In this study, the meals contained 2.0 grams of carbohydrate per kilogram body mass and were consumed three hours before exercise. The exact reasons why these studies produced contradictory results are unclear but may be due to differences in exercise mode, exercise duration, timing and size of the meal, and training status of the participants. Considering that the majority of research indicates that the GI of a meal consumed before exercise alters the rate of fat and carbohydrate oxidation, future research is needed to determine if the GI influences muscle glycogen degradation. As was already mentioned, carbohydrate ingestion may have different effects on muscle glycogen utilization during running as compared to cycling and may decrease the reliance on muscle glycogen in type I muscle fibres during running (Tsintzas et al., 1995; Tsintzas et al., 1996). The delayed release of glucose into the blood following a low GI meal may decrease reliance on muscle

glycogen in type I fibres during running exercise, an effect that is not detectable in studies analyzing mixed muscle biopsy samples that use cycling as the mode of exercise (Febbraio & Stewart, 1996; Febbraio et al., 2000). No studies have analyzed muscle fibre type specific glycogen utilization following low and high GI pre-exercise meals.

1.2.6 The Effect of the Glycemic Index on Exercise Performance

If a low GI meal results in improved fat oxidation, better maintenance of blood glucose, and delayed muscle glycogen depletion during subsequent exercise, then consumption of a low GI pre-exercise meal may be more beneficial for endurance performance (Brand-Miller et al., 1996; Burke et al., 1998a; Siu & Wong, 2004; Walton & Rhodes, 1997). Thomas and colleagues (1991) were the first to demonstrate that consumption of a low GI meal (lentils, GI ≈ 29) increased cycling time to exhaustion compared to a high GI meal (mashed potatoes, GI ≈ 98). Subsequently, numerous studies have investigated the effect of the GI on performance (summarized in Table 1.2). In contrast to the results of Thomas et al. (1991) it can be seen that the majority of studies demonstrate that the GI of a pre-exercise meal appears to have little influence on endurance exercise performance.

The 11 studies listed in Table 1.2 can be grouped into two general categories. In the first category, eight studies use designs where a small meal is consumed 30 to 60 minutes prior to exercise (DeMarco et al., 1999; Febbraio & Stewart, 1996; Febbraio et al., 2000; Kirwan et al., 2001; Sparks et al., 1998; Stannard et al., 2000; Thomas et al., 1991; Thomas et al., 1994). The quantity of carbohydrate consumed was 1.0 gram carbohydrate per kilogram body mass ($g.kg^{-1}$) in seven of the studies and 1.5 $g.kg^{-1}$ in one of the studies. Therefore, assuming a typical 70 kg male, the pre-exercise "meals"

would fall far short of the sport nutrition guidelines that advise a meal containing 140 to 300 grams of carbohydrate (Hargreaves, 2001; Hargreaves et al., 2004; Jeukendrup & Gleeson, 2004; Williams & Serratosa, 2006). In addition to the study of Thomas et al. (1991), only two other studies reported an increase in performance in the low GI compared to the high GI condition when a small meal is consumed within the hour before exercise (DeMarco et al., 1999; Kirwan et al., 2001). All three of these studies used cycling time to exhaustion, the merits and applicability of which can be questioned (see discussion above in 1.2.1). Two studies using exercise time to exhaustion (Stannard et al., 2000; Thomas et al., 1994) and three studies that employed time trials as the performance measure (Febbrio & Stewart, 1996, Febbraio et al., 2000; Sparks et al. 1998) did not demonstrate a beneficial effect of low GI pre-exercise meals, although low GI meals were not detrimental. Importantly, no study that employed a design where a small meal was consumed close to the onset of exercise used running as the mode of exercise. This is likely due to the gastrointestinal distress that is associated when food is consumed too close to the onset of running (Brouns, 1991). Therefore, the results of these eight studies where small meals are consumed within the hour before exercise have limited application to endurance running or sports involving running.

There are three studies that examine the effect of the GI on exercise performance using designs where a meal is consumed two to three hours before exercise. Burke et al. (1998b) studied the effect of low and high GI pre-exercise meals containing 2.0 grams of carbohydrate per kilogram body mass consumed two hours before exercise on cycling time trial performance. This study reported no difference between conditions, but the effects of the GI could not be isolated because high GI glucose drinks were consumed

15 minutes prior to and throughout exercise. Two studies from the same laboratory (Wee et al., 1999; Wu & Williams, 2006) using similar designs (2.0 grams carbohydrate per kilogram body mass consumed three hours before exercise) have examined the effect of the GI on running performance. One study reported no effect of the GI on running time to exhaustion at 70% VO_2max (Wee et al., 1999) and the other reported a significant 7% increase in running time to exhaustion at 70% VO_2max in the low GI compared to the high GI condition (Wu & Williams, 2006). The reasons why contrasting results were reported from the same laboratory are unclear, but may be related to the fact that both males and females participated in the study reporting no effect of the GI (Wee et al., 1999) whereas only males participated in the other (Wu & Williams, 2006). It has been suggested that menstrual cycle phase can influence substrate oxidation and endurance performance in females (Janse de Jonge, 2003; Oosthuyse et al., 2005) and since the low and high GI trials were performed seven days apart, menstrual cycle could have confounded the results of Wee et al. (1999). Furthermore, the subjects in the study of Wee et al. (1999) appeared to be less fit than those of Wu & Williams (2006) (VO_2max of 51.9 for males and 44.7 $ml.kg^{-1}.min^{-1}$ for females versus 60.6 $ml.kg^{-1}.min^{-1}$, respectively). The merits of both of these studies are limited because they used running time to exhaustion, which is considered to be an unreliable measure of performance (Jeukendrup et al., 1996). No study has ever examined the effect of low and high GI pre-exercise meals consumed two to three hours before exercise on a more reliable and applicable measure of performance such as a running time trial. Considering that athletes in sports that involve running would likely consume a large meal at this time

(Wee et al., 1999; Wee et al., 2005; Stevenson et al., 2006) it remains to be determined if the GI of a pre-exercise meal influences running performance.

Table 1.2. Summary of studies that have examined the effect of the glycemic index (GI) of a pre-exercise meal on exercise performance. Abbreviations: carbohydrate (CHO), time to exhaustion (T_{ex}), time trial (TT), maximal oxygen uptake (VO_2max), minutes (min), seconds (sec), no significant difference (N/S). *A high GI CHO solution was also provided immediately before and throughout exercise in each condition.

Reference	**Subjects**	**Meal Conditions**	**CHO content (g CHO per kg body mass)**	**Timing of meal (min before exercise)**	**Exercise Test**	**Results**
Thomas et al., 1991	8 "trained" male cyclists; VO_2max = 55.3 ml/kg/min	Low GI (lentil, GI=29) High GI (potato, GI=98) Glucose (GI=100) Control (water)	1.0	60	Cycling T_{ex} @ ~65-70% VO_2max	↑ T_{ex} by ~21% in low GI (117 min) vs. high GI trial (97 min)
Thomas et al., 1994	6 "trained" male cyclists; VO_2max = 59.5 ml/kg/min	Low GI (lentil, GI=36) Low GI (bran flakes, GI=30) High GI (rice cereal, GI=73) High GI (potato, GI=100)	1.0	60	Cycling T_{ex} @ 65-70% VO_2max	N/S
Febbraio & Stewart, 1996	6 "endurance-trained" males; VO_2max = 62.1 ml/kg/min	Low GI (lentil, GI=29) High GI (potato, GI=80) Control (diet jelly)	1.0	45	15-minute cycling TT (120 min preload at 70% VO_2max)	N/S
Sparks et al., 1998	8 "endurance-trained" males; VO_2max = 67.9 ml/kg/min	Low GI (lentil, GI=29) High GI (potato, GI=80) Control (sweetened drink)	1.0	45	15-minute cycling TT (50 min preload @ 67% VO_2max)	N/S

Table 1.2 cont'd

Reference	Subjects	Meal Conditions	CHO content (g CHO per kg body mass)	Timing of meal (min before exercise)	Exercise Test	Results
Burke et al., 1998b*	6 "trained" male cyclists; VO_2max = 68.6 ml/kg/min	Low GI (pasta, GI=37) High GI (potato, GI=87) Control (diet jelly)	2.0	120	300 kJ TT (120 min preload @ ~70% VO_2max)	N/S
Demarco et al., 1999	10 "trained" males; VO_2max = 61.2 ml/kg/min	Low GI (mixed breakfast, GI = 36) High GI (mixed breakfast, GI = 69)	1.5	30	Cycling T_{ex} @ 100% VO2max (120 min preload @ ~70% VO_2max)	↑ T_{ex} by ~59% in low GI (206.5 sec) vs. high GI trial (129.5 sec)
Wee et al., 1999	5 males; VO_2max = 51.9 ml/kg/min 3 females; VO_2max = 44.7 ml/kg/min	Low GI (lentil, GI not provided) High GI (mixed meal, GI not provided)	2.0	180	Running T_{ex} @ 70% VO_2max	N/S
Stannard et al., 2000	10 "highly trained" males; VO_2max = 71.9 ml/kg/min	Low GI (pasta, GI = 41) High GI (glucose, GI = 100) Control (sweetened water)	1.0	65	Incremental cycling T_{ex} (i.e., VO_2max test)	N/S

Table 1.2 cont'd

Reference	Subjects	Meal Conditions	CHO content (g CHO per kg body mass)	Timing of meal (min before exercise)	Exercise Test	Results
Febbraio et al., 2000	8 "endurance-trained" males; VO_2max = 60.5 ml/kg/min	Low GI (muesli & oats, GI=52) High GI (potato, GI=80) Control (diet jelly)	1.0	30	30-minute cycling TT (120 min preload @ ~70% VO_2max)	N/S
Kirwan et al., 2001	6 "active" males; VO_2max = 54.3 ml/kg/min	Moderate GI (oats, GI = 61) High GI (puffed rice, GI = 82) Control (water)	~1.0 (all subjects consumed 75 g available CHO)	45	Cycling T_{ex} @ 60% VO_2max	↑ T_{ex} by ~23% in Mod GI (165 min) vs. Control trial (134 min) N/S High GI (141 min) vs. Control trial or Mod GI
Wu & Williams, 2006	8 males ; VO2max = 60.6 ml/kg/min	Low GI (bran cereal, fruit, apple juice, GI = 37) High GI (corn flakes, white bread, glucose drink, GI = 77)	2.0	180	Running Tex @ 70% VO2max	↑ T_{ex} by ~7% in low GI (108.8 min) vs. high GI (101.4 min)

1.2.7 Limitations of Previous Research

A critical examination of the GI and exercise literature reveals three important limitations. Firstly, most studies have employed a design where a small amount of carbohydrate (1.0 $g.kg^{-1}$) is ingested 30-60 minutes before exercise following an overnight fast (see Table 1 and discussion above in section 1.2.5). Although this protocol ensures that the onset of exercise coincides with the peak in blood glucose and insulin following a high GI meal (Hargreaves, 2001; Thomas et al., 1991), it may not be applicable to athletes. Most athletes would not prepare for a competition by fasting for 8-12 hours and then consuming such a small meal within the hour before exercise. Consuming a large carbohydrate-rich meal two to four hours before an event is more likely (Burke et al., 1998b; Jeukendrup & Gleeson, 2004; Wee et al., 1999; Wee et al., 2005) and is what sport nutrition guidelines advise (Hargreaves et al., 2004; Williams & Serratosa, 2006). The effect of the GI of a meal consumed at this time point has received little attention. The studies that have isolated for the effects of the GI of a pre-exercise meal have used running time to exhaustion as a measure of performance (Wee et al., 1999; Wu & Williams, 2006) and have produced conflicting results. In addition, the participants in these studies were described as "recreational runners" and may not have been used to prolonged running to exhaustion. A limited number of studies have investigated the metabolic effects of meals with different GIs consumed three hours before exercise. All of these studies report increased carbohydrate oxidation and decreased fat oxidation in the high GI condition (Li et al., 2004; Stevenson et al., 2006; Wee et al., 1999; Wee et al., 2005; Wu & Williams, 2006). One study has directly measured muscle glycogen utilization and has reported an increased rate of muscle

degradation in the high GI compared to the low GI trial (Wee et al., 2005). Unfortunately, exercise only lasted 30 minutes and performance was not assessed in this study (Wee et al., 2005). Therefore, it could not be determined whether the rate of muscle glycogen degradation would have continued to be greater in the high GI condition, possibly leading to premature fatigue. As such, the influence of the GI of a meal ingested two to four hours before exercise on endurance running performance and muscle glycogen metabolism warrants more attention.

A second limitation in the previous research is that some studies have failed to match the macronutrient profile of the pre-exercise foods (Febbraio & Stewart, 1996; Febbraio et al., 2000, Kirwan et al., 2001; Thomas et al., 1991; Thomas et al., 1994). For example, the original GI and exercise study of Thomas et al. (1991) has been criticized because the pre-exercise meals were matched for carbohydrate content only (Wee et al., 1999). The low GI condition (lentils) contained a considerable amount of protein whereas the high GI condition (potato) contained very little protein. Many subsequent studies have used similar designs where the carbohydrate content of the low GI and high GI foods have been matched but the macronutrient profile has been different (Febbraio & Stewart, 1996; Febbraio et al., 2000; Kirwan et al., 2001; Thomas et al., 1994). Since the addition of protein to a carbohydrate source can alter the insulin response (Betts et al., 2005) and has been shown to enhance endurance performance (Ivy et al., 2003; Saunders et al., 2004), differences in protein content between pre-exercise meals may affect metabolism and performance. The exact mechanisms that lead to improved endurance performance when protein is added to carbohydrate remain unclear. Amino acids may provide tricarboxylic acid cycle intermediates to help maintain flux through

this important metabolic pathway (Ivy et al., 2003). Protein may provide branched-chain amino acids that are oxidized for fuel during endurance exercise (Gleeson, 2005). Branched-chain amino acids may help prevent central fatigue by competing with tryptophan at the blood-brain barrier. Decreasing the delivery of tryptophan to the brain may decrease the production of serotonin, a hormone that is thought to decrease arousal and inhibit exercise performance (Gleeson, 2005). Clearly, the protein content of a pre-exercise carbohydrate-rich meal should be taken into consideration when studying the effects of the GI.

The third major limitation in the literature is the fact that almost every study has examined the influence of the GI on continuous steady-state sub-maximal exercise. With the exception of one study (Stannard et al., 2000), all of the investigations discussed previously examined the effect of the GI on metabolism during exercise at 65-70% VO_2max. Stannard et al. (2000) compared a high GI to a low GI meal consumed before a continuous incremental cycling test to exhaustion and noted no difference between trials. However, blood glucose and muscle glycogen availability are not considered limiting factors in exercise of this duration (~15 minutes) and this exercise test lacks applicability to sport performance. Many team sports, for example soccer, hockey, rugby and basketball, involve periods of high-intensity activity interspersed with periods of low-intensity activity and rest. Muscle glycogen is depleted at very high rates during intermittent exercise (MacDougall et al., 1977; Saltin, 1973). Consequently, decreased muscle glycogen availability contributes to fatigue late in the game (Bangsbo et al., 2006; Jacobs et al., 1982; Mohr et al., 2003; Mohr et al., 2005; Saltin, 1973; Spencer et al., 2005). Of the many team sports, soccer appears to involve the largest endurance

component. Matches consist of 90 minutes of intermittent running, and players run a total of 9-12 km throughout the game (Reilly, 2000; Spencer et al., 2005). Muscle glycogen is an important fuel source for soccer players (Bangsbo et al., 2006; Kirkendall, 2004; Nicholas et al., 1999; Saltin, 1973; Spencer et al., 2005) and at the end of a game, muscle glycogen stores can be almost fully depleted (Agnevik, 1970 from Kirkendall, 2004; Jacobs et al., 1982; Mohr et al., 2003; Mohr et al., 2005). Not surprisingly, dietary carbohydrates have been shown to be important for soccer players. Saltin (1973) showed that players who consumed a low carbohydrate diet for three days prior to a soccer match had lower levels of muscle glycogen before and during the match and performed worse than players who consumed a high carbohydrate diet. Specifically, this study reported that players on the low carbohydrate diet had lower levels of muscle glycogen at half-time, covered less distance in the second half, and performed less high speed running compared to players on the high carbohydrate diet (Saltin, 1973). Similarly, Bangsbo et al. (1992) demonstrated that consuming a high carbohydrate diet during the week before a field-based soccer test resulted in improved performance when compared to a moderate carbohydrate diet. As such, strategies to increase muscle glycogen availability and/or delay its degradation would appear to be beneficial for soccer players.

1.2.8. Carbohydrate Ingestion and Soccer Performance

Despite the importance of carbohydrates in the diet of soccer players, research that examines the effects of game day nutrition is somewhat limited (Kirkendall et al., 1988; Leatt & Jacobs, 1989; Muckle, 1973; Nicholas et al., 1995; Nicholas et al., 1999; Ostojic & Mazic, 2002; Zeederberg et al., 1996). A number of field studies have

reported improved performance when soccer players consume liquid carbohydrates immediately before and during soccer matches. Muckle (1973) gave players from an English professional soccer team glucose syrup (quantity of carbohydrates was not specified) 30 minutes prior to each match for 20 games and then had them consume nothing before the match for the next 20 games. When consuming the glucose syrup, the team scored more goals and conceded fewer during the 20-game period. Also, players had more touches of the ball and a greater number of shots on goal during the games when glucose was consumed before the match. However, during the 20-game period where glucose was provided before the games players were supplemented with glucose syrup during training sessions and were given supplemental glucose syrup on the day before matches. Thus, the independent effects of consuming a high-carbohydrate diet on the day before the match and the effects of the pre-game glucose ingestion could not be separated. Further confounding the results was the failure to report dietary information for the 20-game period when no glucose was consumed before matches.

In another field study, Leatt and Jacobs (1989) had players on one team consume a glucose drink (containing ~35 grams of carbohydrate) and players on the other team consume a placebo immediately before and at halftime of a soccer game. They reported a decreased reliance on muscle glycogen in the glucose condition but because players were on different teams exercise intensity could not be matched between conditions. Unfortunately, the impact of increased glycogen availability at the end of the match in the glucose condition could not be determined because performance was not assessed in this study. Using a similar study design, Ostojic and Mazic (2002) had players from one team consume a carbohydrate drink and players from another consume a placebo

immediately before and every 15 minutes during an actual soccer match. At the end of the game, players from the team that consumed the carbohydrate drink performed better on soccer-dribbling and precision tests but no differences between conditions were reported for sprinting power or a co-ordination test. Once again, the results are confounded because the players in the experimental and placebo conditions were on different teams and the outcome of the match could have affected the amount of work performed.

Kirkendall et al. (1988) used a crossover design and reported a 20% improvement in overall distance covered and a remarkable 40% improvement in the distance covered at running and sprinting speeds during the second half when players consumed a glucose polymer drink before and at halftime of matches compared to a placebo condition. Despite the within-subjects study design, the results of this field investigation are still limited because the circumstances during the experimental and placebo condition matches would have been different for each individual player. Overall, field studies that examine carbohydrate consumption immediately before and/or during soccer matches generally report evidence of improved performance. However, inherent problems with field study design make it difficult to link improved performance directly to the carbohydrates consumed. When players in the carbohydrate and placebo conditions are on different teams or the carbohydrate and placebo conditions are administered during different games to the same players, it is virtually impossible to control for differences in exercise intensity between conditions.

Laboratory studies can overcome the inability of field studies to match exercise intensity between conditions. A number of laboratory investigations have reported

favourable effects associated with consuming carbohydrate beverages before and during exercise tests that are designed to simulate soccer matches. Nicholas et al. (1999) reported a decreased reliance on muscle glycogen when players consumed a carbohydrate drink compared to placebo immediately before and at 15-minute intervals during a 90-minute shuttle running test. There was no effect of the carbohydrate drink on average 15-metre sprint times, although the merits of this performance criterion are difficult to determine. The authors only presented the mean sprint times for the entire test (~66 sprints) and provided no information about the changes in sprint times over the course of the 90-minute test or the ability to sustain maximal sprinting speeds for longer than 15 metres (which took subjects ~2.4 seconds to complete in this study). Using a similar study design, these same authors (Nicholas et al., 1995) had previously reported increased time to exhaustion on a repeated sprint test performed at the end of the 90-minute shuttle run test when subjects consumed a carbohydrate-electrolyte beverage compared to placebo.

It can be seen that the majority of field and laboratory studies demonstrate a beneficial effect of ingesting carbohydrates immediately before and/or during a soccer match. However, all of these previous investigations compared the effects of a carbohydrate beverage to a sweetened placebo or water consumed immediately (i.e., < 30 minutes) before and/or during an actual or simulated soccer match. The effects of pre-game meals on metabolism and performance during games have not been studied.

1.2.9. High-Intensity Intermittent Exercise and the Glycemic Index

The metabolic environment during high-intensity intermittent exercise is quite different from that of steady-state exercise. During high-intensity exercise, a marked

increase in circulating catecholamines and neural feed-forward processes enhance liver glycogenolysis to the extent that hepatic glucose output tends to exceed muscle glucose uptake (Jeukendrup & Gleeson, 2004; Kreisman et al., 2000; Kreisman et al., 2001; Marliss & Vranic, 2002). As a result, hypoglycemia does not generally develop during exercise at intensities greater than 80% VO_2max (Achten & Juekendrup, 2003; Coggan et al., 1995; Jeukendrup & Gleeson, 2004). Indeed, hypoglycemia is not usually considered to be a contributing factor to fatigue in sports involving long duration, high-intensity intermittent activity (Glaister, 2005; Leatt & Jacobs, 1989; Mohr et al. 2003; Mohr et al., 2005) although extremely low blood glucose concentrations (<3.0 mmol.l^{-1}) at the end of a soccer match have been reported in some subjects in one study (Ekblom, 1986). Therefore, improved maintenance of blood glucose after a low GI meal (DeMarco et al., 1999; Thomas et al., 1991; Thomas et al., 1994) may not be as important during high-intensity intermittent exercise. However, the sustained release of glucose after a low GI meal may act similar to a carbohydrate drink and decrease the reliance on muscle glycogen in type I muscle fibres (Nicholas et al., 1999; Tsintzas et al., 1995; Tsintzas et al., 1996). The sustained release of glucose after a low GI meal may also help to maintain carbohydrate oxidation when muscle glycogen becomes low. Oxidation of carbohydrates is needed to fuel high-intensity efforts and blood glucose becomes an increasingly important fuel as muscle glycogen levels decline (Coggan & Coyle, 1991). Thus, in the later stages of a game, when muscle glycogen levels are low (Saltin, 1973), blood glucose may be a very important fuel for high-intensity activity.

The effects of the GI of a pre-exercise carbohydrate meal may have implications for lipid metabolism during intermittent activity. Fatty acid oxidation would be an

important energy source for soccer players during the low-intensity and rest periods of the game. The large increase in insulin from a high GI carbohydrate source inhibits fatty acid oxidation during exercise for up to four hours after ingestion, even if circulating insulin levels have returned to normal (Montain et al., 1991). Therefore, a high GI meal that is ingested before a soccer game may lead to decreased fatty acid oxidation in the low-intensity and rest periods, increasing the reliance on muscle glycogen and blood glucose. No study has ever examined the effect of the GI of pre-game meals on metabolism or performance during long duration, high-intensity intermittent exercise.

1.3 Statement of the Problem

The effect of the GI of a pre-exercise meal on intermittent exercise is currently unknown. As the GI has been shown to affect substrate utilization in most studies (DeMarco et al., 1999; Febbraio et al., 2000; Guezennec et al., 1993; Sparks et al., 1998; Thomas et al., 1991; Wee et al., 1999; Wee et al., 2005; Wu et al., 2003; Wu & Williams, 2006) and may also influence performance during continuous endurance exercise (DeMarco et al., 1999; Kirwan et al., 2001; Thomas et al., 1991; Wu & Williams, 2006), the effect of the GI on long duration, high-intensity intermittent exercise resembling the activity pattern in team sports warrants further study. Muscle glycogen is depleted at very high rates during high-intensity intermittent exercise (MacDougall et al., 1977) and depletion of muscle glycogen contributes to fatigue during the late stages of games (Saltin, 1973). Therefore, the effect of the GI on substrate utilization during exercise resembling team sport games has important implications. The primary purpose of this study was to examine the effect of low and high GI pre-exercise meals on metabolism and performance during soccer-specific

intermittent exercise. A low GI-high protein meal (lentils), two high GI meals (high GI-high protein and high GI-low protein), and a control (no meal) condition were studied in a repeated crossover design. The low GI-high protein and high GI-high protein meals were matched for macronutrient content whereas the high GI-low protein meal was matched for energy content but contained more carbohydrate and less protein. The high GI-low protein condition was included in order to address a secondary aim of this study; that is, to examine whether the protein content of a carbohydrate-rich pre-exercise meal influences metabolism or performance. Some studies have shown that adding protein to a carbohydrate source consumed during exercise improves performance over carbohydrates alone (Ivy et al., 2003; Saunders et al., 2004). However, when the energy content of the carbohydrate + protein and carbohydrate conditions are matched there appears to be no further benefit to performance (Romano-Ely et al., 2006; Van Essen & Gibala, 2006). The effect of adding protein to a pre-exercise meal has not been addressed. Early studies on the effect of the GI on exercise performance failed to match the protein content of meal conditions (Thomas et al., 1991; Thomas et al., 1994; Febbraio & Stewart, 1996) which could have confounded the results. Therefore, by incorporating two high GI meals that were matched for energy content but differed in carbohydrate and protein content, this study could shed light on whether increasing the protein content of a pre-exercise meal influences exercise metabolism and performance. A low GI condition that differed in protein content was not included to decrease participant burden associated with completing five experimental trials in the repeated crossover.

Meals were consumed two hours before the start of a 90-minute soccer match simulation. Blood samples were collected before and during the simulated soccer match to assess major blood borne energy substrates (glucose and free fatty acids) and glucoregulatory hormones (insulin, epinephrine and norepinephrine). Expired gas samples were collected throughout exercise to estimate the rates of carbohydrate and fat oxidation. Performance was assessed by measuring the distance covered during a repeated sprint test during the last 15 minutes of the match.

1.4 Hypotheses

I. The insulin response to both the high GI-high protein and high GI-low protein meals will be greater when compared to low GI-high protein or control. Correspondingly, serum free fatty acid concentration will be lower in both high GI conditions versus low GI-high protein or control. As a result, carbohydrate oxidation will be higher and fat oxidation will be lower following the high GI pre-exercise meals when compared to low GI-high protein or control.

II. In the low GI-high protein condition, the greater rate of fat oxidation coupled with the ongoing release of glucose from the gastrointestinal tract will preserve endogenous carbohydrate stores (i.e., muscle and liver glycogen, blood glucose) for the later stages of exercise. Since increased carbohydrate stores have been associated with improved performance during the late stages of soccer games (Saltin, 1973) the distance covered during the repeated sprint test during the last 15 minutes of the soccer match simulation will be improved in the low GI-high protein condition when compared to the high GI conditions.

III. The distance covered on the repeated sprint test during the last 15 minutes of the soccer match simulation will be greater after consumption of a low GI-high protein, high GI-high protein, or high GI-low protein pre-exercise meal when compared to control (no meal) due to increased carbohydrate availability.

IV. There will be no difference in the distance covered on the repeated sprint test between the high GI-high protein and high GI-low protein conditions because these conditions are matched for energy content.

V. Circulating catcholamines (serum epinephrine and norepinephrine) will be greater during exercise in the control condition compared to low GI-high protein, high GI-high protein, and high GI-low protein as a result of greater physiological stress associated with exercising in the fasted state.

2. METHODS

2.1 Participants

Eighteen male athletes were originally recruited to participate. From these original participants, five failed to complete the study. Two participants withdrew after completing one experimental condition due to injuries sustained during training which were unrelated to the study. Three participants withdrew after preliminary testing due to time constraints or injuries unrelated to the study. Therefore, thirteen participants completed the entire study (age, 22.3 ± 3.3 years; body mass, 74.8 ± 6.5 kg; height, 179.5 ± 4.4 cm; maximal oxygen uptake [VO_2max], 55.0 ± 4.6 $ml.kg^{-1}.min^{-1}$; peak treadmill speed [Vmax], 17.8 ± 1.8 $km.hr^{-1}$). Seven of the participants were players on the University of Saskatchewan Huskies varsity men's soccer team (four defenders, one midfielder, and two goalkeepers). Three participants were club level soccer players (two defenders and one midfielder) and three were competitive middle distance runners with recreational soccer experience. Runners were included because they were fit enough to complete 90 minutes of exercise and were familiar with intermittent running (e.g., interval training). All participants had experience with treadmill running prior to the study. The study protocol was approved by the University of Saskatchewan Biomedical Research Ethics board (see Appendix I) and participants provided written informed consent before any data was collected (Appendix II).

2.2 Experimental Design

Participants were required to make six visits to the laboratory. The first two visits were for preliminary testing that consisted of a maximal oxygen uptake (VO_2max) test on a treadmill and a familiarization session with the treadmill soccer match simulation (details of these exercise tests are described below). Following preliminary testing, each participant participated in four experimental trials separated by seven days in a randomized crossover design. A different pre-exercise meal condition was administered in each trial. The experimental conditions were: low GI-high protein (boiled red lentils; GI = ~26), high GI-high protein (instant mashed potato, white bread, and egg whites; GI = ~76), high GI-low protein (instant mashed potato and white bread; GI = ~76), and control (no meal). All GI values were obtained from Foster-Powell et al. (2002). GI values for the mixed high GI meals were calculated according to the methods of Wolever and Jenkins (1986). To increase palatability, participants were allowed to add a small amount (~50 millilitres) of crushed tomato to the low GI-high protein meal and tomato ketchup to the high GI meals. The experimental conditions were administered in a randomized counter-balanced fashion. The study was single-blind; that is, all personnel conducting the exercise tests and evaluating results were blinded to experimental conditions but the participants knew what meals they consumed. This was accomplished by having a research assistant prepare the meals and supervise consumption in a separate room.

2.2.1 Details of Experimental Meal Conditions

The energy and macronutrient profile for the three test meals is presented in table 2.1. The low GI-high protein meal (boiled red lentils, Small Football Lentils, Arbel Pulse and Grain, Regina, SK) was designed to provide 1.5 grams of available

carbohydrate per kilogram of body mass. Although this is slightly below published guidelines that recommend consuming approximately 2.0 grams of carbohydrate per kilogram body mass from a large meal prior to exercise (Hargreaves, 2001; Hargreaves et al., 2004; Gleeson & Jeukendrup, 2004), pilot testing indicated that participants could consume approximately 75% of a lentil or instant mashed potato meal that contained this quantity of carbohydrate. The high GI-high protein meal (instant mashed potato, [McCain Instant Mashed Potatoes, McCain Foods, Florenceville, NB], white bread [Wonder Enriched White Bread, Weston Bakeries Limited, Toronto, ON] , and egg whites [Naturegg Simply Egg Whites, Burnbrae Farms, Upton, QC]) also provided 1.5 grams of available carbohydrate per kilogram of body mass. Egg whites were incorporated into this meal to match the protein content of lentils (0.42 grams of protein per gram of carbohydrate; nutrient values are from the United States Department of Agriculture [USDA] National Nutrient Database or from information provided by the manufacturers). A small amount of margarine was also added to the mashed potatoes to match the fat content of lentils (0.036 grams of fat per gram of carbohydrate). Therefore, the low GI-high protein and high GI-high protein meals both provided 1.5 grams of carbohydrate per kilogram of body mass, 0.63 grams of protein per kilogram of body mass, and 0.054 grams of fat per kilogram of body mass. The energy content of the meal was therefore approximately nine kilocalories per kilogram of body mass. The high GI-low protein meal (instant mashed potato and white bread) contained approximately 1.9 grams of available carbohydrate per kilogram of body mass. The protein content was lower (~0.26 grams per kilogram of body mass) and fat content similar (0.054 grams per kilogram of body mass) when compared to the other two meal conditions. The high GI-

low protein meal was matched for energy content with the low GI-high protein and high GI-high protein meals.

Table 2.1. Energy content and macronutrient profile of the three test meals (based on a 70 kg participant). A control condition (no meal) was also employed. GI values for individual foods are from Foster-Powell et al. (2002). GI values for the high GI meals were calculated using the mixed meal method from Wolever and Jenkins (1986).

	Low GI-high protein (lentils)	High GI-high protein	High GI-low protein
Energy (kilocalories)	~632	~632	~632
Carbohydrate (grams)	105.0	105.1	131.2
Protein (grams)	44.3	44.0	18.0
Fat (grams)	3.9	3.9	3.9
Glycemic Index (GI)	~26	~76	~76

2.2.2 Details of Exercise Tests

2.2.2.1 Maximal Oxygen Uptake (VO_2max) Test

VO_2max and peak treadmill speed (Vmax) were determined using an incremental running test to exhaustion on a treadmill (Vacu Med, Model 13622, Ventura, CA) according to the methods of Harling et al. (2003). A five-minute warm-up at eight kilometers per hour followed by five minutes of stretching was performed prior to the test. The test began at ten kilometers per hour and a computer loaded with the appropriate software (Vacu Med, TurboFit 5.05, Ventura, CA) controlled the velocity of the treadmill such that it increased by one kilometer per hour every minute until volitional fatigue. The treadmill incline remained constant at one percent. During the test, expired minute ventilation (VE), VO_2, VCO_2 and respiratory exchange ratio (RER) were measured breath-by-breath using open circuit indirect calorimetry (Sensor Medics, Vmax Series 29, Anaheim, CA). VO_2max was defined as the highest 20-second average

for VO_2. Heart rate was measured continuously using a Polar S610i heart rate monitor (Polar Electro Oy, Kempele, Finland) and maximal heart rate was defined as the highest 5-second average. Vmax was defined as the highest treadmill speed that was maintained for one complete minute (Harling et al., 2003). A velocity-based treadmill test was used as opposed to a traditional progressive incline test because Vmax was used to individualize treadmill speeds for the soccer match simulation and repeated sprint test (see description of these tests below).

2.2.2.2 Treadmill Soccer Match Simulation

The soccer match simulation consisted of two 45-minute halves separated by a 15-minute break (i.e., halftime). The aforementioned treadmill and software package were used to simulate the activity pattern of a soccer game by alternating between periods of rest, walking, jogging, running, and sprinting. The proportion of time spent at each speed was based on time-motion analysis of professional soccer players (Ali & Farrally, 1991). This research categorized movement patterns during games and demonstrated that players spend approximately 7% of the game standing still, 56% of the game walking (~ 6 $km.h^{-1}$), 30% of the game jogging (~ 10 $km.h^{-1}$), 4% of the game running (~ 17 $km.h^{-1}$), and 3% of the game sprinting (~23 $km.h^{-1}$). The treadmill soccer match simulation was based on the protocol of Drust et al. (2000) with slight modifications to account for differences in the treadmill and software package used in our laboratory. Drust et al. (2000) divided one 45-minute half of a soccer match into two 22.5 minute sections consisting of six walking intervals, six jogging intervals, three runs, and eight sprints presented in a random order. The treadmill used in the present study was able to accelerate faster and therefore one 45-minute half of the soccer

simulation employed in the present study was separated into three 15-minute sections, each consisting of six walking intervals, six jogging intervals, three runs and eight sprints presented in the same random order as that of Drust and colleagues (2000). The order of the intervals for one 15-minute section is presented in figure 2.1. Ninety-five second standing periods were incorporated into the protocol in between each 15-minute section to account for the proportion of time spent standing during actual soccer matches (7%, Ali & Farrally, 1991). Pilot testing determined the time required for the transitions (i.e., acceleration and deceleration) between each speed. Based on the order of the intervals the total time required for transitions was calculated and then subtracted from the total time available for each section (i.e., 15 minutes) to obtain the total usable time for the intervals. The length of the intervals for each speed (walking, jogging, running, and sprinting) was then determined by multiplying the proportion of time spent at each speed by the total usable time and dividing it by the number of intervals at that speed (details and full calculations for the development of the soccer match simulation are presented in Appendix III). The lengths of the intervals (not including acceleration and deceleration time) were: 66 seconds (walking), 36 seconds (jogging), 10 seconds (running), and 3 seconds (sprinting). Including the time it took for acceleration and deceleration the lengths of the intervals were: ~72 seconds (walking), ~42 seconds (jogging), ~17 seconds (running), and ~13 seconds (sprinting).

During the familiarization trial, the speed of the running and sprinting intervals were adjusted according to participants' individual abilities. It was determined that 23 kilometres per hour was too fast for participants to run on the treadmill and the sprint speed was therefore decreased to 21 kilometres per hour (acceleration and deceleration

times were adjusted accordingly). Three subjects requested the speed of the sprint be decreased further to 20 kilometres per hour. The running speed was also decreased by one kilometre per hour to 16 kilometres per hour in these three subjects. The intensity of the intervals expressed relative to each participant's Vmax is presented in table 2.2.

Table 2.2. The intensity of the walking, jogging, running, and sprinting intervals used in the treadmill soccer match simulation expressed relative to peak treadmill speed (Vmax). Values are means ± SD.

Interval	**% Vmax**
Walking (6 $km.h^{-1}$)	34.0 ± 3.3
Jogging (10 $km.h^{-1}$)	56.6 ± 6.6
Running (16-17 $km.h^{-1}$)	93.3 ± 6.7
Sprinting (20-21 $km.h^{-1}$)	114.9 ± 8.3

In order to assess exercise performance, a repeated sprint test was performed during the sixth 15-minute section of the soccer match simulation. Therefore, the 90-minute soccer match simulation consisted of five identical 15-minute sections (with a 15-minute halftime after the third section) plus a repeated sprint test during the final 15 minutes. This test consisted of five 1-minute sprints with two minutes and thirty seconds of recovery in between. Each sprint started at the participant's individual Vmax and the participant was allowed to control the speed of the treadmill by verbally instructing a researcher to increase or decrease the speed. The participants were kept blind to the speed and distance during the sprints but were allowed to see the elapsed time. A researcher informed the participant after 15, 30, 45, and 50 seconds and counted down the last five seconds of each sprint. The treadmill was stopped at the end of each sprint and the subject stood still for 15 seconds and then walked at five kilometers per hour for two minutes and 15 seconds before the speed was increased again for the next sprint. A white line was drawn on the treadmill belt and the distance covered during each sprint

was measured by recording the number of treadmill belt revolutions using a manual click counter and multiplying by the length of the belt. This allowed for greater precision of the distance covered during the sprints when compared to the treadmill display. One minute sprints were chosen because pilot testing demonstrated that this allowed the participant sufficient time to gauge their speed and adjust it accordingly by instructing the researcher to increase or decrease the speed. Pilot testing also indicated that participants were generally able to complete one minute sprints at a speed slightly above their Vmax, making individual Vmax an ideal speed for standardizing the start of the sprints. Two and a half minutes of rest was chosen because this allowed for 10 minutes of standing and walking in the 15-minute section which corresponds to the proportion of time spent in these resting activities during actual soccer games (Ali & Farrally, 1991).

2.3 Experimental Protocol

On testing days, participants reported to the laboratory in the morning after an overnight fast. It was originally planned to insert an intravenous catheter into a forearm or dorsal hand vein for repeated blood sampling but this technique was abandoned after the first two experimental trials because of difficulties in keeping the catheter patent and the propensity for the catheter to become dislodged during high speed running. Therefore, venous blood samples were not taken at baseline or during the postprandial period. A baseline finger tip capillary blood sample was taken for determination of blood glucose (Accu-chek Compact Plus, Roche Diagnostics, Mannheim, Germany) and blood lactate (Accutrend, Roche Diagnostics, Mannheim, Germany) using commercial analyzers. Participants then had twenty minutes to consume one of the test meals. In the

control condition they rested quietly during this time. Capillary blood samples were obtained at 15, 30, 60, and 120 minutes after completion of the meal. Participants were allowed to rest quietly, read, or study during the postprandial period. Water was provided *ad libitum* in the first trial and was matched in subsequent trials. Exactly 120 minutes after completing the meal a venous blood sample was obtained by venipuncture. Participants were then fitted with a heart rate monitor and performed a five-minute warm-up on the treadmill at eight kilometres per hour. After some light stretching, the soccer match simulation began. Expired gas samples were collected for seven-minute periods from the third to the tenth minute of the first, third and fifth 15-minute sections (i.e., during minutes 3-10, 33-40, and 63-70). A venous blood sample was obtained at the end of the first 15-minute section, at halftime (i.e., at 45 minutes), and at the end of the match (i.e., at 90 minutes). This provided four time points for venous blood sampling, which was deemed the maximal number of venipuncture samples to take from a participant on one testing day. The sample taken 15 minutes into the match would allow for analysis of the early exercise response and allowed for comparison to previous studies reporting the presence of rebound hypoglycemia at this time point (Foster et al., 1979; Jentjens & Jeukendrup, 2002). The 45 and 90 minute samples allowed for analysis at the mid and end points of exercise and were ideal for comparison to previous research on the metabolic responses to soccer games (e.g., Leatt & Jacobs, 1989). Capillary blood samples were taken at the same time points as the venous blood samples plus an additional sample was taken at the end of the fifth 15-minute block (i.e., at 75 minutes). This allowed for the analysis of blood glucose and lactate concentration immediately prior to the repeated sprint test. Water was provided *ad libitum* during the standing

periods at the end of every 15-minute section in the first trial and was matched on subsequent trials. Ratings of perceived exertion (RPE) were obtained at the end of each 15-minute section using a scale from 6 (very, very light) to 20 (very, very hard) (Borg, 1975). The experimental protocol is depicted in Table 2.3 and a graphical representation is available in Appendix IV.

2.3.1 Venous blood sample collection and analysis

Venous blood samples were collected while the subject was seated. Whole blood was collected into 10 millilitre tubes (BD Vacutainer SST) and according to manufacturer's instructions were allowed to clot for 30 minutes prior to being centrifuged at 3000 revolutions per minute at room temperature. Serum was then transferred into four separate 1.5 millilitre microcentrifuge tubes and stored at -80º Celsius until analysis. Serum insulin was analyzed using an enzyme linked immunosorbent assay (ELISA) kit (Insulin EIA, Alpco Diagnostics, Salem, NH, USA). An ELISA kit was used to measure serum epinephrine and norepinephrine (BA 10-1500, Rocky Mountain Diagnostics, Colorado Springs, CO, USA). The results for serum catecholamines are presented together as total serum epinephrine plus norepinephrine. Free fatty acids were measured using a commercial assay kit (NEFA HR(2), Wako Diagnostics, Richmond, VA, USA) adapted for a 96-well microtiter plate (Falcon Microtest 96, Becton Dickinson, Franklin Lakes, NJ, USA) according to the manufacturer's instructions except that the two incubations were carried out at room temperature for 15 minutes (Baur et al., 2006). Serum glucose was measured using a colorimetric assay kit (Quantichrom Glucose Assay Kit, Bio Assay Systems, Hayward, CA, USA). All analyses were performed using a Biotek Synergy HT microplate reader

with Gen5 software (Biotek Instuments, Winooski, VT, USA). The coefficients of variation for the insulin, epinephrine, norepinephrine and free fatty acid assays were all ≤10 %. The coefficient of variation for the glucose assay was ≤ 5 %. Details of assay protocols are presented in Appendix V.

2.3.2 Expired gas sample collection and analyses

At each time point, expired gas samples were collected for a seven-minute period. VO_2, VCO_2, and RER values were measured by the metabolic cart. VO_2 and VCO_2 were used to calculate carbohydrate and fat oxidation rates based on published equations adapted for high intensity exercise (equations 2.1 and 2.2, respectively; Jeukendrup & Wallis, 2005). The contribution of protein was considered negligible and was ignored.

$$\text{Carbohydrate oxidation } (g.min^{-1}) = 4.210 \text{ X } VCO_2 - 2.962 \text{ X } VO_2 \quad (2.1)$$

$$\text{Fat oxidation } (g.min^{-1}) = 1.695 \text{ X } VO_2 - 1.701 \text{ X } VCO_2 \quad (2.2)$$

2.3.3 Dietary and physical activity controls

In order to minimize any potential diet-induced variability in resting muscle substrates or exercise metabolism, participants were asked to complete a 24-hour diet record prior to the first experimental trial. A trained nutrition researcher went over records with each participant to ensure accuracy. Diet records were photocopied and returned to participants and they were instructed to consume the same types and quantities of food for 24 hours prior to each experimental trial. Compliance was assessed upon arrival to the laboratory on each testing day. To limit any potential effects of prior physical activity on exercise metabolism or performance, participants were asked to refrain from strenuous physical activity on the day before each trial.

Participants were asked to record their physical activity for 24 hours prior to the first testing day. These records were photocopied and returned with instructions to follow this physical activity pattern prior to subsequent trials.

2.4 Statistics

Repeated sprint performance was analyzed by a 4 (meal condition) × 5 (sprints) repeated measures ANOVA. Capillary blood glucose and lactate, VO_2, heart rate, RPE, serum measures, and expired gas parameters were all analyzed by a 4 (meal condition) × time repeated measures ANOVA. Total fat and total carbohydrate oxidized were analyzed by a one-factor (meal condition) repeated measures ANOVA. The significance level was set at $p \leq 0.05$. Tukey's HSD post hoc tests were used when significance was found. All results are reported as means ± SD. All analyses were carried out on Statistica version 7.0 (StatSoft Inc., Tulsa, OK). ANOVA tables and full results of all post-hoc tests are presented in Appendix VI.

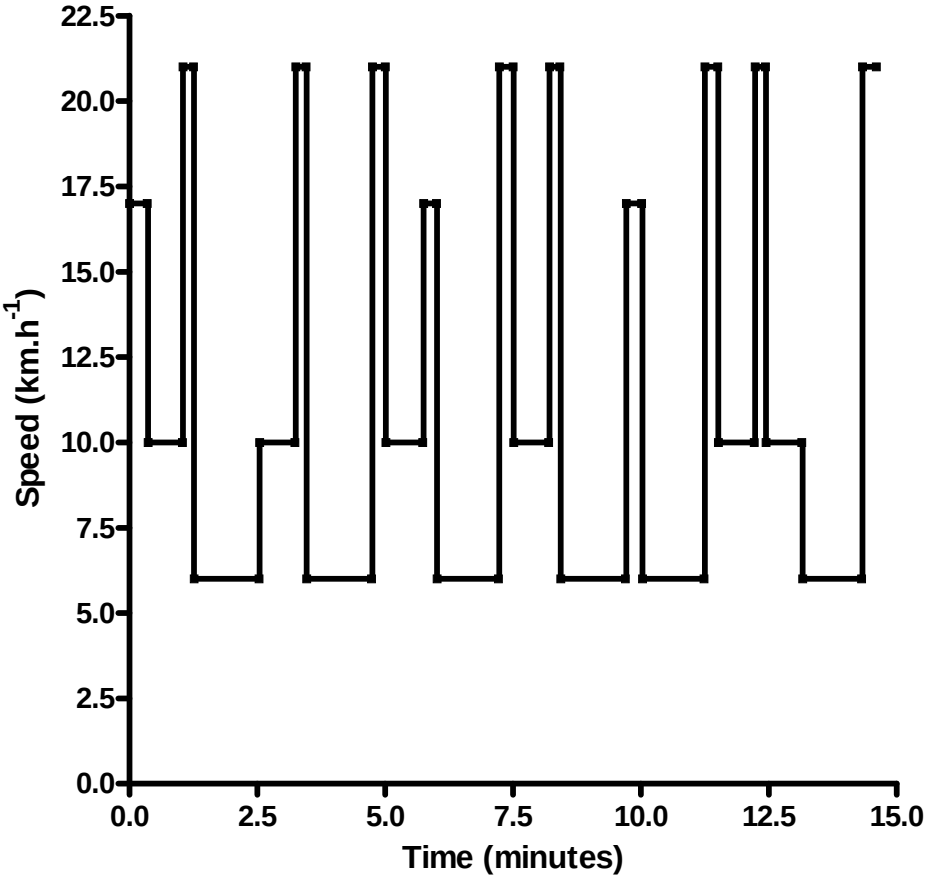

Figure 2.1. Graphical representation of one 15-minute section of the soccer match simulation. The treadmill was controlled by a computer so that the speed alternated between walking (6 $km.h^{-1}$), jogging (10 $km.h^{-1}$), running (17 $km.h^{-1}$) and sprinting (21 $km.h^{-1}$) based on the protocol of Drust et al. (2000). The proportion of time spent at each speed was based on time-motion analyses of professional soccer matches (Ali & Farraly, 1991). The 90-minute match consisted of five identical 15-minute sections plus the repeated sprint test in the last 15 minutes of the match. There were 95 second standing periods in between each section and a 15-minute halftime after the third section.

Table 2.3. Time and events schedule of the experimental protocol

	Time (minutes)															
	Pre-exercise						First Half				Half-time	Second Half				
Measure	**-140**	**-120**	**-105**	**-90**	**-60**	**0**	**3**	**15**	**30**	**33**	**45-60**	**75**	**78**	**90**	**90-105**	**105**
Finger tip blood sample (glucose and lactate)	X		X	X	X	X		X			X			X		X
CHO meal (1.5 $g.kg^{-1}$)	X															
Venipuncture blood sample (glucose, FFA, hormones)						X		X			X					X
Expired gas collection							X			X			X			
RPE								X	X		X	X		X		X
Repeated Sprint Test															X	

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Expired Gas Analyses

3.1.1.1 Respiratory Exchange Ratio (RER)

There was a significant main effect of meal condition on RER ($p = 0.029$, figure 3.1). Post hoc analyses revealed that average RER was significantly higher in the high GI-low protein condition compared to control (0.994 ± 0.027 vs.0.971 ± 0.032). As expected, a main effect of time was also detected ($p < 0.001$) with post hoc analyses revealing a significantly lower RER in collection periods two (minutes 33-40, 0.977 ± 0.011) and three (minutes 63-70, 0.963 ± 0.009) compared to collection period one (minutes 3-10, 1.01 ± 0.010). Collection period three was also significantly lower than two.

3.1.1.2 Carbohydrate Oxidation

For the rate of carbohydrate oxidation, there was a significant main effect of meal condition ($p = 0.019$, figure 3.2). Post hoc analyses revealed that carbohydrate oxidation was significantly greater in high GI-low protein compared to control (3.10 ± 0.50 vs. 2.83 ± 0.47 $g.min^{-1}$). There was also a main effect of time, as would be expected ($p < 0.001$). Post hoc analyses revealed that the rate of carbohydrate oxidation was lower during collection period two (2.92 ± 0.14 $g.min^{-1}$) and three (2.79 ± 0.10 $g.min^{-1}$) compared to collection period one (3.19 ± 0.12 $g.min^{-1}$). There was a significant main

effect of meal condition for total carbohydrate oxidized ($p = 0.020$, Figure 3.3) with the total amount of carbohydrate oxidized being significantly greater for high GI-low protein (279 ± 45 g) compared to control (254 ± 42 g). There were no other significant differences between meal conditions for carbohydrate oxidation.

3.1.1.3 Fat Oxidation

There was a main effect of meal condition for the rate of fat oxidation ($p = 0.03$, figure 3.4). Post hoc analyses showed that fat oxidation was significantly lower in high GI-low protein compared to control (0.064 ± 0.082 vs 0.142 ± 0.096 $g.min^{-1}$). As expected, there was a main effect of time ($p < 0.001$) with post hoc analyses revealing a greater rate of fat oxidation during collection periods two (0.106 ± 0.042 $g.min^{-1}$) and three (0.156 ± 0.038 $g.min^{-1}$) compared to collection period one (0.044 ± 0.024 $g.min^{-1}$). Collection period three was also significantly greater than two (0.156 ± 0.038 vs. 0.106 ± 0.042 $g.min^{-1}$). There was a significant main effect of meal condition on the total fat oxidized ($p = 0.031$, Figure 3.5). The total fat oxidized during exercise was significantly less in the high GI-low protein condition (5.7 ± 7.4 g) compared to control (12.8 ± 8.6 g). There were no other differences in fat oxidization between meal conditions.

3.1.1.4 Oxygen Uptake (VO_2)

There were no differences between meal conditions for average absolute VO_2 during exercise ($p = 0.475$, Table 3.1). There was a significant main effect of time ($p = 0.006$) with post hoc tests revealing that average VO_2 during collection period three (63-70 minutes) was significantly greater than collection period one (3-10 minutes) (2.58 ± 0.06 vs. 2.52 ± 0.06 $l.min^{-1}$). For the entire soccer match simulation, VO_2 averaged 62.4 ± 0.01, 62.6 ± 0.01, 63.2 ± 0.01, and 62.7 ± 0.01 percent of VO_2max in the control, low

GI-high protein, high GI-high protein, and high GI-low protein conditions, respectively. There were no differences in statistical outcomes when VO_2 was expressed relative to VO_2max.

3.1.2 Finger tip capillary blood

3.1.2.1 Glucose

There was a significant meal condition × time interaction for finger tip blood glucose concentration ($p < 0.001$, Figure 3.6). Post hoc analyses revealed that blood glucose was significantly higher in the high GI-low protein and high GI-high protein compared to low GI-high protein and control at 15 (8.8 ± 1.4, 8.6 ± 1.4, 5.6 ± 0.5, and 5.0 ± 0.3 $mmol.l^{-1}$ for high GI-low protein, high GI-high protein, low GI-high protein, and control, respectively), 30 (8.0 ± 1.3, 7.8 ± 1.4, 5.6 ± 0.7, and 5.2 ± 0.5 $mmol.l^{-1}$), and 60 (6.3 ± 0.8, 6.1 ± 0.7, 5.4 ± 0.6, and 5.0 ± 0.2 $mmol.l^{-1}$) minutes after the meal. There were no significant differences in finger tip blood glucose concentration between meal conditions at 120 minutes post meal or at any time point during exercise.

3.1.2.2 Lactate

There were no differences between meal conditions for finger tip blood lactate concentration ($p = 0.367$) nor was there a meal condition × time interaction ($p = 0.906$). There was a significant main effect of time ($p < 0.001$, Table 3.2). Post hoc tests revealed that blood lactate concentration was significantly greater at the end of the repeated sprint test compared to all resting/postprandial and other exercise time points.

3.1.3 Serum Measures

3.1.3.1 Glucose

There were no significant differences in serum glucose concentration between meal conditions ($p = 0.172$, Figure 3.7). The meal condition × time interaction was also not significant ($p = 0.106$). There was a significant main effect of time ($p < 0.001$), such that serum blood glucose concentration increased during exercise and was significantly higher at all time-points during exercise compared to immediately before exercise (i.e., at time 0).

3.1.3.2 Free Fatty Acids

A significant main effect for meal condition was noted for free fatty acid concentration ($p < 0.001$, Figure 3.8). Post hoc analyses revealed that free fatty acid concentration was significantly higher in the control trial (0.604 ± 0.137 meq.l^{-1}) compared to low GI-high protein (0.383 ± 0.076 meq.l^{-1}), high GI-high protein (0.389 ± 0.120 meq.l^{-1}), and high GI-low protein (0.382 ± 0.091 meq.l^{-1}; average of all four time-points for each condition). As expected there was a time main effect ($p < 0.001$) such that free fatty acid concentration increased over time throughout exercise (Figure 3.8).

3.1.3.3 Insulin

There was a significant meal × time interaction for serum insulin ($p < 0.001$, figure 3.9). Post hoc analyses revealed that serum insulin concentration was significantly higher immediately before exercise (time 0) in the high GI-low protein condition (23.5 ± 4.4 mU.l^{-1}) compared to high GI-high protein (12.9 ± 1.7 mU.l^{-1}), low GI-high protein (10.1 ± 1.4 mU.l^{-1}), and control (5.6 ± 1.0 mU.l^{-1}). There were no significant differences between conditions at any time-point during exercise.

3.1.3.4 Epinephrine plus norepinephrine

There was a significant meal condition × time interaction for serum epinephrine plus norepinephrine ($p < 0.001$, Figure 3.10). Post hoc tests showed that serum epinephrine plus norepinephrine concentration was significantly higher at the end of exercise in the high GI-low protein condition (17.8 ± 2.7 $nmol.l^{-1}$) compared to low GI-high protein (12.6 ± 1.6 $nmol.l^{-1}$) and control (11.7 ± 2.0 $nmol.l^{-1}$). Serum epinephrine plus epinephrine was also significantly higher at the end of exercise in the high GI-high protein condition (15.6 ± 2.6 $nmol.l^{-1}$) compared to control (11.7 ± 2.0 $nmol.l^{-1}$). There were no differences between conditions at any other measurement time.

3.1.4 Exercise Performance

For the repeated sprint test, there was a significant meal condition × sprint interaction ($p = 0.05$, figure 3.11). Post hoc analyses revealed that the distance covered in sprint one was significantly greater in the low GI-high protein compared to control condition (310.5 ± 40.0 vs. 295.1 ± 32.4 m). The distance covered in sprint two was significantly greater in low GI-high protein (305.1 ± 35.2 m) and high GI-high protein (307.7 ± 31.6 m) compared to control (291.7 ± 35.4 m). There were no significant differences between conditions in sprints three, four or five.

3.1.5 Rating of Perceived Exertion (RPE)

There was a significant main effect of meal condition on RPE ($p = 0.005$, Figure 3.12). Post hoc analyses revealed that the average RPE for the entire soccer match simulation was lower in the low GI-high protein (13.0 ± 1.6) compared to control (13.9 ± 1.2) and high GI-low protein (13.8 ± 1.4). There was also a significant time main effect ($p < 0.001$) such that RPE tended to increase over time. Post hoc tests revealed that RPE was significantly higher after the third (12.9 ± 1.6), fourth (13.2 ± 1.8), fifth

(13.9 ± 1.9) and sixth (17.4 ± 1.4) 15-minute section compared to the first (11.4 ± 1.6). RPE was significantly higher after the fifth (13.9 ± 1.9) and sixth (17.4 ± 1.4) 15-minute sections compared to the second (12.2 ± 1.6). RPE after the sixth (17.4 ± 1.4) 15-minute section was also significantly greater than the third (12.9 ± 1.6), fourth (13.2 ± 1.8), and fifth (13.9 ± 1.9) sections.

3.1.6 Heart Rate

For heart rate, there was a significant main effect of time ($p < 0.001$, Table 3.3). Post hoc analyses revealed that average heart rate generally increased over time throughout the soccer match simulation, as would be expected (full results of post hoc tests in Table 8). There were no differences between meal conditions in average heart rate ($p = 0.365$). Heart rate throughout the entire soccer match simulation averaged 74.5 ± 0.04, 74.5 ± 0.04, 75.6 ± 0.04, and 75.7 ± 0.05 percent of maximal heart rate in the control, low GI-high protein, high GI-high protein, and high GI-low protein conditions, respectively. When heart rate data was expressed as a percentage of maximum the results of the statistical analyses were the same as those with absolute heart rate.

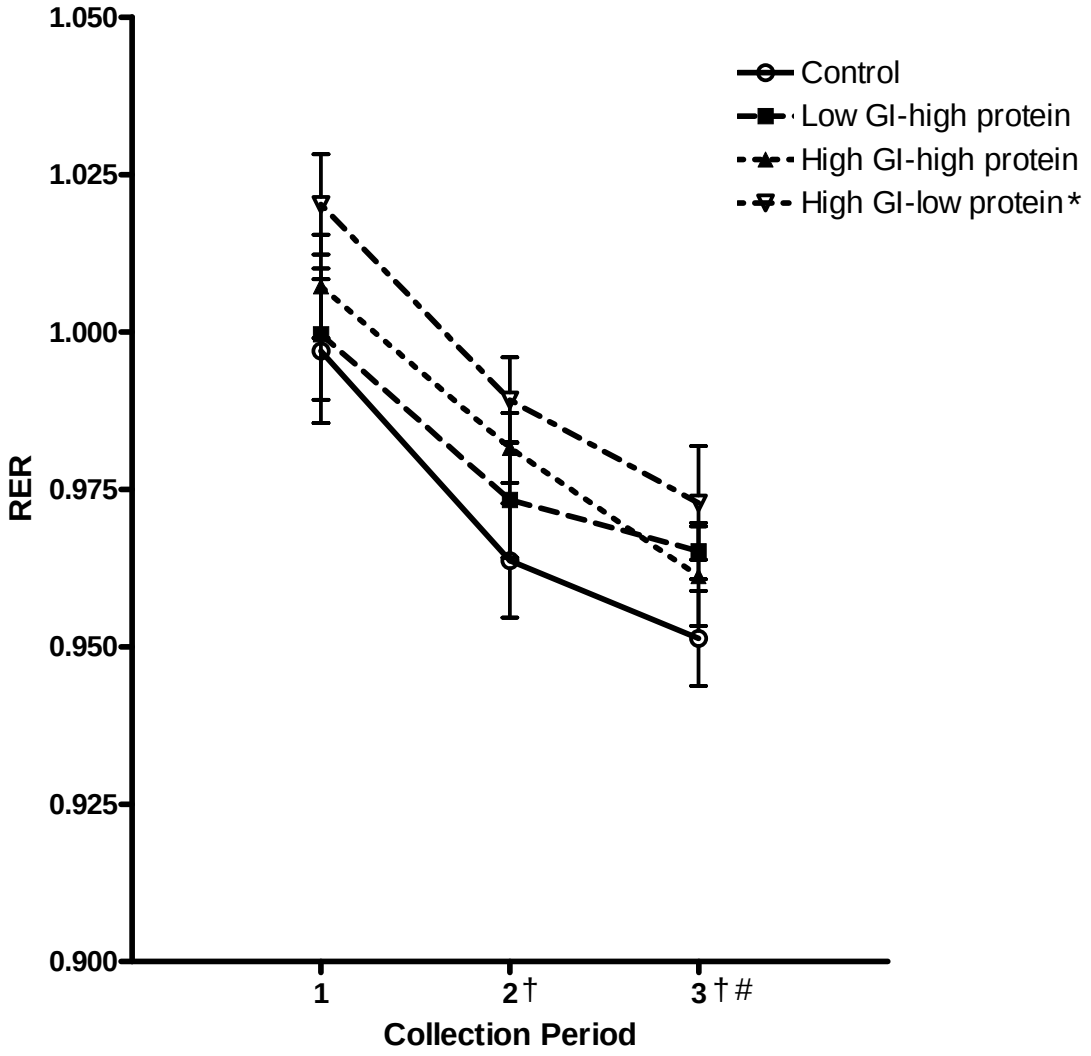

Figure 3.1. Respiratory exchange ratio (RER) during collection period 1 (3-10 minutes), 2 (33-40 minutes), and 3 (63-70 minutes) of the soccer match simulation. * High GI-low protein significantly greater than control (main effect of meal condition, $p < 0.05$). † Collection periods 2 and 3 significantly lower than collection period 1 (time main effect, $p < 0.05$). # Collection period 3 significantly lower than collection period 2 (time main effect, $p < 0.05$).

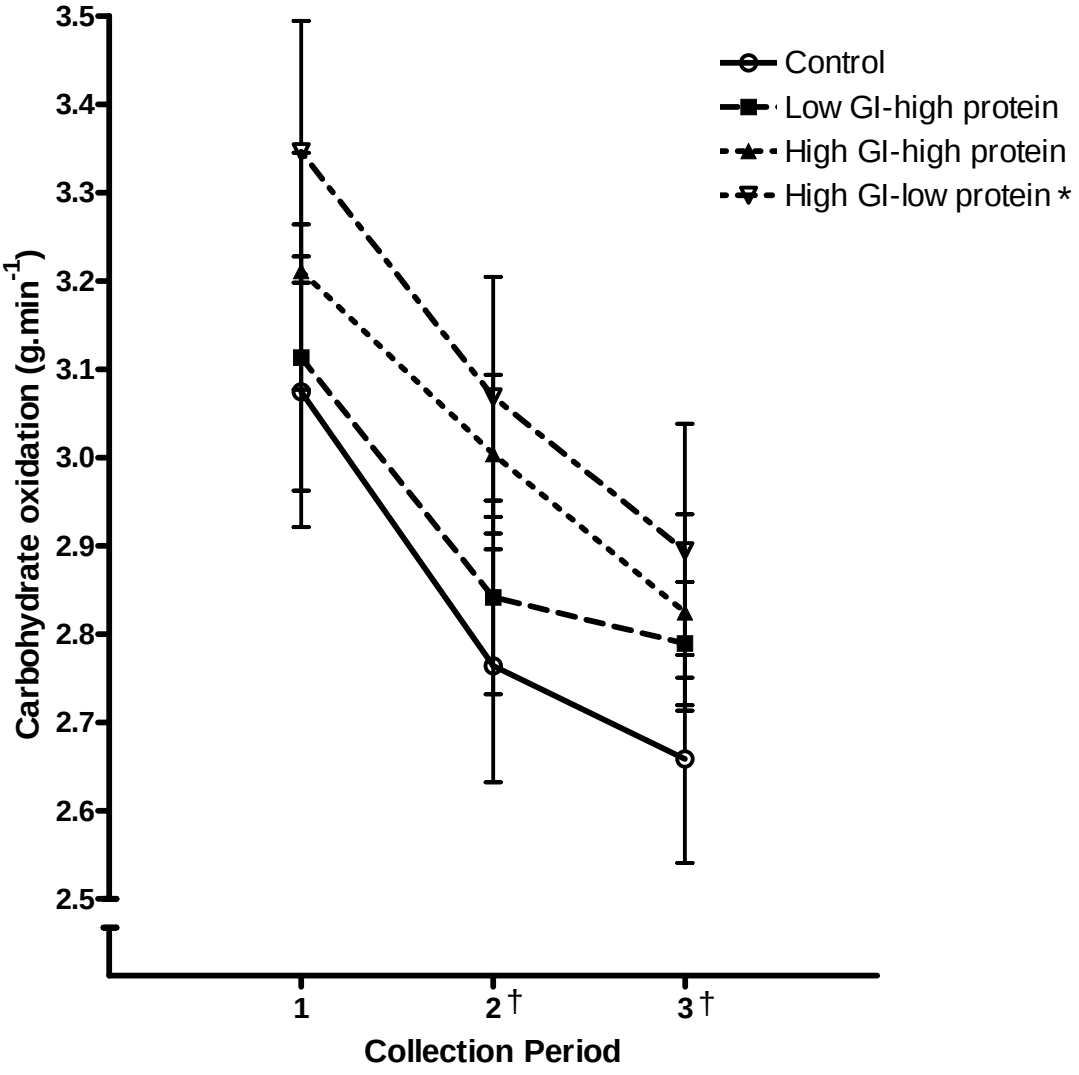

Figure 3.2. Average carbohydrate oxidation ($g.min^{-1}$) during collection period 1 (3-10 minutes), 2 (33-40 minutes), and 3 (63-70 minutes) of the soccer match simulation. * High GI-low protein significantly greater than control (main effect of meal condition, $p < 0.05$). † Collection periods 2 and 3 significantly lower than collection period 1 (time main effect, $p < 0.05$).

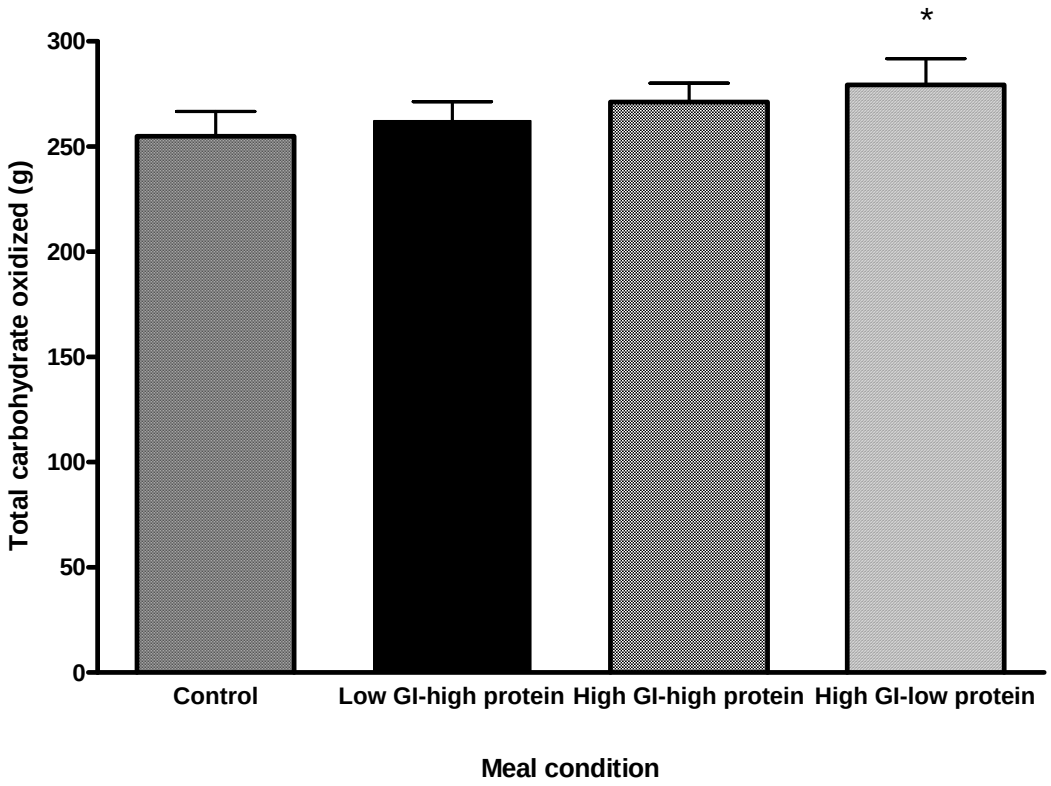

Figure 3.3. Total carbohydrate oxidized (grams) throughout the entire soccer match simulation. * High GI-low protein significantly greater than control ($p < 0.05$).

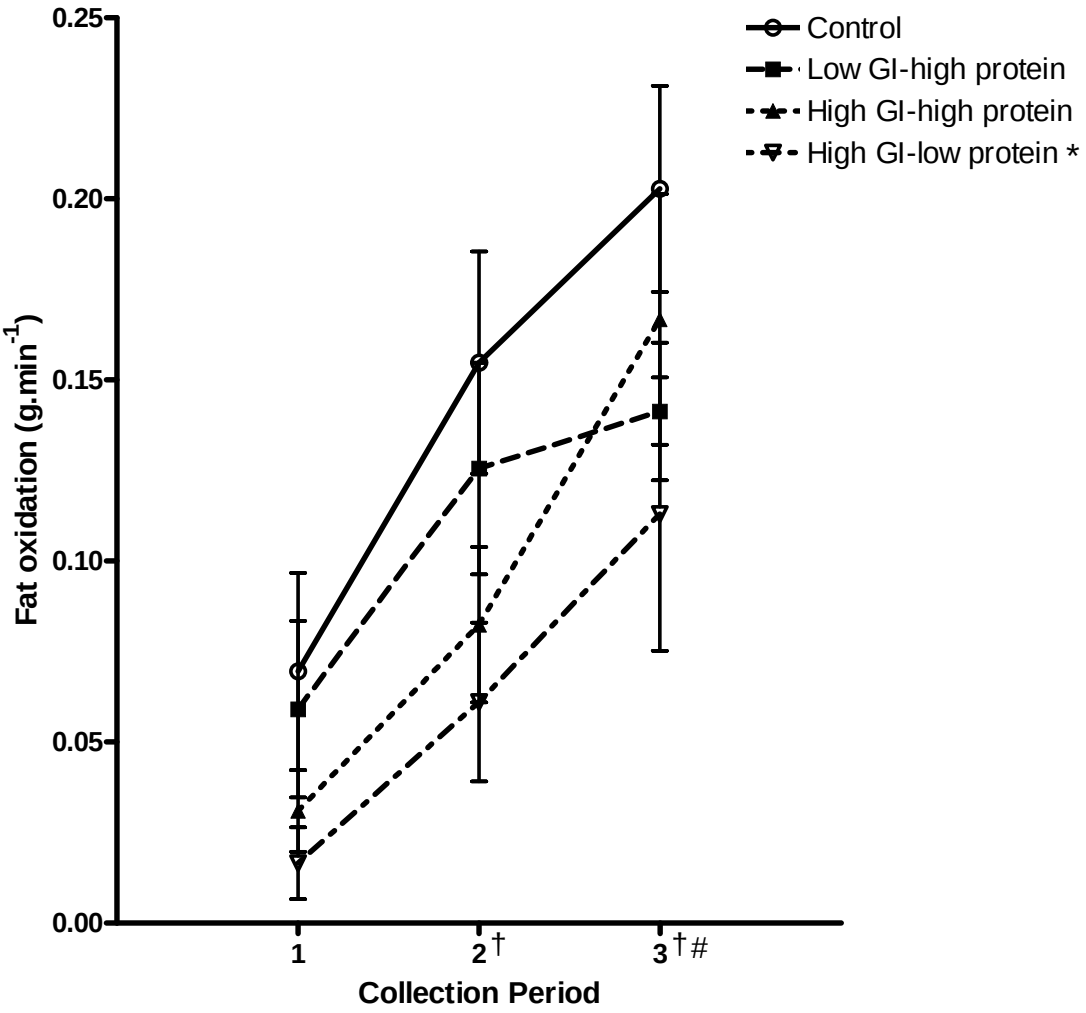

Figure 3.4. Average fat oxidation ($g.min^{-1}$) during collection period 1 (3-10 minutes), 2 (33-40 minutes), and 3 (63-70 minutes) of the soccer match simulation. * High GI-low protein significantly less than control (main effect of meal condition, $p < 0.05$). † Collection periods 2 and 3 significantly greater than collection period 1 (time main effect, $p < 0.05$). # Collection period 3 significantly greater than collection period 2 (time main effect, $p < 0.05$).

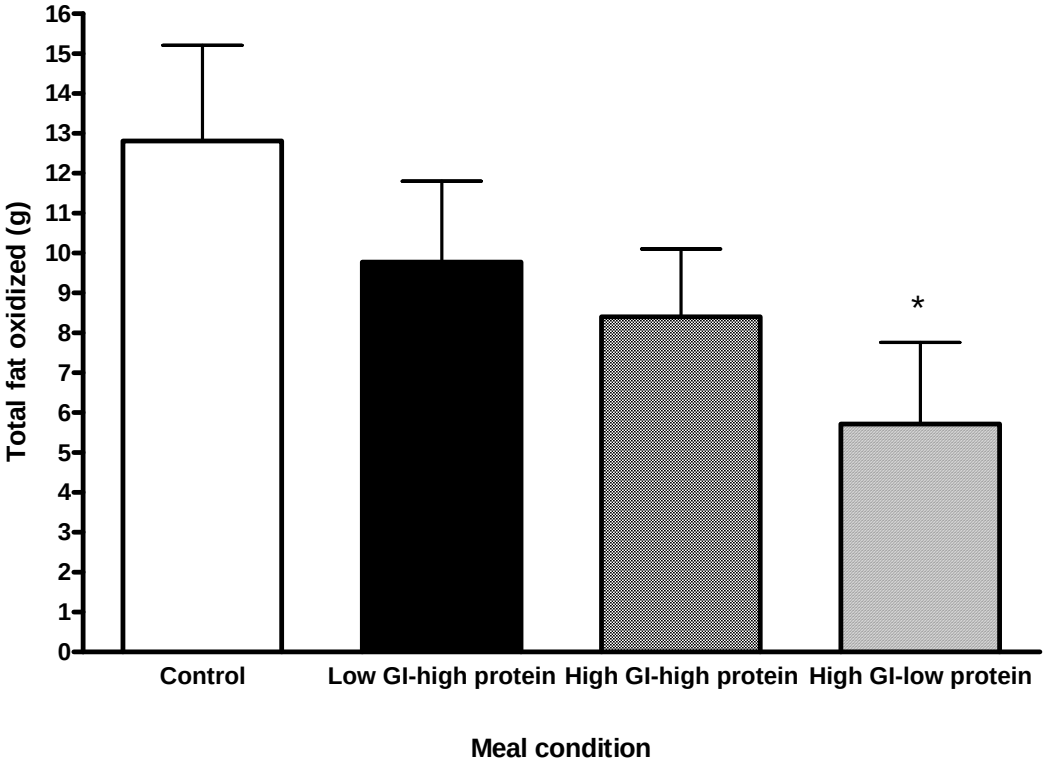

Figure 3.5. Total fat oxidized (grams) throughout the entire soccer match simulation. * High GI-low protein significantly less than control ($p < 0.05$).

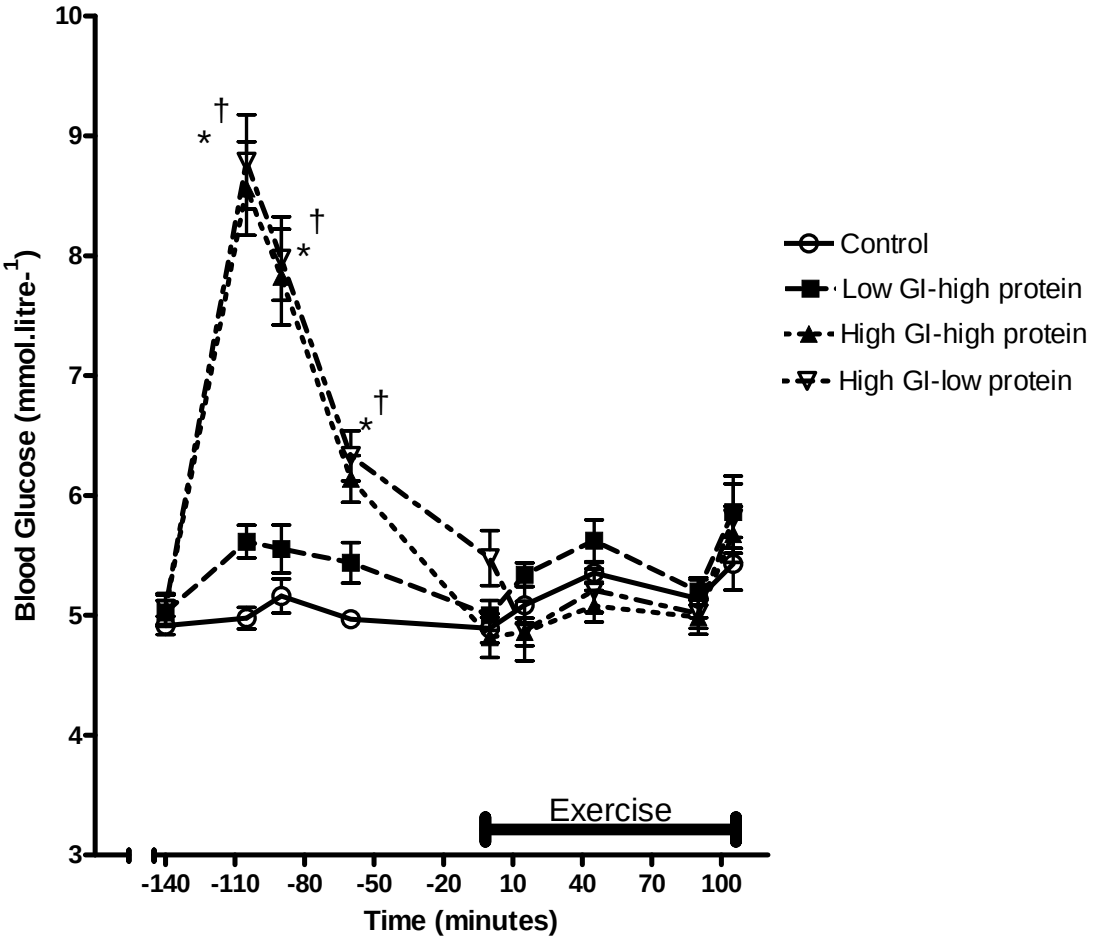

Figure 3.6. Blood glucose concentration (mmol.l^{-1}) at baseline (-140 minutes), during the postprandial period (-105 to 0 minutes), and throughout exercise (0 to 105 minutes). * high GI-high protein significantly greater than low GI-high protein and control ($p < 0.001$). † high GI-low protein significantly greater than low GI-high protein and control ($p < 0.001$).

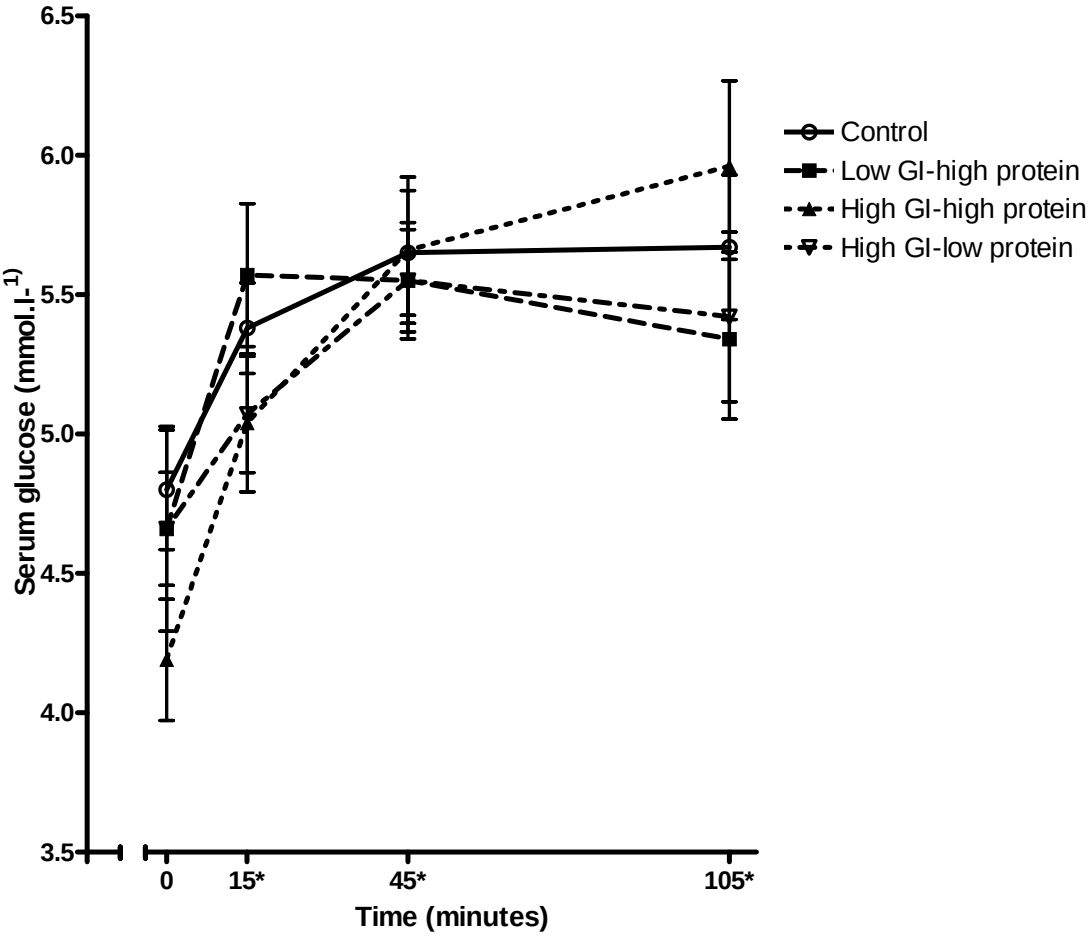

Figure 3.7. Serum glucose concentration (mmol.l^{-1}) throughout the soccer match simulation. *significantly different than time 0 (time main effect, $p < 0.05$).

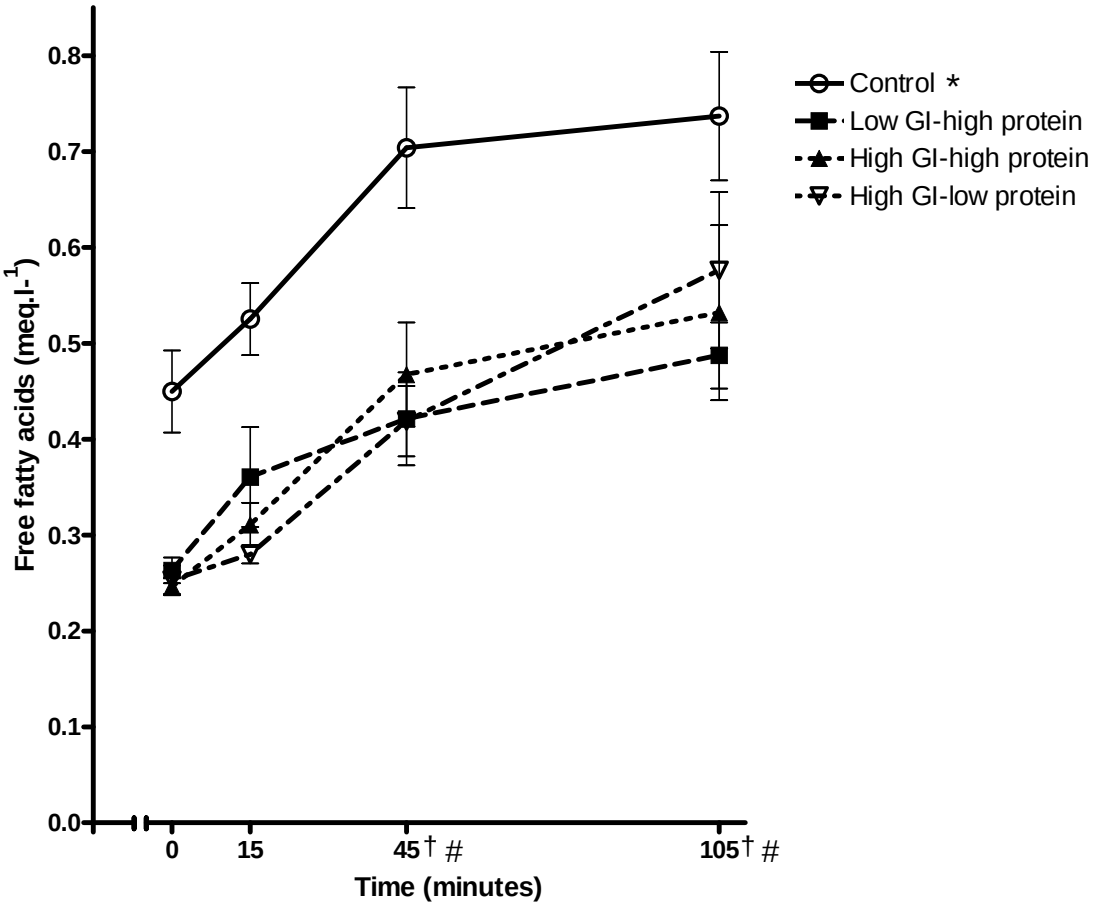

Figure 3.8. Serum free fatty acid concentration (FFA, meq.l^{-1}) throughout the soccer match simulation. * Control significantly greater than low GI-high protein, high GI-high protein, and high GI-low protein (main effect of meal condition, $p < 0.05$). † 45 and 105 minutes significantly greater than time 0 (time main effect, $p < 0.05$). # 45 and 105 minutes significantly greater than 15 minutes (time main effect, $p < 0.05$).

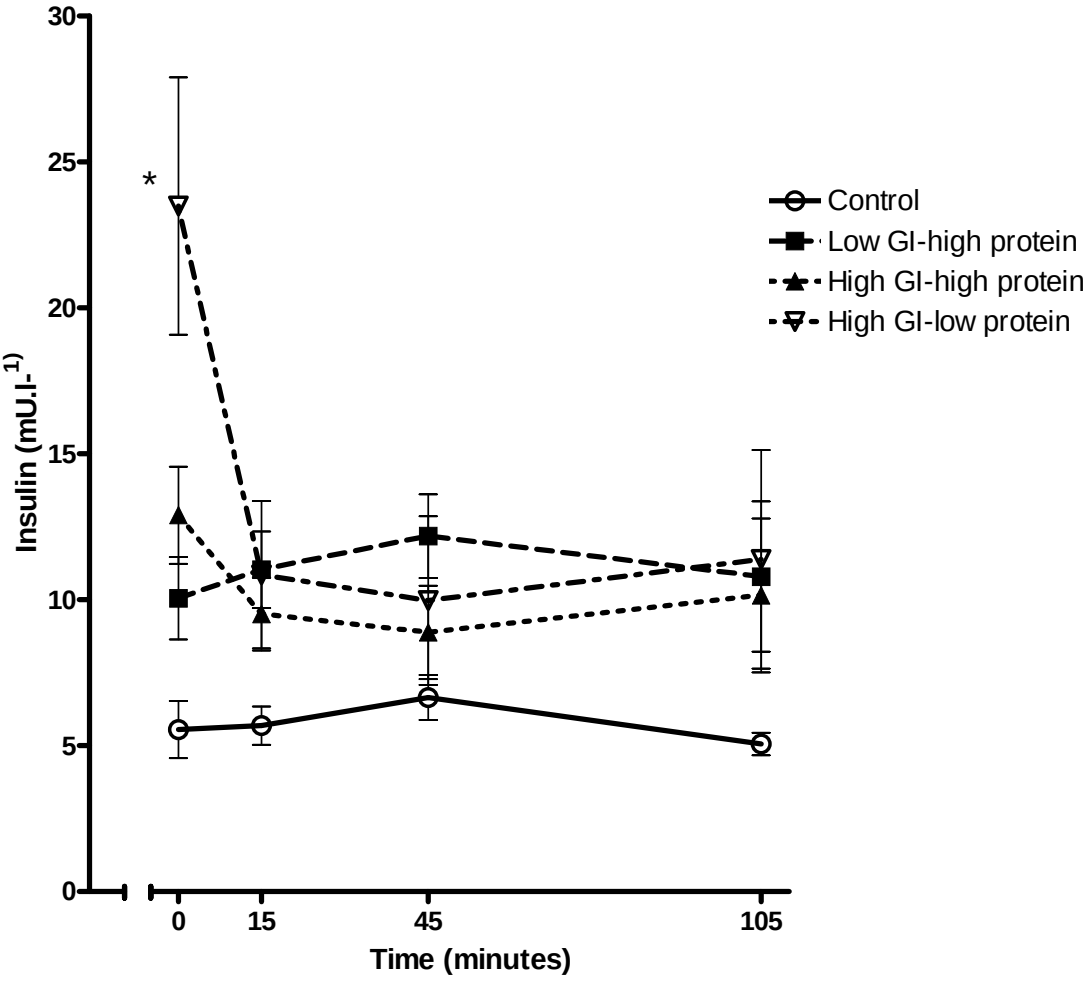

Figure 3.9. Serum insulin concentration ($mU.l^{-1}$) throughout the soccer match simulation. * High GI-low protein significantly greater than control, low GI-high protein, and high GI-high protein ($p < 0.01$).

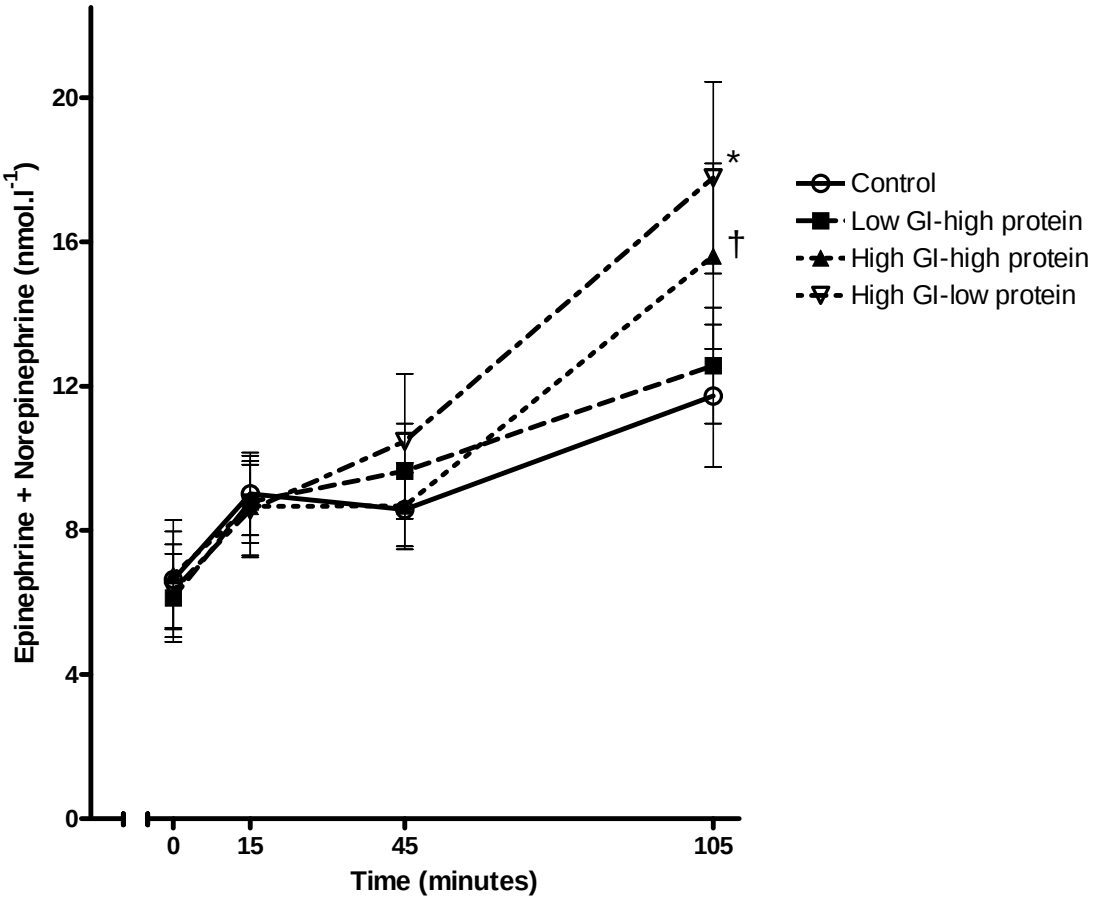

Figure 3.10. Serum epinephrine plus norepinephrine concentration (nmol.l^{-1}) throughout the soccer match simulation. * High GI-low protein significantly greater than low GI-high protein and control ($p < 0.001$). † High GI-high protein significantly greater than control ($p < 0.05$).

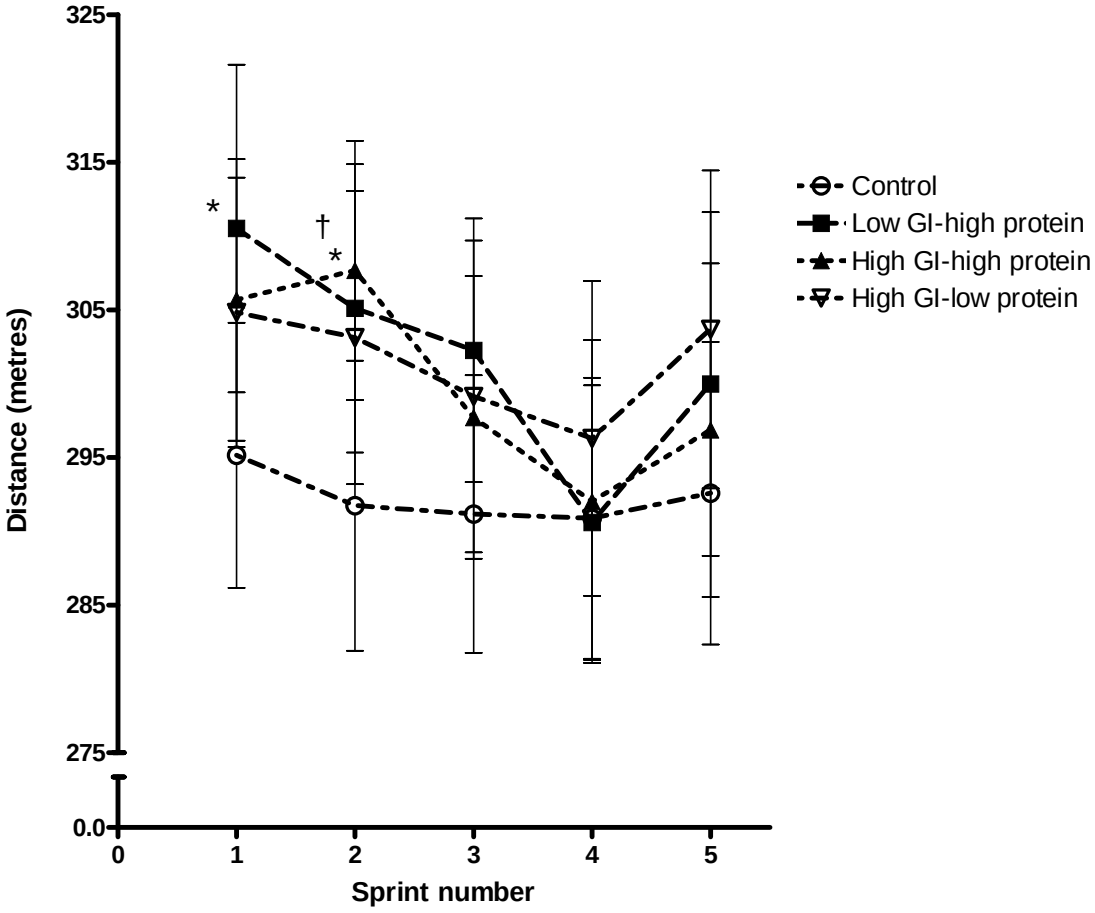

Figure 3.11. Distance covered (metres) during all five sprints of the repeated sprint test. * Low GI-high protein significantly greater than control ($p < 0.05$). † High GI-high protein significantly greater than control ($p < 0.05$).

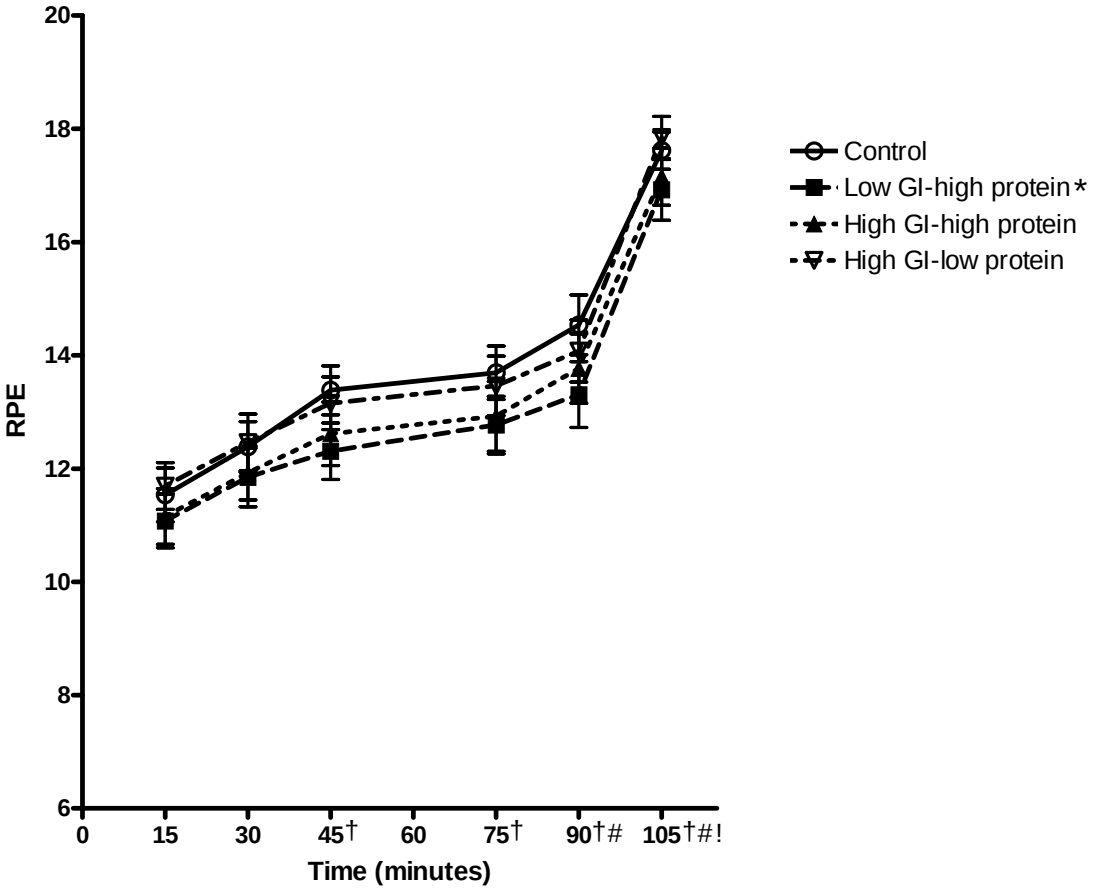

Figure 3.12. Ratings of perceived exertion (RPE) throughout the soccer match simulation. * Low GI-high protein significantly less than control and high GI-low protein (main effect of meal condition, $p < 0.05$). † significantly greater than 15 minutes (time main effect, $p < 0.05$). # significantly greater than 30 minutes (time main effect, $p < 0.05$). ! significantly greater than 45, 75, and 90 minutes (time main effect, $p < 0.05$).

Table 3.1. Oxygen uptake (VO_2, in $l.min^{-1}$) during collection periods 1, 2 and 3 in the control, low GI-high, high GI-high protein, and high GI-low protein conditions.

Meal Condition	Collection period 1 (3-10 minutes)	Collection period 2 (33-40 minutes)	Collection period 3 (63-70 minutes)*
Control	2.51 ± 0.23	2.53 ± 0.24	2.56 ± 0.25
Low GI-high protein	2.52 ± 0.19	2.52 ± 0.17	2.58 ± 0.19
High GI-high protein	2.53 ± 0.19	2.59 ± 0.23	2.62 ± 0.23
High GI-low protein	2.53 ± 0.26	2.56 ± 0.27	2.57 ± 0.26

Values are means ± SD. *Collection period 3 significantly greater than collection period 1 (time main effect, $p < 0.05$).

Table 3.2. Blood lactate concentration ($mmol.l^{-1}$) throughout the postprandial period (-140 to 0 minutes) and during the soccer match simulation (15, 45, 90, and 105 minutes) in the control, low GI-high protein, high GI-high protein, and high GI-low protein conditions.

Meal Condition	-140	-105	-90	-60	0	15	45	90	105*
Control	1.7 ± 0.5	1.4 ± 0.6	1.5 ± 0.6	1.5 ± 0.6	1.8 ± 0.5	2.6 ± 1.2	2.8 ± 1.4	2.5 ± 10	7.0 ± 3.1
Low GI-high protein	1.6 ± 0.6	1.7 ± 0.6	1.7 ± 0.5	1.6 ± 0.5	2.0 ± 0.5	2.5 ± 1.2	2.3 ± 0.8	2.7 ± 0.8	7.8 ± 4.3
High GI-high protein	1.5 ± 1.0	1.5 ± 0.5	1.9 ± 1.0	1.9 ± 0.9	1.4 ± 0.5	2.4 ± 0.9	2.3 ± 1.2	2.2 ± 0.7	6.5 ± 3.1
High GI-low protein	1.7 ± 0.7	1.6 ± 0.5	1.6 ± 0.5	1.7 ± 0.5	2.0 ± 1.2	2.5 ± 0.9	2.7 ± 1.8	2.9 ± 1.5	7.1 ± 3.1

Values are means ± SD. *Blood lactate concentration significantly greater at 105 minutes versus all other time points (time main effect, $p < 0.05$)

Table 3.3. Average heart rate (HR) throughout each 15-minute section of the soccer match simulation in the control, low GI-high protein, high GI-high protein, and high GI-low protein conditions. The 15-minute halftime occurred between minutes 46-60.

Meal Condition	0-15	16-30*	31-45*†	60-75*	76-90*†‡	91-105*
Control	140 ± 10	143 ± 9	145 ± 9	146 ± 10	148 ± 10	148 ± 9
Low GI-high protein	139 ± 11	144 ± 10	146 ± 10	143 ± 10	147 ± 10	150 ± 11
High GI-high protein	142 ± 9	145 ± 10	148 ± 10	147 ± 10	149 ± 9	150 ± 11
High GI-low protein	142 ± 11	144 ± 12	148 ± 12	145 ± 10	149 ± 12	152 ± 11

Values are means ± SD. *significantly greater than 0-15 minutes, † significantly greater than 16-30 minutes, ‡ significantly greater than 60-75 minutes (time main effect, $p < 0.05$).

3.2 Discussion

To our knowledge, this is the first study to compare the effects of low and high GI pre-exercise meals on metabolism and performance during prolonged, high-intensity intermittent exercise. With regards to exercise metabolism, the major finding was that a pre-exercise meal with a high GI and low protein content (i.e., high GI-low protein) resulted in increased reliance on carbohydrate oxidation and decreased reliance on fat oxidation compared to fasted control during soccer-specific intermittent exercise. With respect to exercise performance, the major finding was that the distance covered on the first two of five sprints performed during the last 15 minutes of a 90-minute soccer match simulation was significantly greater when a low GI-high protein pre-exercise meal was consumed compared with fasted control. Also, the distance covered on the second of five sprints was significantly greater following a high GI-high protein pre-exercise meal compared to the fasted condition. There were no significant differences during the repeated sprint test between low GI-high protein, high GI-high protein, and high GI-low protein meal conditions nor were there any differences between high GI-low protein and fasted control. Related to the performance results, overall ratings of perceived exertion (RPE) were lower in the low GI-high protein condition when compared to both the control and high GI-low protein conditions. The following discussion will address each of these principal findings in relation to previous research and with specific attention paid to potential physiological mechanisms.

3.2.1 Effects of the GI on metabolism during intermittent exercise

The greater rate of carbohydrate oxidation and decreased rate of fat oxidation in the high GI-low protein condition compared to fasted control is in partial agreement

with the first hypothesis, which stated that carbohydrate oxidation would be greater and fat oxidation would be lower following both high GI meals compared to the low GI-high protein meal or control condition. This hypothesis was based on previous demonstrations of increased carbohydrate oxidation and decreased fat oxidation during continuous, moderate intensity exercise following high compared to low GI meals (Febbraio et al., 2000; Stevenson et al., 2006; Thomas et al., 1991; Wee et al., 1999; Wee et al., 2005; Wu et al., 2003; Wu & Williams, 2006). High GI pre-exercise meals result in large increases in circulating insulin which may contribute to accelerated muscle glucose uptake and rebound hypoglycemia, decreased fat oxidation, and increased muscle glycogen utilization during the subsequent exercise period (Thomas et al., 1991; Wee et al., 2005).

3.2.1.1 Rebound hypoglycemia

This study did not demonstrate evidence of accelerated muscle glucose uptake at the onset of exercise following either high GI meal as rebound hypoglycemia was not present in any of the subjects. This is in contrast to many previous studies which demonstrate a fall in blood glucose at the onset of moderate intensity exercise following a high GI pre-exercise meal (Febbraio & Stewart, 1996; Febbraio et al., 2000; Stannard et al., 2000; Thomas et al., 1991; Wee et al., 2005; Wu et al., 2003). Most of these studies have provided the pre-exercise meal within the hour before exercise (Febbraio & Stewart, 1996; Febbraio et al., 2000; Stannard et al., 2000; Thomas et al., 1991). The longer postprandial period in the present study (two hours) could have provided more time for circulating insulin to return to basal levels following the high GI meals. This appeared to be the case in the high GI-high protein condition, as serum insulin at the

start of exercise was not different between high GI-high protein, low GI-high protein, or control. However, serum insulin was still significantly elevated two hours post-meal in the high GI-low protein condition. In spite of this, no evidence of rebound hypoglycemia was present at the onset of exercise in this condition. Therefore, the failure to notice evidence of rebound hypoglycemia was likely due to the differences in exercise intensity in this study compared with previous research. Previous research has investigated moderate intensity exercise and only a slight rise in circulating catecholamines would be expected at the onset of exercise (e.g., Schneider et al., 2000). Catecholamines play a role in glucoregulation during intense exercise by increasing hepatic glucose output and inhibiting pancreatic insulin secretion (Kreisman et al., 2003; Marliss & Vranic, 2002). The soccer-specific intermittent exercise protocol used in this study consisted of periods of low- (i.e., walking), moderate- (i.e., jogging), and high- (i.e., running and sprinting) intensity exercise and it would be the expected that the catecholamine response would be greater during this type of exercise compared to continuous moderate intensity exercise. Indeed, the average serum epinephrine plus norepinephrine concentration after 15 minutes of the soccer-specific exercise protocol (when rebound hypoglycemia would most likely be found) was ~8.8 $nmol.l^{-1}$. Differences in sample collection procedures and training status of participants aside, this is approximately 2.5 times higher than the total catecholamine concentration reported by others during moderate intensity exercise at the lactate threshold (Schneider et al., 2000). Therefore, increased secretion of catecholamines at the start of high-intensity intermittent exercise appeared to prevent the occurrence of rebound hypoglycemia in this study.

3.2.1.2 Effect of meals on substrate oxidation

Despite no evidence of rebound hypoglycemia at the onset of exercise, the high GI-low protein pre-exercise meal did increase the reliance on carbohydrate and decrease the reliance on fat oxidation during the exercise period when compared to fasted control. The potential reasons why this meal altered substrate oxidation during high-intensity intermittent exercise are most likely related to the elevated insulin response associated with consuming a large, high GI meal. The serum insulin concentration at the onset of exercise was significantly higher in the high GI-low protein condition when compared to all other conditions. This indicates that this meal resulted in the greatest metabolic perturbation in the postprandial period. Insulin has been shown to suppress adipose tissue lipolysis and reduce free fatty acid oxidation (Horowitz et al., 1997). The elevated insulin response following the high GI-low protein meal may have impaired fat oxidation either through decreased availability or utilization of circulating free fatty acids. As we did not see any differences in serum free fatty acid concentrations at any time point during exercise between high GI-low protein, high GI-high protein, or low GI-high protein meal conditions (see figure 3.8) it is likely that the residual effects of hyperinsulinemia decreased free fatty acid oxidation and not availability. Montain et al. (1991) have shown that free fatty acid oxidation during exercise is impaired for up to four hours after consumption of a carbohydrate load, presumably due to the substantial increase in circulating insulin. Furthermore, Horowitz et al. (1997) have demonstrated that the insulin induced reduction in lipid oxidation during exercise is still present even when an exogenous lipid preparation is infused to artificially elevate circulating free fatty acid concentration. The impairment in whole body fat oxidation is thought to occur at the level of long chain fatty acid transport, either at the sarcolemma or mitochondrial

membrane (Coyle et al., 1997; Siddossis et al., 1996). This impairment persists even when circulating insulin concentration declines throughout exercise (Horowitz et al., 1997; Montain et al., 1991). The increased rate of carbohydrate oxidation and decreased rate of fat oxidation during exercise following the high GI-low protein meal can therefore most likely be attributed to the increased insulin response and residual suppression of free fatty acid oxidation.

Although there was a tendency for carbohydrate oxidation to be higher and fat oxidation to be lower following the high GI-high protein meal compared to control, the carbohydrate and fat oxidation results were only significantly different between high GI-low protein and control (see figures 3.2 – 3.5). The reasons why differences in substrate oxidation were seen following the high GI-low protein but not the high GI-high protein meal are not entirely clear. The protein content of high GI-high protein could have altered the GI as addition of amino acids has been suggested to lower the GI of a carbohydrate source (Walton & Rhodes, 1997). This lowering of the GI by co-ingestion of amino acids is primarily mediated by the ability of amino acids to further elevate circulating insulin, resulting in improved glucose disposal (Spiller et al., 1987). The two-hour glucose area under the curve provides some evidence that the GI was slightly higher in the high GI-low protein compared to the high GI-high protein condition (191.9 vs. 160.6 $mmol.l^{-1}$.min in the high GI-low protein and high GI-high protein, respectively). The calculated GI using the mixed-meal method (Wolever & Jenkins, 1986) was not different between these conditions because the addition of egg whites, which contain a negligible amount of carbohydrate (1.77 grams per 250 millilitre serving), did not affect the calculations for the high GI-high protein meal. It should be

noted that the glycemic load of the high GI-low protein meal was higher as a result of the increased carbohydrate content. The high GI-low protein meal contained ~1.9 whereas the high GI-high protein meal contained 1.5 grams of carbohydrate per kilogram body mass, resulting in glycemic loads of ~99.7 and 79.8, respectively (based on test meals for a 70 kilogram subject). Ultimately, it is most likely that the increased carbohydrate content and glycemic load in the high GI-low protein condition resulted in a greater insulin response, which in turn had more pronounced and longer lasting effects on fatty acid oxidation. This explanation is supported by the significantly greater insulin concentration at the start of exercise in the high GI-low protein condition compared to the high GI-high protein condition.

Previous studies have reported differences in substrate oxidation during continuous, moderate intensity exercise between low and high GI pre-exercise meal conditions (Wee et al., 1999; Wee et al., 2005; Stevenson et al., 2006). The first hypothesis was not fully supported in that substrate oxidation during the soccer-specific intermittent exercise period was not different between the high GI conditions and the low GI-high protein condition. At the onset of exercise, the serum insulin concentration in the low GI-high protein condition was not significantly different from the high GI-high protein condition, although both were significantly lower that the high GI-low protein. However, it is very likely that the overall insulin response (e.g., insulin area under the curve) following both high GI meals would have been significantly greater than the insulin response following the low GI-high protein meal (Thomas et al., 1991). The overall insulin response in the postprandial period is probably more important for regulating carbohydrate and fat oxidation during exercise rather than the insulin

concentration at the start of exercise (Horowitz et al., 1997; Montain et al., 1991; Stevenson et al., 2006). Unfortunately, the overall insulin response could not be determined in the present study because of the failure to obtain blood samples in the postprandial period. The use of indwelling venous catheters was abandoned after experiencing difficulty keeping the catheters in place and patent during the postprandial and exercise periods. In spite of this limitation, several investigations using the same low GI and high GI meals as this study have shown that the overall insulin response after consumption of lentils is much lower than that of potatoes (Thomas et al., 1991; Thomas et al., 1994). Therefore, there is no reason to believe that the insulin responses following the high GI meals were not substantially greater than the insulin response following the low GI-high protein meal. As such, factors other than insulin must have contributed to the lack of a difference in carbohydrate and fat oxidation between low and high GI pre-exercise meals.

Although serum free fatty acid concentration was significantly higher in the fasted condition, there were no differences in serum free fatty acids between low and high GI conditions immediately prior to, or during, exercise. This is in contrast to many previous studies that have noted significantly higher levels of circulating free fatty acids in low GI compared to high GI conditions at the start and throughout exercise (Febbraio et al., 2000; Sparks et al., 1998; Stevenson et al., 2006; Wee et al., 2005). The lack of a difference in free fatty acids at the start of exercise might have been the result of inherent differences in the experimental protocol in this study compared to others. Most studies providing small meals within the hour before exercise notice higher free fatty acid levels at the start of exercise in the low GI compared to high GI conditions

(Febbraio et al., 2000; Sparks et al., 1998; Thomas et al., 1991; Thomas et al., 1994). Other studies providing meals containing 2.0 grams of carbohydrate per kilogram body mass three hours before exercise also have reported higher free fatty acid levels at the start of exercise in the low GI compared to high GI (Stevenson et al., 2006; Wee et al., 1999; Wee et al., 2005). Since meals containing 1.5 (low GI-high protein, high GI-high protein) and 1.9 (high GI-low protein) grams of carbohydrate per kilogram body mass were provided two hours before exercise in this study, it is difficult to make direct comparisons with previous research. It is interesting to note, however, that studies providing 2.0 grams of carbohydrate per kilogram body mass three hours before exercise do not report differences between conditions two hours after consumption of the meals (Stevenson et al., 2006; Wee et al., 2005; Wu & Williams, 2006). Therefore, we may have failed to detect a difference in serum free fatty acid concentration between low and high GI conditions prior to exercise as a result of the timing of the meal and blood sampling.

Previous research has also demonstrated increased availability of free fatty acids throughout exercise following low GI compared to high GI meals (Febbraio et al., 2000; Sparks et al., 1998; Stevenson et al., 2006; Wee et al., 2005; Wu & Williams, 2006). The lack of a difference in free fatty acids between low and high GI conditions throughout exercise in this study could also be due to the timing and size of meal or could be the result of the greater catecholamine response seen with high-intensity compared to moderate intensity exercise. Catecholamines act to increase adipose tissue lipolysis thereby increasing the availability of free fatty acids (Horowitz & Klein, 2000). Therefore, higher catecholamine levels in response to the soccer-specific intermittent

exercise in this study may have stimulated lipolysis to an extent that any residual effects of the high GI meals on adipose tissue were overridden.

Differences in metabolic regulation between high-intensity intermittent and continuous, moderate intensity exercise may be another reason why the substrate oxidation results differ from previous studies. Previous studies that show differences in carbohydrate and fat oxidation between low and high GI pre-exercise meals have required subjects to exercise at ~70% VO_2max (DeMarco et al., 1999; Febbraio & Stewart, 1996; Febbraio et al., 2000; Sparks et al., 1998; Thomas et al., 1991; Thomas et al., 1994). During continuous, moderate intensity exercise at ~70% VO_2max, muscle glycogen and blood glucose account for ~75% of the overall energy supply (Van Loon et al., 2001). Free fatty acids are also a significant source of energy during exercise at this intensity, contributing to ~20% of the total energy supply (Van Loon et al., 2001). Muscle glycogen stores decline as exercise progresses and blood glucose becomes an increasingly more important fuel source (Coggan & Coyle, 1991). Fatigue during this type of exercise is thought to occur when muscle glycogen and/or blood glucose levels are reduced to an extent that the required energy demands can no longer be met (Bergstrom et al., 1967; Hawley et al., 1994; Nybo, 2003). In contrast, muscle glycogen, and not blood glucose or free fatty acids, is the primary fuel source during high-intensity exercise (Saltin, 1973). The pronounced catecholamine response during high-intensity exercise acts to stimulate hepatic glucose release (Kreisman et al., 2000; Kreisman et al., 2001; Kreisman et al., 2003; Marliss & Vranic, 2002). An increase in circulating catecholamines also activates muscle glycogenolysis and may decrease muscle glucose uptake (Jansson et al., 1986). Consequently, hepatic glucose output tends to exceed

muscle glucose uptake during exercise above ~85% VO_2max and blood glucose concentration can actually rise during high-intensity exercise (Marliss & Vranic, 2002; McConnell et al., 2000). The time main effect for serum glucose in this study supports an increase in blood glucose during high-intensity intermittent exercise, as serum glucose concentration was significantly higher at all time points during exercise compared to rest (time 0). Intermittent bouts of high-intensity exercise, such as the activity pattern of soccer, result in substantial depletion of muscle glycogen but tend not to lead to hypoglycemia (MacDougall et al., 1977; Mohr et al., 2003; Mohr et al., 2005; Saltin, 1973). Fatigue during this type of exercise is therefore primarily the result of depletion of muscle glycogen and not a decrease in blood glucose (Leatt & Jacobs, 1989; Mohr et al., 2003; Mohr et al., 2005; Saltin, 1973). Indeed, our results show that after 90 minutes of intermittent exercise in the fasted condition blood glucose concentration was still above 5 $mmol.l^{-1}$. The greater catecholamine response to high-intensity exercise also stimulates adipose tissue lipolysis to mobilize free fatty acids (Horowitz & Klein, 2000). Therefore, the potential for low and high GI pre-exercise meals to influence blood glucose and free fatty acid concentration may not be as important during high-intensity intermittent exercise when compared to moderate intensity exercise.

In summary, the size and timing of the meal, the greater catecholamine response, as well as fundamental differences in metabolic regulation between moderate intensity and high-intensity intermittent exercise may be why, in contrast to previous studies, substrate oxidation was not significantly different between low and high GI conditions in this study. Although the differences between low and high GI meal conditions were

not statistically significant, it should be noted that the general trend was for fat oxidation to be higher and carbohydrate oxidation to be lower in the low GI-high protein compared to the high GI conditions (see figures 3.2 – 3.5).

3.2.1.3 Effect of meals on circulating catecholamines

An interesting finding with regards to both high GI meal conditions was the significant meal × time interaction for the catecholamine response (epinephrine plus norepinephrine). At the end of exercise, catecholamine levels were significantly higher in the high GI-low protein condition compared to low GI-high protein and control and were significantly higher in the high GI-high protein compared to control. This was in contrast to the fifth hypothesis which stated that serum catecholamine levels would be greater in the fasted control condition compared to all other conditions. Catecholamines act peripherally to mobilize fuel sources and locally on skeletal muscle to increase both carbohydrate and fat metabolism. Catecholamines activate glycogen phosphorylase to increase muscle glycogenolysis (Jansson et al., 1986) and stimulate hormone-sensitive lipase to increase intramuscular triglyceride lipolysis (Talanian et al., 2006). As carbohydrate would have been the primary fuel during the high-intensity repeated sprint test, the higher catecholamine response in the high GI conditions may indicate that a greater stimulation of glycogen phosphorylase was needed to activate muscle glycogenolysis. This could have been the result of decreased availability of muscle glycogen due to increased reliance on this fuel during the preceeding exercise period. Wee et al. (2005) have previously reported increased reliance on muscle glycogen during moderate intensity running following high compared to low GI pre-exercise meals. It has been suggested that when muscle glycogen concentration is lowered to a

critical level, a feedback loop is in place to increase norepinephrine secretion in an effort to mobilize fuel sources (Weltan et al., 1998). Therefore, the finding of an increased catecholamine response in both high GI conditions could have been the result of lower muscle glycogen levels triggering this feedback loop. Also, it has been reported that blood lactate concentration tends to rise when circulating catecholamine levels increase (Schneider et al., 2000) and this is thought to be the result of increased stimulation of non-oxidative glycogen utilization. Therefore, if ample muscle glycogen were available, a rise in blood lactate might have been expected to accompany the sharp increase in catecholamines during the repeated sprint test in the high GI conditions. The lack of a difference in blood lactate concentration after the repeated sprint test despite the increase in catecholamine levels provides some associative evidence that muscle glycogen may have been lower at the end of exercise in the high GI conditions. However, if decreased muscle glycogen contributed to the increased catecholamine levels in the high GI conditions, it would be expected that catecholamine levels would have also been higher in the fasted condition because low levels of muscle glycogen during exercise would most likely occur in this condition. Previous research has reported higher circulating catecholamine levels at the end of exhaustive endurance exercise in the fasted compared to carbohydrate-fed state (De Bock et al., 2007). As such, it is presently unclear why catecholamine levels were not similarly elevated at the end of exercise in the fasted control condition. Free fatty acid availability was significantly greater in the control condition and this may have affected the muscle glycogen-catecholamine feedback loop (Weltan et al., 1998). Further research is required to

substantiate the findings of the increased catecholamine concentration at the end of prolonged, high-intensity intermittent exercise following high GI pre-exercise meals.

3.2.2 Effects of the GI on intermittent exercise performance

It is commonly believed that increasing fat utilization and sparing carbohydrate is associated with increased endurance capacity (Graham, 2000; Lambert et al., 1997; Pitsiladis et al., 1999; Williams & Serratosa, 2006). Therefore, a pre-exercise meal that increases the reliance on carbohydrate and decreases the reliance on fat during exercise may be detrimental to exercise performance (Foster et al., 1979; Thomas et al., 1991). Since high GI meals can increase carbohydrate oxidation and decrease fat oxidation during exercise (Stevenson et al., 2006; Wee et al., 1999; Wee et al., 2005), low GI pre-exercise meals have been suggested to be optimal for endurance performance (Burke et al., 1998a; Siu & Wong, 2004; Thomas et al., 1991). Previous research examining the effects of low and high GI pre-exercise meals on exercise performance has focused on prolonged, continuous exercise. The results of these studies are mixed, with most showing no effect of the GI on performance (Burke et al., 1998b; Febbraio & Stewart, 1996; Febbraio et al., 2000; Sparks et al., 1998; Stannard et al., 2000; Thomas et al., 1994; Wee et al., 1999) and a few showing increased endurance capacity following low GI compared to high GI meals (DeMarco et al., 1999; Kirwan et al., 2001; Thomas et al., 1991; Wu & Williams, 2006). This study is the first to our knowledge that examines the effects of low and high GI pre-exercise meals on prolonged, high-intensity intermittent exercise. Intermittent exercise performance was assessed with a repeated sprint test consisting of five 1-minute sprints with 2.5 minutes recovery during the last 15 minutes of a 90-minute treadmill soccer match simulation. This test was chosen

because lower muscle glycogen stores have been associated with decreased distance covered and decreased time spent sprinting during the last 15 minutes of competitive soccer matches (Saltin, 1973). The last 15 minutes of soccer games also appear to be critical to the outcome, as a disproportionate number of goals are scored in this period (Reilly, 2000). Therefore, if a low GI pre-exercise meal resulted in increased reliance on fat and decreased reliance on carbohydrate during soccer-specific intermittent exercise, muscle glycogen stores during the late stages of the game could be preserved and the distance covered on repeated sprints may be greater. If a player could cover more distance at a high intensity during this period of the game, it is reasonable to assume that he/she could have more of an impact on the outcome (Reilly, 2000). This was the basis for the second hypothesis, which stated that repeated sprint performance would be improved in the low GI-high protein compared to the high GI conditions. This hypothesis was not supported by the results from the repeated sprint test. The distance covered in sprints one and two was significantly greater in the low GI-high protein condition compared to fasted control but there were no differences between low GI-high protein, high GI-high protein, and high GI-low protein conditions. The third hypothesis stated that performance would be improved in the low GI-high protein, high GI-high protein, and high GI-low protein when compared to control. This was partially supported by the results from the repeated sprint test. Along with the improvements in performance in sprints one and two in the low GI-high protein condition versus control, the distance covered in sprint two was greater in the high GI-high protein condition compared to control. Taken together, these results could be interpreted to suggest a potential performance advantage during high-intensity intermittent exercise following a

low GI-high protein pre-exercise meal. Repeated sprint performance was improved on two of five sprints in the low GI-high protein condition versus control but on only one of five sprints in the high GI-high protein condition versus control and there were no differences in repeated sprint performance in high GI-low protein condition versus control.

The majority of studies that have examined the effect of the GI on exercise performance have utilized performance tests that range from 15 to ~90 minutes (Burke et al., 1998b; DeMarco et al., 1999; Febbraio & Stewart, 1996; Febbraio et al., 2000; Kirwan et al., 2001; Sparks et al., 1998; Stannard et al., 2000; Thomas et al., 1991; Thomas et al., 1994; Wee et al., 1999; Wu & Williams, 2006). These performance tests would rely primarily on the capacity of the aerobic system to resynthesize ATP to fuel muscle contraction. The performance test in the present study involved five repeated one-minute sprints in which subjects were instructed to work at the maximum possible intensity. Therefore, a significant portion of the energy would have come from non-oxidative metabolism. Thus, it is difficult to make direct comparisons between the performance results from this study and most of the previous investigations. However, the study of DeMarco et al. (1999) provides some basis for comparison. In their study, subjects cycled at ~70% VO_2max for 120 minutes and then rode to exhaustion at 100% VO_2max. They found that cycling time to exhaustion was increased by 59% in the low GI compared to high GI condition. The exercise intensity of their performance test was similar to the intensity of the sprints during the repeated sprint test of this study (~120% VO_2max, based on the speeds achieved during the sprints). Therefore, our results lend some support to those of DeMarco et al. (1999) in that high-intensity exercise performed

after an extended period of exercise was improved following low GI pre-exercise meals. However, due to the differences in exercise mode, duration, intensity, and the nature of the performance test, further research is needed to confirm whether low GI meals provide performance benefits for high-intensity exercise performed at the end of a prolonged exercise trial.

The physiological basis for the performance improvement in the first two sprints in the low GI-high protein condition versus control cannot be ascertained from the present study. One explanation is that muscle glycogen content at the start of the repeated sprint test was greater in the low GI-high protein condition compared to the fasted control. Increased muscle glycogen stores have been associated with increased muscle power and repeated high-intensity exercise (Balsom et al., 1999; Maughan, 1988). Muscle glycogen stores could be greater in the low GI-high protein condition compared to control because of increased exogenous carbohydrate availability following ongoing digestion and absorption of the meal throughout exercise and the rest periods (i.e., standing periods and halftime). The steady release of glucose from the low GI-high protein meal may have acted to spare endogenous carbohydrate stores (DeMarco et al., 1999). Although carbohydrate provision in the high GI-high protein condition was matched and carbohydrate provision was greater in the high GI-low protein condition when compared to the low GI-high protein condition the pattern of exogenous carbohydrate availability would have been much different between high and low GI conditions. The ongoing release of glucose from the gastrointestinal tract into circulation during exercise in the low GI-high protein condition may have contributed exogenous carbohydrate to the blood glucose pool, similar to the effects of ingesting carbohydrates

during exercise. Whether increasing the availability of exogenous carbohydrate can spare muscle glycogen is a topic of some debate, although there is evidence that muscle glycogen utilization is decreased in type I muscle fibres during running exercise when exogenous glucose is ingested (Tsintzas & Williams, 1998). Whether ongoing release of glucose from a low GI pre-exercise meal can spare muscle glycogen during exercise could not be determined in the present study as this would require multiple muscle biopsies and/or stable isotope glucose tracer techniques. In addition to muscle glycogen sparing, the ongoing digestion and absorption of glucose following the low GI-high protein meal could have supported muscle glycogen resynthesis in inactive muscle fibres during the low intensity and rest periods thereby increasing muscle glycogen availability for the late stages of the match. Previous studies have suggested that consuming carbohydrate drinks during intermittent exercise can contribute to glycogen resynthesis in inactive muscle fibres during low intensity and rest periods (Kuipers et al., 1987; Tsintzas & Williams, 1998). It would be interesting to investigate whether exogenous glucose from a low GI pre-exercise meal can be incorporated into skeletal muscle glycogen during intermittent exercise. Incorporation of stable isotope glucose tracers into the test meals would be required to determine whether this type of resynthesis was taking place during the low intensity and rest periods. The influence of ingested carbohydrate to spare muscle glycogen or increase muscle glycogen resynthesis during low intensity and rest periods may largely depend on whether circulating insulin concentration is elevated as a result of the carbohydrate consumption (Tsintzas & Williams, 1998; Yaskapelkis et al., 1993). We did not see any significant differences between conditions in serum insulin concentration throughout exercise, although there

was a trend for the absolute value to be higher in the low GI-high protein condition at halftime (45 minutes) compared to the other conditions (12.18, 6.66, 8.88, 9.98 $mU.l^{-1}$ in low GI-high protein, control, high GI-high protein, high GI-low protein, respectively). Therefore, it is possible that muscle glycogen was spared and/or resynthesized during rest periods in the low GI-high protein condition as a result of the slightly higher insulin concentration at 45 minutes into exercise. In summary, the improved performance on the first two sprints in the low GI-high protein compared to the fasted control condition may have been related to increased muscle glycogen availability. The ongoing digestion and absorption of glucose following the low GI-high protein meal may have acted to spare muscle glycogen or promote muscle glycogen resynthesis in the low intensity and rest periods.

The finding that performance was only improved on the first two sprints in the low GI-high protein condition versus control cannot be readily explained. Muscle glycogen content may have been lowered to a critically low level after two sprints in the low GI-high protein condition, with muscle glycogen content at or below this critically low level in all conditions for sprints three to five. Evidence from a number of studies using nuclear magnetic resonance imaging support the notion that critically low levels of muscle glycogen can limit muscle contractile performance (Shulman & Rothman, 2001). These studies show that high energy turnover, such as would be required during sprinting, cannot be supported when skeletal muscle glycogen stores drop below a threshold level (Shulman & Rothman, 2001). The critically low value is currently unknown and it is unlikely that it would be conserved across different muscle groups and fibre types or between individuals (Rauch et al., 2005). Due to the mixed fibre type

composition of human skeletal muscle and the number of muscle groups involved in sprint running, it would be very difficult to confirm whether a critically low level of muscle glycogen was limiting performance in this type of exercise. Nevertheless, it is possible that increased muscle glycogen availability contributed to improved performance in sprints 1 and 2 in the low GI-high protein condition, but after sprint 2, muscle glycogen had reached a critically low level such that it was similarly limiting sprint performance in all conditions. This is supported by the finding that the distance covered in sprints 3 to 5 was not different between conditions.

The increased performance in sprint two in the high GI-high protein condition compared to control is somewhat puzzling. This study employed two high GI conditions in an attempt to address whether the protein content of a pre-exercise meal could influence metabolism or performance. A few recent studies have reported improved exercise time to exhaustion when protein is added to a carbohydrate drink that is consumed during exercise (Ivy et al., 2003; Saunders et al., 2004) although the effects of adding protein to a pre-exercise meal has received little attention. The findings of Ivy et al. (2003) and Saunders et al. (2004) are somewhat confounded because the energy content of the carbohydrate-protein beverages was greater than the carbohydrate-only beverages. More recent results have shown that if the energy content is matched between conditions, the combination of carbohydrate and protein is not superior to carbohydrate alone (Romano-Ely et al., 2006; Van Essen & Gibala, 2006). The design utilized in the present study therefore employed two high GI meals that were matched for energy content, one with lower carbohydrate and higher protein (high GI-high protein) and the other with higher carbohydrate and lower protein (high GI-low protein).

The results of the repeated sprint test show that the distance covered in sprint two was significantly greater in the high GI-high protein compared to control but there were no differences between high GI-low protein and control. This indirectly suggests a performance advantage for the high GI pre-exercise meal with the higher protein content which is somewhat in contrast to the fourth hypothesis which stated that there would be no difference in performance between these conditions. The reasons for this are unclear, but may be related to alterations in metabolism following the high GI-low protein condition. The increased reliance on carbohydrate oxidation and decreased reliance on fat oxidation during exercise following the high GI-low protein meal may have placed greater demands on muscle glycogen, thus decreasing the availability of this important energy source in the later stages of exercise. Alternatively, the extra protein in the high GI-high protein may have provided benefits. Addition of amino acids to a carbohydrate beverage that is consumed during endurance exercise has been associated with decreased markers of muscle damage (Romano-Ely et al., 2006; Saunders et al., 2004). Exercise-induced muscle damage has been shown to impair intermittent sprint performance (Twist & Eston, 2005) so it is possible that performance was improved in the high GI-high protein condition because greater amino acid availability reduced muscle damage. It was not the aim of this study to assess muscle damage and therefore this explanation is largely speculative.

3.2.3 Effect of the GI on ratings of perceived exertion

The third novel finding of this study was that ratings of perceived exertion, using the Borg RPE scale (Borg, 1975), were lower in the low GI-high protein condition compared to the control and the high GI-low protein condition. Previous authors have

reported that RPE is lower when carbohydrate availability is increased (Sherman et al. 1991) and therefore the lower RPE in the low GI-high protein condition may have been related to increased muscle glycogen availability. Many previous studies examining the effect of the GI on exercise performance have not reported RPE results (Febbraio et al., 2000; Stannard et al., 2000; Wee et al., 1999; Wee et al., 2005) and all but one study that does report RPE has found no differences between low and high GI conditions (Burke et al., 1998b; Stevenson et al., 2006; Wu & Williams, 2006). DeMarco et al. (1999) reported lower RPE throughout 120 minutes of cycling at 70% VO_2max following a low GI compared to a high GI pre-exercise meal. As this study was also the only study to report an improvement in high-intensity exercise performance in the low GI condition (see discussion above in section 3.2.2) this provides evidence that performance improvements following low GI pre-exercise meals are possibly linked to feelings of perceived exertion throughout exercise.

The role the central nervous system plays in muscular fatigue has become increasingly clear in recent years (Noakes, 2001; Nybo, 2003). Some authors have even suggested a feedback mechanism that links glycogen content in skeletal muscle to the brain (Rauch et al., 2005). Although associative, using RPE to gauge subjective feelings of exertion provides some insight into these central fatigue processes (Edwards et al., 2007). A lower perception of effort in the low GI-high protein condition could have allowed subjects to increase motor output to a greater extent at the beginning of the repeated sprint test, thus increasing motor unit activation. Increased motor unit activation could have increased muscle power and improved sprint performance. A measure of motor unit activation, such as electromyography, and/or a measure of

voluntary activation, such as the interpolated twitch technique, would have provided support for this explanation but unfortunately were not a part of the experimental protocol. RPE is not designed to directly assess central fatigue and therefore this interpretation should be viewed with caution. The magnitude of the difference in overall RPE was quite small (0.8-0.9 RPE units lower in low GI-high protein compared to control and high GI-low protein) and may not have been substantial enough to elicit changes in higher order brain processing. Nevertheless, the lower RPE in the low GI-high protein compared to control or high GI-low protein conditions provide some evidence that a low GI pre-exercise meal is more beneficial for reducing feelings of physical exertion throughout exercise.

3.2.4 Strengths and Limitations

This study was unique in that it was the first to our knowledge to examine the effects of low and high GI pre-exercise meals on metabolism and performance during prolonged, high-intensity intermittent exercise. Previous studies in this area have examined continuous, moderate intensity exercise. The results of these studies may not be relevant to team sport athletes who are required to perform intermittent activity for extended periods of time. In addition, most studies have provided pre-exercise meals within the hour before exercise (DeMarco et al., 1999; Febbraio & Stewart, 1996; Febbraio et al., 2000; Kirwan et al., 2001; Sparks, 1998; Stannard, 2000; Thomas et al., 1991; Thomas et al., 1994) which may not be practical for athletes due to the potential for gastrointestinal distress. Therefore, pre-exercise meals were provided two hours before a soccer-specific intermittent exercise protocol to more closely represent how an athlete prepares for competition. Pilot work demonstrated that blood glucose

concentration had returned to baseline within two hours after consuming a high GI meal and it was noted that subjects reported no significant gastrointestinal distress two hours after consuming both low and high GI meals (see Appendix VII for blood glucose and gastrointestinal symptoms data from the pilot study). Therefore, two hours before exercise was chosen as the ideal time to consume the pre-exercise meals. Ingesting a pre-competition meal at this time point is more realistic and practical than consuming a small meal 30 to 60 minutes before starting to exercise and makes the results of this study more applicable to athletes.

A motorized treadmill soccer match simulation was used in this study to mimic the demands of a competitive soccer match. This approach could be considered both a strength and a limitation of this study. Using a treadmill allowed for accurate determination of speeds and the time spent at each speed during the soccer match simulation. This ensured that exercise intensity and total work done during the soccer match simulation was the same across the four meal conditions and enabled valid interpretation of expired gas, RPE, and blood data. However, the treadmill did not allow for rapid accelerations and decelerations, changes in direction, or backwards and sideways running that are characteristics of the actual activity pattern in soccer games. Many studies that examine soccer-specific exercise utilize shuttle running protocols where subjects must run back and forth between cones set 20 metres apart in response to audible beeps (e.g., Edwards et al., 2007; Nicholas et al., 1995; Nicholas et al., 1999). In these protocols, the exercise intensity usually alternates between low intensity jogging (55% Vmax) and high intensity running (95% Vmax) and the subject must gauge their speed accordingly to get from one cone to the other. Although this exercise protocol

requires the subject to stop and start quickly and change direction, it is difficult to ensure that the exercise intensity is the same between trials done on separate days. It is quite possible that subjects could run faster, especially during the low intensity periods, and arrive at the cone before the beep. Because of this, the treadmill protocol was chosen in the present study to ensure that exercise intensity would be identical between trials and therefore provide accurate metabolic data. However, despite accurately portraying the speeds and proportion of time spent during walking, jogging, running, and sprinting activities, the treadmill soccer match simulation lacked many activities and movement patterns that would occur during an actual soccer game including sideways and backwards running, changes of direction, jumping, tackling, and kicking. In addition, playmaking and tactical decisions did not have to be made during the simulated match. As such, direct application of this study to the sport of soccer may be limited.

The lack of a direct measurement of muscle glycogen is a limitation in this study. Not having a measurement of this important energy substrate preempted much of the explanation of results to be theoretical and speculative in nature. The original study design included a percutaneous muscle biopsy sample immediately prior to the repeated sprint test in order for muscle glycogen availability to be determined in the *vastus lateralis* muscle. However, ethical and practical limitations prevented the collection of this biopsy in the exercise physiology laboratory. It is the author's intention to continue to recruit additional subjects to complete the same experimental protocol with the inclusion of a muscle biopsy in order to assess muscle glycogen content. This procedure is currently being performed at Saskatoon City Hospital by a medical doctor and data collection is expected to be completed in the fall of 2007.

The failure to obtain blood samples during the postprandial period also presents a potential limitation. The original aim was to include blood sampling from an indwelling catheter in an anterior forearm or dorsal hand vein throughout the postprandial period and during exercise. This technique was abandoned because of difficulty keeping the catheter patent and the propensity of the catheter to become dislodged during high-intensity running exercise. Cycling or moderate intensity running are usually the modes of exercise in studies using indwelling venous catheters for blood sampling. These activities do not involve the same high speed arm movements that occur during the sprinting portions of the soccer match simulation. Having blood samples from the postprandial period and at more time points during exercise would have allowed for more accurate determination of the metabolic response to each meal and possibly shed more light as to why substrate oxidation was affected in the high GI-low protein condition only.

In order to estimate carbohydrate and fat oxidation, published equations based on indirect calorimetry principles were used (Jeuekendrup & Wallis, 2005). These equations give valid representations of whole body carbohydrate and fat oxidation during exercise below the ventilatory threshold, when CO_2 given off at the lungs reflects CO_2 released from the exercising tissues (Jeukendrup & Wallis, 2005). Research shows that these equations may be valid above the ventilatory threshold but only during exercise up to an intensity of approximately 85% VO_2max (Romijn et al., 1992). Exercise intensity during the running and sprinting portions of the soccer match simulation were approximately 90 and 120% VO_2max, respectively. Expired CO_2 probably overestimated CO_2 produced from oxidative metabolism, thereby violating one

of the assumptions of using these equations (Jeukendrup & Wallis, 2005). However, it has been argued that longer gas collection periods during intermittent exercise will give a valid representation of CO_2 flux because the body will undergo periods of relative hypoventilation during the low intensity and rest periods in an attempt to retain CO_2 and maintain a steady bicarbonate pool (Bangsbo et al., 1992). Previous research has reported expected results using expired gas data to estimate carbohydrate and fat oxidation following dietary carbohydrate manipulations prior to soccer-specific intermittent exercise (Bangsbo et al., 1992). Bangsbo et al. (1992) used multiple five-minute gas collection periods during a 45-minute field based soccer test and reported increased carbohydrate oxidation and decreased fat oxidation following a three day high-carbohydrate diet compared to a three day low-carbohydrate diet, as would be expected. Therefore, the three 7-minute gas collection periods used in this study, coupled with the precise control of exercise intensity between trials, should have produced valid results.

4. SUMMARY AND CONCLUSIONS

4.1 Summary

This study examined the effect of low and high GI pre-exercise meals on metabolism and performance during soccer-specific intermittent exercise. Low GI-high protein, high GI-high protein, and high GI-low protein meals were provided two hours before a 90-minute treadmill soccer match simulation. A fasted control condition was also employed. Blood and expired gas samples were collected in order to assess markers of carbohydrate and fat metabolism. Intermittent exercise performance was assessed by the distance covered on a repeated sprint test during the last 15 minutes of the match.

The major findings were that a high GI-low protein meal containing 1.9 grams of carbohydrate per kilogram body mass resulted in a greater postprandial glycemic response and significantly higher insulin levels at the start of exercise compared to all other conditions. The high GI-low protein condition also resulted in significantly greater carbohydrate oxidation and significantly lower fat oxidation during exercise compared to fasted control. There were no differences in circulating insulin concentration at the onset of exercise nor were there any differences in carbohydrate or fat oxidation during exercise following low GI-high protein or high GI-high protein meals containing 1.5 grams of carbohydrate per kilogram body mass.

Performance on five repeated one-minute sprints during the last 15 minutes of the 90-minute soccer match simulation was not different following the high GI-low

protein meal compared to the fasted state whereas there were slight improvements in performance in the low GI-high protein and high GI-high protein conditions compared to fasting. The distance covered on the first two sprints was significantly greater in the low GI-high protein condition compared to fasted control and the distance covered on sprint two was significantly greater in the high GI-high protein compared to fasted control.

Ratings of perceived exertion throughout exercise were significantly lower in the low GI-high protein condition compared to the high GI-low protein and fasted conditions.

4.2 Conclusions

A large, high GI-low protein meal consumed two hours before soccer-specific intermittent exercise increased the reliance on carbohydrate and decreased the reliance on fat oxidation during exercise compared to fasted control. Performance on a repeated sprint test during the last 15 minutes of a 90-minute soccer match simulation was not improved in the high GI-low protein condition whereas small improvements in sprint performance were seen following low GI-high protein and high GI-high protein meals compared to fasted control. This suggests that the alteration in substrate oxidation following the high GI-low protein meal negated any performance benefits of carbohydrate consumption, possibly due to increased reliance on muscle glycogen during the exercise period prior to the repeated sprint test. Ratings of perceived exertion throughout exercise were lower in the low GI-high protein condition compared to high GI-low protein and fasted control, indicating that a low GI-high protein pre-exercise meal may provide a beneficial effect through reduced perception of effort.

4.3 Recommendations for Future Research

It could not be determined in the present study whether the alteration in substrate oxidation following the high GI-low protein meal increased reliance on muscle glycogen. Future research should examine the effects of low and high GI pre-exercise meals with different carbohydrate and protein contents on muscle glycogen metabolism during intermittent exercise. It could also not be determined whether the ongoing digestion and absorption of the low GI meal throughout exercise contributed to the blood glucose pool and influenced muscle glycogen utilization and/or promoted muscle glycogen synthesis during the low-intensity and rest periods. Incorporation of stable isotope labeled glucose tracers into pre-exercise meals would be required to test this hypothesis.

The majority of research on pre-competition nutrition has focused on manipulating carbohydrate and fat content (Graham, 2000; Hargreaves et al., 2004) and little is known regarding the addition of protein to a pre-exercise meal. In light of our findings of slight improvements in performance in both meal conditions that contained considerable amounts of protein, future research is required to determine whether the addition of protein to a carbohydrate-rich pre-exercise meal is beneficial for improving exercise performance.

As is the case with most studies examining the effects of pre-exercise meals on metabolism and performance, subjects in this study consumed the test meals in the morning after an overnight fast. Many competitions occur in the evening, and the pre-competition meal would not be consumed in the overnight fasted state. Studies that examine the effects of pre-competition meals that are consumed in the late afternoon or

evening (i.e., when subjects are not fasted) are required to increase the applicability of research in this area.

Carbohydrate-containing sports drinks have been reported to improve soccer-related running performance (e.g., Kirkendall et al., 1988; Nicholas et al., 1995; Nicholas et al., 1999) and soccer nutrition guidelines advise consuming carbohydrate drinks during games (Williams & Serratosa, 2006). Carbohydrate drinks were not provided to subjects in this study in order to isolate the effects of the pre-exercise meals. Future research should examine the influence on low and high GI pre-exercise meals when carbohydrate drinks are provided during exercise.

The potential for high GI carbohydrate sources to inhibit fat oxidation during exercise may have important health implications. Maximizing fat oxidation and promoting a negative lipid balance appears to be a critical factor for individuals attempting to decrease body fat through exercise (Bennard et al., 2005). Although fat oxidation is maximized by exercising in the fasted state, exercising in this condition may be uncomfortable and impractical. Exercise may feel easier and intensity may be greater if carbohydrates are consumed prior to exercise. The results of this study and others (e.g., Stevenson et al., 2006; Thomas et al., 1991) suggest that consuming high GI carbohydrate sources impairs fat oxidation during exercise. Consuming low GI carbohydrates prior to exercise may be the ideal situation to maximize exercise intensity and fat oxidation.

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Appendix I – Certificate of Research Ethics Board Approval

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB) 13-Nov-2006

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Philip D. Chilibeck	Kinesiology	05-198

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT

College of Kinesiology
105 Gymnasium Place
Saskatoon SK S7N 5C2

SUB-INVESTIGATOR(S)

Gordon A. Zello, Albert A. Vandenberg, Maureen G. Reed, Huw Rees, Heather Sirounis

STUDENT RESEARCHER(S) Jonathan Little, Dawn Ciona

SPONSORING AGENCIES

SASKATCHEWAN PULSE GROWERS

TITLE

The Effects Of High And Low Glycemic Index Meals On Soccer Performance

ORIGINAL APPROVAL DATE	STUDY APPROVAL EXPIRY
16-Dec-2005	30-Nov-2007

APPROVAL OF — APPROVED ON

- Addition of M.D. for collection of muscle biopsies (Dr. Heather Sirounis) — 13-Nov-2006
- Addition of a student researcher (Dawn Ciona)
- Change in the title of the consent form.
- Change in one of the meal conditions
- Change in the timing of the meals given before the exercise test.
- Elimination of the test of motor unit recruitment
- Research Participation Information and Consent Form Version 4 (02-Nov-2006)
- Addition of questionnaire about how subjects feel after consuming their meals and about general dietary practices

CERTIFICATION

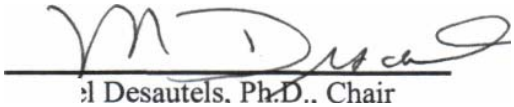
The study is acceptable on scientific and ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research study, and for ensuring that the authorized research is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved protocol or consent process.

FIRST TIME REVIEW AND CONTINUING APPROVAL

The University of Saskatchewan Biomedical Research Ethics Board reviews above minimal studies at a full-board (face-to-face meeting. Any research classified as minimal risk is reviewed through the delegated (subcommittee) review process. The initial Certificate of Approval includes the approval period the REB has assigned to a study. The Status Report form must be submitted within one month prior to the expiry date that appears on the current Certificate of Approval. The researcher shall indicate to the REB any specific requirements of the sponsoring organizations (e.g. requirement for full-board review and approval) for the continuing review process deemed necessary for that project. For more information visit http://www.usask.ca/research/ethics_review/#.

REB ATTESTATION

In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.



Michel Desautels, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

Please send all correspondence to:

Ethics Office
University of Saskatchewan
Room 305 Kirk Hall, 117 Science Place
Saskatoon SK S7N 5C8

Appendix II – Consent Form

Research Participant Information and Consent Form

Title: The effects of high and low glycemic index meals on soccer performance.

Sponsor: Saskatchewan Pulse Growers

Names of Researchers: Principal Investigator: Philip D. Chilibeck, Ph.D., College of Kinesiology, University of Saskatchewan, phone: 966-1072 or 343-6577, Co-investigators: Jonathan Little, B.Sc. (student researcher, supervised by Phil Chilibeck), College of Kinesiology, University of Saskatchewan, phone: 966-1123, Gordon Zello, Ph.D., College of Pharmacy and Nutrition, University of Saskatchewan, phone: 966-5825, Dawn Ciona, B.Sc., (student researcher, supervised by Gordon Zello), phone: 966-5831, Albert Vandenberg, Ph.D., Department of Plant Sciences, University of Saskatchewan, 966-8786, Dr. Heather Sirounis, phone: 652-1211, Dr. Huw Rees, M.D., 610 Queen St., 244-4433.

Introduction: You are being invited to participate in a research study because we want to compare the effectiveness of consuming different meals (i.e., lentils, white bread, potatoes, egg whites) on running performance that simulates a soccer game.

Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.

If you wish to participate, you will be asked to sign this form. Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. If you do decide to take part in this study, you are free to withdraw at any time without giving any reasons for your decision and your refusal to participate will not affect your relationship with university instructors, your academic evaluations, or any other services at the university. Please take time to read the following information carefully and, if you choose, discuss it with your family, friends, and doctor before you decide.

Purpose of the study: The purpose of the study is to compare the effectiveness of a lentil meal (i.e. boiled lentils) to potato meals (i.e. potato, white bread and egg whites or potato and white bread) for improving running performance that simulates a soccer match on a treadmill. Lentils are digested more slowly in the body than potatoes and therefore may provide energy to your exercising muscles for a longer period of time. In our study we will be comparing lentils to a potato, white bread and egg white meal, a meal of potato and white bread, and a condition where no food is consumed before a treadmill running test. We will also be comparing the effectiveness of consuming these meals on recovery from the running test. A total of 20 participants will be involved in this study.

Possible benefits of the study: Information from this study can be used by soccer players and coaches to increase endurance performance during soccer matches. These benefits are not guaranteed.

Procedures:

If you agree to be in this study the following will happen:

You will initially be given a questionnaire (the physical activity readiness questionnaire) which assesses whether you are at a health risk from participating in exercise. If there are possible health risks, with your permission we will send an additional form to your family physician for approval to allow you to participate in the study.

The study involves a total of 10 visits to our lab. The procedures to be done at each visit are as follows:

Visit #1:

You will have your maximal aerobic capacity determined on a treadmill test. This test determines your aerobic fitness. The length of this test can vary from about six minutes to 15 minutes, depending on your level of physical fitness. The test begins with running at an easy pace on the treadmill. The treadmill's speed is increased every minute (i.e. the treadmill gets faster and faster). This is done until you reach exhaustion. During this test you will be breathing through a mouthpiece connected to a computer that measures your maximal oxygen consumption. The maximal oxygen consumption is used to determine your level of physical fitness.

Visit #2:

At least 24 hours after visit #1, you will perform a "practice run" of the simulated soccer match on the treadmill. The speeds on the treadmill will vary to match the speeds you run/jog/walk during a soccer match. This will involve 5-10 second intervals of running alternating with 60 to 120 second intervals of walking or jogging. These intervals will be alternated for 45 minutes (simulating the first half of a soccer game). You will then be given a 15 minute break (simulating a half time break in a soccer game). After the break, you will continue doing the treadmill exercise (i.e. alternating intervals of running with walking/jogging) for 30 minutes. For the final 15 minutes of the treadmill test (simulating the final 15 minutes of a game) you will be required to run for 5 intervals of 1 minute, alternating with walking for 2.5 minutes. The 1 minute intervals will be done at your own pace and we will measure the amount of "distance" you cover. You should attempt to go at the fastest pace you can for each of the five 1 minute intervals during the last 15 minutes of the treadmill test.

Visits #3 and #4:

At least one week after visit #2, you will return to the lab for visit #3. You will be required to fast for at least eight hours prior to visit number 3. For two days prior to visit number 3 you will be required to keep a diary of all the food you consume and the amount of physical activity you perform. During these two days you will not be allowed to consume any meat products (including red meat, poultry and fish). The reason for this

is that consumption of meat products affects one of the measurements we will be doing during this visit.

For this visit you will be randomized (by chance by a computer) to one of four meal conditions: 1) A lentil meal; 2) a potato, white bread, and egg white meal; 3) a potato and white bread meal; or 4) you will not receive a meal.

You will consume 250 grams of the meal once you arrive at the lab, after the eight hour fast. Two hours after you finish the meal you will perform the exact simulated soccer match on the treadmill that you performed during visit #2. You will be allowed to consume as much water as you wish during this test. After the simulated soccer match you will be provided with another 250 grams of the meal, which you will be required to eat over the next 2 hours after you leave the lab. You will be allowed to drink as much water as you want during this 2 hour period. You will be asked to record other foods that you eat for the rest of the day.

During the treadmill test the following measurements will be made:

- Approximately 10 mL of blood will be drawn from a catheter that is inserted into a vein at the top of your hand. This will be done at the start of the test and every 15 minutes during the test (for a total of 80 mL of blood). The purpose of the blood collection is to measure fats and carbohydrates so we can estimate the type of fuel sources that are being made available to your muscles.

- You will be required to breathe into a mouthpiece that feeds into a tube connected to a computer for 8-minute periods starting at minutes 3, 33, and 78 of the test.. The gases that we collect during this test (i.e. the oxygen you consume and the carbon dioxide you exhale) will be used to estimate the proportion of fat and carbohydrate your muscles are using during the exercise test.

- Every 15 minutes of the test (i.e., immediately before the blood samples are drawn) you will be asked to rate how the exercise feels on a scale of one to twenty, with “one” being “easy” and “twenty” being “very hard”.

- Before the final 15 minutes of running/jogging on the treadmill (i.e. the portion of the test that is done at your own pace) a muscle biopsy will be taken from your quadriceps (i.e. the large muscle at the front of your thigh). This procedure is done under local anesthetic, which will be injected into your thigh. A small incision will be made through your skin (approximately 1 cm long). A biopsy needle will be inserted into the incision and into the mid-portion of your thigh muscle to extract a small piece of muscle. This will allow us to measure the amount of glycogen in your muscle. “Glycogen” is the main storage form of carbohydrate in your muscle and it is thought that endurance performance depends on the level of glycogen in your muscle. Dr. Huw Rees, M.D. or Dr. Heather Sirounis, will be doing the biopsy procedure. A “butter fly” bandage

will be applied after the biopsy so that the incision is covered during the final 15 minutes of running/jogging on the treadmill.

After the treadmill test, the following measurements will be made:

- We will require you to collect urine in a plastic container for 24 hours after the test and return this to us the next day. We want to measure a marker for muscle protein breakdown in the urine to assess your muscles' ability to recover from the treadmill test. Meat consumption affects the level of this marker; therefore, you will not be allowed to consume meat (i.e. red meat, poultry, and fish) for two days before the treadmill test and for 24 hours after the treadmill test.

- When you return your urine container we will perform an exercise test on one of your legs that requires you to do 50 knee extensions with maximal effort on a device that measures your force output. This test lasts about one minute. The purpose of this test is to measure how well your leg muscles have recovered from the treadmill test from the previous day.

Visits #5 and #6

At least one week after visit #4, you will come back to the lab for a repeat of the testing described for visits #3 and #4, but this visit will involve a different meal condition (i.e. randomly assigned from the remaining three meal conditions) and a different leg will be biopsied. For two days before this visit, you will be required to consume the same foods and perform the same physical activities you recorded during the two days prior to visit #3. During the treadmill test you will be given the same amount of water that you consumed during the treadmill test you performed at visit #3. You will not be allowed to consume meat for two days prior to visit #5 and for 24 hours after this visit.

Visits #7 and #8

At least one week later you will again repeat the testing described for visits #3 and #4 but with a different meal condition than visits #3 and #5 (i.e. randomly assigned from the remaining two meal conditions) and a different leg will be biopsied. For two days before this visit, you will be required to consume the same foods and perform the same physical activities you recorded during the two days prior to visit #3. During the treadmill test you will be given the same amount of water that you consumed during the treadmill test you performed at visit #3.You will not be allowed to consume meat for two days prior to visit #7 and for 24 hours after this visit.

Visits #9 and #10

At least one week later you will again repeat the testing described for visits #3 and #4 but with a different meal condition than visits #3, #5, and #7 (i.e. lentil, mashed potato and white bread, mashed potato with egg whites and white bread, or no meal) and a

different leg will be biopsied. For two days before this visit, you will be required to consume the same foods and perform the same physical activities you recorded during the two days prior to visit #3. During the treadmill test you will be given the same amount of water that you consumed during the treadmill test you performed at visit #3.You will not be allowed to consume meat for two days prior to visit #9 and for 24 hours after this visit.

We give you a questionnaire about how you feel (e.g., fullness, taste) when you are consuming the different test meals. Following the study we will give you a questionnaire about your general dietary practices.

Foreseeable risks, side effects or discomfort:

The exercise may result in muscle pulls or strains. You will be given a proper warm-up prior to exercising and this will minimize this risk and all exercise tests will be administered by qualified exercise trainers. If any serious pulls or strains occur, you will be withdrawn from the study.

Exercise on an empty stomach (i.e. during the "no meal" condition) may be quite fatiguing and may result in you feeling faint. You will be monitored closely during the exercise test so if this happens the test will be immediately stopped.

There may be some discomfort/pain during the drawing of blood, the muscle biopsy, or the electrical stimulus used to get a maximal contraction of the calf muscle. The discomfort during the biopsy will be minimized by giving a local anesthetic before the biopsy. There is a risk of bruising and infection with the drawing of blood and the muscle biopsy, but care will be taken to minimize these risks. The biopsy will leave a small scar, but this will fade over time.

There may be unforeseen and unknown risks during the study, or after the study has been completed.

Research-Related Injury: There will be no cost to you for participation in this study. You will not be charged for any research procedures. In the event you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no additional cost to you. By signing this document you do not waive any of your legal rights. You will be compensated for your time commitment to the study, for travel to our lab, and parking expenses.

Confidentiality: While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

Voluntary Withdrawal: Your participation in this research is entirely voluntary. You may withdraw from this study at any time. If you decide to enter the study and to withdraw at any time in the future, there will be no penalty or loss of benefits to which you are otherwise entitled.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis.

Who to Contact for Questions or Concerns: If you have questions concerning the study you can contact Dr. Philip Chilibeck at 966-1072, 343-6577, or 230-3849 (24 hour cell) or Jonathan Little (student researcher) at 966-1123.

If you have any questions about your rights as a research subject or concerns about this study, you should contact the Chair of the Biomedical Research Ethics Board, c/o the Office of Research Services, University of Saskatchewan at (306) 966-4053.

By signing below, I confirm the following:

- I have read this research subject information and consent form and I understand the contents of this form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive. I understand that if I am a student a decision not to participate will not affect my academic evaluations.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me (if applicable).
- I have read this form and I freely consent to participate in this study.
- I will receive a dated and signed copy of this form.
- I agree that my family physician can be contacted about my participation in this study:

_____Yes _____No

Participant's Name (printed):_____

Participant's Signature:_____ Date: _____

Name of Individual conducting the consent process
(printed):_____

Signature of Individual conducting the consent process:_____

Date: _____

Appendix III – Development of the soccer match simulation

Order of Treadmill Speeds for each 15-minute section (Drust et al., 2000)

Interval	Speed ($km.h^{-1}$)
1	17
2	10
3	21
4	6
5	10
6	21
7	6
8	21
9	10
10	17
11	6
12	21
13	10
14	21
15	6
16	17
17	6
18	21
19	10
20	21
21	10
22	6
23	21

Speed transition trials (pilot testing on unloaded treadmill)

Transition ($km.h^{-1}$)	Mean of 10 trials (seconds)	SD	# transitions per 15-min section	Total transition time per 15-min section (column B X D)
6 to 10	9.8	0.9	1	9.8
6 to 17	10.7	0.9	1	10.7
6 to 21	12.65	0.6	4	50.6
10 to 17	6.9	0.6	1	6.9
10 to 21	9.2	0.6	3	27.6
10 to 6	5.9	0.3	1	5.9
17 to 6	6.8	0.2	2	13.6
17 to 10	6.2	0.6	1	6.2
21 to 6	10.6	0.4	3	31.8
21 to 10	7.95	0.7	3	23.85
TOTAL TIME REQUIRED FOR TRANSITIONS (seconds) = sum of column E				**187**

Calculations for time spent at each speed

Total Time per 15-min block (seconds) = 900

Total usable time (seconds) = total time - transition time = 900 - 187 = 713 seconds

Proportion of usable time spent at each speed (Ali & Farrally, 1991)

	Time (s) (usable time X % time spent)	# intervals per 15-min section	Time per discrete bout (time at speed / # intervals)
Walk (56%)	399.28	6	66.5
Jog (30%)	213.9	6	35.7
Run (4%)	28.52	3	9.5
Sprint (3%)	21.39	8	2.7

Calculations for time spent standing

Standing (7% total game)

2 standing periods per half @ 15 and 30 minutes = 4 standing periods

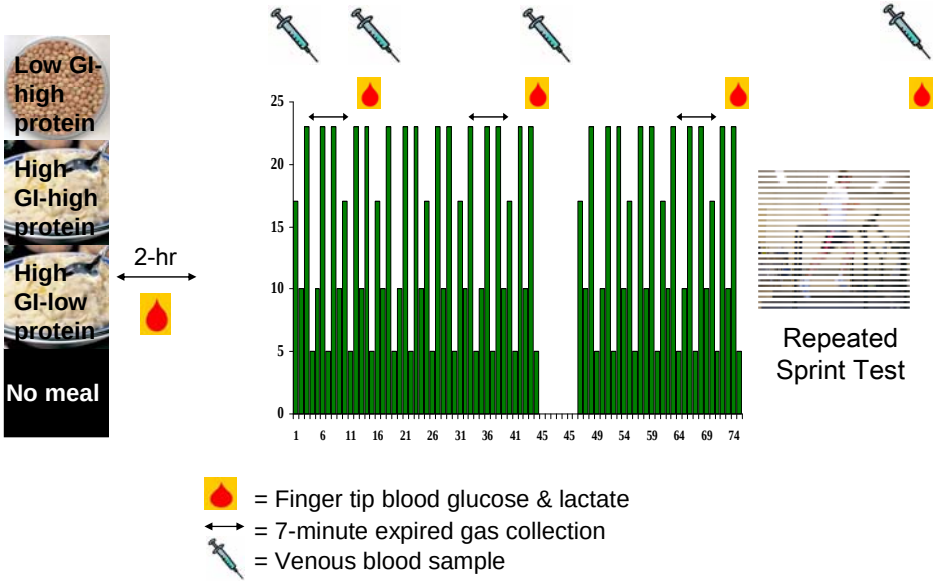
Time spent standing = 90 min*0.07 = 6.3 min

6.3 min divided by 4 = 1.575 min = 94.5 s

Therefore, each standing period is ~95 seconds.

Appendix IV – Graphical representation of the experimental protocol

Experimental Protocol



Appendix V – Details of assay protocols

Free fatty acid colorimetric assay (NEFA HR(2), Wako Diagnostics, Richmond, VA):

Code No. 999-34691
991-34891
993-35191

Wako

HR Series NEFA-HR (2)

(ACS·ACOD method)

Intended use

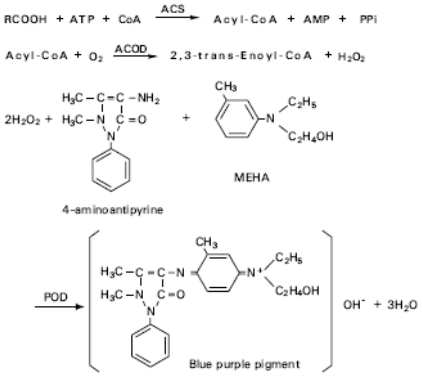
The HR Series NEFA-HR (2) is an in vitro enzymatic colorimetric method assay for the quantitative determination of non-esterified fatty acids (NEFA) in serum.

Summary and explanation of the test

Extraction methods are widely used for the colorimetric determination of non-esterified fatty acids (NEFA) in serum. NEFA are converted to their copper salts that are extracted into an organic solvent. The salts are then complexed with a dye for the purpose of colorimetric measurement [1,2,3,4]. Alternatively, extracted NEFA are titrated with standard alkali to an acid-base indicator endpoint [5,6]. These approaches are time consuming, hazardous and not easily automated. Wako has made extensive studies of NEFA quantitation and has succeeded in developing an original enzymatic method which is accurate, precise, simple and fast. The need for an extraction step has been eliminated and the method can be automated.
The Wako enzymatic method relies upon the acylation of coenzyme A (CoA) by the fatty acids in the presence of added acyl-CoA synthetase (ACS). The acyl-CoA thus produced is oxidized by added acyl-CoA oxidase (ACOD) with generation of hydrogen peroxide. Hydrogen peroxide, in the presence of peroxidase (POD), permits the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple colored product which can be measured colorimetrically at 550 nm.

Principle of the method

Non-esterified fatty acids (NEFA) in serum, when treated with acyl-CoA synthetase (ACS) in the presence of adenosine triphosphate (ATP) and CoA, form the thiol esters of CoA known as acyl-CoA along with the byproducts adenosine monophosphate (AMP) and pyro-phosphate (PPi). In the second portion of the procedure, the acyl-CoA is oxidized by added acyl-CoA oxidase (ACOD) to produce hydrogen peroxide which in the presence of added peroxidase (POD) allows for the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple colored end product with an absorption maximum at 550 nm. Hence the amount of NEFA in the sample can be determined from the optical density measured at 550 nm.



Reagents

(1) Color Reagent A
When reconstituted
0.53 U/mL Acyl-coenzyme A synthetase (ACS) (*Pseudomonas* sp.)
0.31 mmol/L Coenzyme A (CoA, Candida)
4.3 mmol/L Adenosine triphosphate (ATP) (*Bacterium* sp.)
1.5 mmol/L 4-aminoantipyrine
2.6 U/mL Ascorbate oxidase (pumpkin)
0.062% Sodium azide (as Color Reagent A Solution)
Store at 2-10 C.

(2) Solvent A
50 mmol/L phosphate buffer, pH 7.0
0.05% Sodium azide
Store at 2-10℃.

(3) Color Reagent B
When reconstituted
12 U/mL Acyl-coenzyme A oxidase (ACOD) (*Arthrobacter* sp.)
14 U/mL Peroxidase (POD) (horseradish)
Store at 2-10℃.

(4) Solvent B
2.4 mmol/L 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA)
Store at 2-10℃.

Warnings and precautions

(1) For in vitro diagnostic use.
(2) Not to be used internally in humans or animals.
(3) Do not use reagents past the expiration date stated on each reagent container label.
(4) Do not use the reagerts described above for any purpose other than described herein.
(5) Do not use reagents which were frozen in error. Such reagents may give false results.
(6) If the reagents come in contact with the mouth, eyes or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
(7) Color Reagent A and Solvent A contain sodium azide as a preservative (0.062% as reconstituted solution). Sodium azide may react with copper or lead plumbing to form explosive compounds. Even though the reagents contain minute quantities of sodium azide, drains should be well flushed with a large amount of water when discarding the reagents.
(8) The vials are sealed under vaccum. Slowly remove the stopper in order not to release the powder in the vial.
(9) Be careful not to cut yourself with the aluminium cap when removing it from the vial.

Physical or chemical indications of instability

The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent instability.

Instruments

The reagent is designed to be used on commercially available automated analyzers such as Beckman SYNCHRON CX5® analyzer.
Refer to the operating manual for a description of instrument operation, specifications and calibration.

Specimen collection and preparation

Use serum as a specimen. Blood should be collected in the early morning after the patient has fasted for at least 12 hours. Collection of only a few mL of blood from the antecubital vein into a plain evacuated tube will be quite satisfactory.
After the blood has been allowed to clot, the serum should be separated by centrifugation as soon as possible. Please note that serum is the specimen of choice, but alternate collections may be acceptable.
Any specimen containing heparin is unsuitable for this analysis. Hence, any patient receiving heparin therapy, or any specimen collected in a heparinized collection vessel is unsuitable for this analysis.
Specimens that are noticeably icteric, hemolyzed or lipemic may yield inaccurate results unless a specimen blank is also analyzed.

Warning/Biohazard

It is recommended that specimen collection be carried out in accordance with NCCLS Document M29-T2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Procedure for Beckman SYNCHRON CX5®

Materials supplied

Refer to the section entitled "Reagents."

Materials required but not supplied

Beckman SYNCHRON CX5® analyzer
NEFA Standard Solution
Quality control material
All analyzer applications should be validated in accordance with NCEP and CLIA recommendations. For further assistance call Wako Diagnostics Technical Service Department at 1-877-714-1924.

Reagent preparation

Color Reagent A Solution :
Add one bottle of Solvent A to one vial of Color Reagent A. Mix gently by inverting the vial until the contents are completely dissolved. Reconstituted solution is stable for 10 days at 2-10℃.

Color Reagent B Solution :
Add one bottle of Solvent B to one vial of Color Reagent B. Mix gently by inverting the vial until the contents are completely dissolved. Reconstituted solution is stable for 3 weeks at 2-10℃.

Test procedure

Parameter setting (Beckman SYNCHRON CX5®)

Reagent	HR Series NEFA-HR (2)
Test name	NEFA
Reaction Type	ENDPOINT2
Reaction Direction	Positive
Unit	mEq/L
Decimal Position	X.XX
Calculation Factor	0
Math Model	LINEAR
Cal. Time Limit	9999hr
No. of Calibrator	2
Cal#1	saline/0.00/*2
Cal#2	Calibrator/*1/*2
Cal#3	
Cal#4	
Cal#5	
Cal#6	
Primary Wavelength	560 nm
Secondary Wavelength	670 nm
Sample Volume	4 μL
Primary Inject R1	225 μL
Primary Inject R2	
Secondary Inject Reagent	75 μL
Add Time	592 sec.
Calibrators	
Multi point Span 1-2	0.00
2-3	
3-4	
4-5	
5-6	
6-1	
RGT Blank Start Read	528 sec.
End Read	560 sec.
Low/High Abs	−1.5/1.5
Reaction Start Read	576 sec.
End Read	608 sec.
Low/High Abs	−1.5/1.5
Usable Range Low/High Limit	0/9999
Substrate Depletion	
Initial Rate	99.999
Delta Abs.	1.5

*1 : Input the assigned value of the calibrator.
*2 : Input the position of the calibrator.

Results

The final results are automatically calculated and printed in concentration. The results are given in mEq/L.

Calibration

The HR Series NEFA-HR (2) assay must be calibrated using the NEFA Standard Solution.

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. The values obtained for controls should fall within the manufacturer's acceptable ranges. If values are to be established for unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and acceptable range.

Limitations of the procedure

The linearity of HR Series NEFA-HR (2) is 0.01-4.00 mEq/L. If the NEFA value exceeds 4.00 mEq/L, dilute the sample 1 + 2 with saline, repeat the assay, and multiply the result by 3.

Expected values

The expected normal range for serum NEFA from fasting patients is 0.1 - 0.6 mEq/L. Since expected values are affected by age, sex, diet and geographical factors, each laboratory should establish its own expected values for this procedure.

Performance characteristics

Accuracy

The accuracy of this method was demonstrated by a recovery study.

No.	Added value	Expected value	Measured value	Obtained value	Recovery
	(mEq/L)	(mEq/L)	(mEq/L)	(mEq/L)	(%)
1	0.15	0.42	0.42	0.15	100.0
2	0.30	0.57	0.58	0.31	103.3
3	0.59	0.86	0.89	0.62	105.1

No.	Added value	Expected value	Measured value	Obtained value	Recovery
	(mEq/L)	(mEq/L)	(mEq/L)	(mEq/L)	(%)
1	0.39	1.43	1.43	0.39	100.0
2	0.65	1.69	1.69	0.65	100.0
3	0.78	1.82	1.82	0.78	100.0

No.	Added value	Expected value	Measured value	Obtained value	Recovery
	(mEq/L)	(mEq/L)	(mEq/L)	(mEq/L)	(%)
1	0.59	2.61	2.62	0.60	101.7
2	1.18	3.20	3.16	1.14	96.6
3	1.77	3.79	3.87	1.85	104.5

Precision

Within-run precision

Sample #	Replicates	Mean (mEq/L)	SD	CV (%)
1	20	0.51	0.0038	0.75
2	20	0.96	0.0059	0.61

Total precision

Number of assay days	Mean (mEq/L)	SD	CV (%)	S_{WT}	S_T
20	0.548	0.0181	0.03	0.0015	0.0041
20	1.082	0.0776	0.37	0.0053	0.0531

Sensitivity

The minimum detectable level of this method is estimated to be 0.0014 mEq/L.

Correlation

A group of 97 serum samples with NEFA concentration ranging from 0.10 to 1.73 mEq/L was assayed by the described procedure and by a commercially available method. Comparison by values yielded a correlation coefficient of 0.992 and the regression equation was $y = 1.027x + 0.041$.

Specificity (Beckman SYNCHRON CX5®)
(Additive Study)

Hemoglobin (mg/dL)	None	100	200	300	400	500
NEFA (mEq/L)	0.48	0.47	0.45	0.44	0.42	0.40

Ascorbic acid (mg/dL)	None	10	20	30	40	50
NEFA (mEq/L)	2.16	2.15	2.14	2.13	2.14	2.11

Free Bilirubin (mg/dL)	None	10	20	30	40	50
NEFA (mEq/L)	1.74	1.69	1.65	1.60	1.57	1.56

Conjugated Bilirubin (mg/dL)	None	8	16	24	32	40
NEFA (mEq/L)	2.08	1.98	1.87	1.77	1.67	1.56

References

(1) Duncombe, W. G. : Clin. Chim. Acta 9. 122 (1964).
(2) Itaya, K., and Ui, M. : J. Lipid Res. 6, 16 (1965).
(3) Novak, M. : J. lipid Res. 6 431 (1965).
(4) Elphick, M. D. : J. Clin. Pathol. 21, 567.
(5) Trout, D. L., Estes, Estes, E. H. and Friedbekg, S. J. :J. Lipid Res. 1 : 199 (1960).
(6) Dole, V.P. and Meinertz, H. : J. Biol. Chem. 235, 2595 (1960).

Ordering information

Code No.	Product	Package
999-34691	HR Series NEFA-HR (2) Color Reagent A	4 × for 50 mL
995-34791	HR Series NEFA-HR (2) Solvent A	4 × 50 mL
991-34891	HR Series NEFA-HR (2) Color Reagent B	4 × for 25 mL
993-35191	HR Series NEFA-HR (2) Solvent B	4 × 25 mL
997-76491	Wako NEFA Linearity Set	10 mL
276-76491	NEFA Standard Solution	4 × 10 mL

Insulin ELISA (Insulin EIA, Alpco Diagnostics, Salem, NH):

INTENDED USE

Mercodia Insulin ELISA provides a method for the quantitative determination of human insulin in serum or plasma.

SUMMARY AND EXPLANATION OF THE TEST

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesised in the ß-cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form C-peptide and insulin. Both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain and B chain (21 and 30 amino acids respectively). The two chains are linked together by two inter-chain disulphide bridges. There is also an intra-chain disulphide bridge in the A chain.

Secretion of insulin is mainly controlled by plasma glucose concentration, and the hormone has a number of important metabolic actions. Its principal function is to control the uptake and utilisation of glucose in peripheral tissues via the glucose transporter. This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycaemic hormones including glucagon, epinephrine (adrenaline), growth hormone and cortisol.

Insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM) and some other conditions such as hypopituitarism. Insulin levels are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and acromegaly.

PRINCIPLE OF THE PROCEDURE

Mercodia Insulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microtitration well. A simple washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- The contents of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- The Stop solution in this kit contains 0.5 M H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All patient speciments should be handled as of capable of transmitting infections.

TEST PROCEDURE

All reagents and samples must be brought to room temperature before use.
Prepare a calibration curve for each assay run.

1. Prepare sufficient microplate wells to accomodate Calibrators and samples in dulpicate.
2. Pipette 25 µl each of Calibrators and samples into appropriate wells.
3. Add 100 µl of Enzyme Conjugate to each well.
4. Incubate on a plate shaker for 1 hour at room temperature (18–25°C)
5. Wash plate 6 times with automatic plate washer
   or
   Aspirate the reaction volume and fill each well completely with 350 µl Wash Buffer. Discard liquid completely. Repeat 5 times.
   After final wash, invert and tap the plate firmly against absorbent paper.
6. Add 200 µl Substrate TMB into each well
7. Incubate for 15 minutes at room temperature (18–25°C)
8. Add 50 µl Stop Solution to each well.
   Place plate on a shaker for approximately 5 seconds to ensure mixing.
9. Read optical density at 450 nm and calculate results.
   Read within 30 minutes

Epinephrine/Norepinephrine ELISA (BA 10-1500, Rocky Mountain Diagnostics, Colorado Sprints, CO):

Introduction and principle of the test

Catecholamine is the name of a group of aromatic amines (noradrenaline, adrenaline, dopamine and their derivatives) which act as hormones and neurotransmitter, respectively. Adrenaline and noradrenaline are formed from dopamine. They act on the cardiac musculature and the metabolism (adrenaline) as well as on the peripheral circulation (noradrenaline) and help the body to cope with acute and chronic stress. An increased production of catecholamines can be found with tumours of the chromaffine system (pheochromocytoma, neuroblastoma, ganglioneuroma). An increased or decreased concentration of the catecholamines can also be found with hypertension, degenerative cardiac diseases, schizophrenia and manic-depressive psychosis. The measurement of dopamine and its derivatives is of special diagnostic value with children who are suspected to have a neuroblastoma.
The assay kit provides materials for the quantitative measurement of adrenaline and noradrenaline in urine and plasma. Adrenaline and noradrenaline are extracted using a cis-diolspecific affinity gel, then acylated to N-acyladrenaline and N-acylnoradrenaline after this converted enzymatically during the detection procedure into N-acylmetanephrine and N-acylnormetanephrine, respectively.
The competitive EIA kit uses the microtiter plate format. Adrenaline and noradrenaline, respectively, are bound to the solid phase of the microtiter plate. Acylated adrenaline and noradrenaline from the sample and solid phase bound adrenaline and noradrenalline compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase catecholamine is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm with the amount of antibody bound to the solid phase catecholamine being inversely proportional to the catecholamine concentration of the sample.

Sample preparation, extraction and acylation

Each 10 µl of standards, Control 1 & 2 and urine samples, respectively, are extracted. Each 300 µl of plasma samples are extracted.

1. Pipette 10 µl of Standard A - F, 10 µl of Control 1 & 2 and 10 µl of urine samples into the respective wells of the Macrotiter Plate. Add 250 µl of distilled water to these wells to correct for volume. Pipette 300 µl of plasma sample into the respective wells for extracting adrenaline and noradrenaline.
2. Pipette 50 µl of Assay Buffer into all wells
3. Pipette 50 µl of Extraction Buffer into all wells.
4. Cover the plate with adhesive foil and incubate 30 min. at room temperature on an orbital shaker (600 - 900 rpm).
5. Remove the foil and discard. Immediately decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
6. Pipette 1 ml of diluted Washbuffer Concentrate into all wells, cover with adhesive foil and incubate for 5 minutes at room temperature on an orbital shaker (600 - 900 rpm).
7. Remove the foil and discard. Immediately decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
8. Repeat step 6., discard and remove residual liquid by tapping the inverted plate on a paper towel.
9. Pipette 150 µl of Acylation Buffer into all wells.
10. Pipette 25 µl of Acylation Reagent into all wells.
11. Incubate the plate without foil for 15 minutes at room temperature on an orbital shaker (600 - 900 rpm).
12. Decant the plate immediately and remove residual liquid (see 5.).
13. Pipette 1 ml of diluted Washbuffer Concentrate into all wells.
14. Incubate the plate with foil for 10 minutes at room temperature on an orbital shaker (600 - 900 rpm).
15. Decant the plate immediately and remove residual liquid (see 5.).
16. Pipette 150 µl of Hydrochloric Acid (0.025) into all wells to elute adrenaline and noradrenaline.
17. Cover the plate with adhesive foil and incubate for 10 minutes at room temperature on an orbital shaker (600 - 900 rpm). **Caution: Do not decant the supernatant thereafter!**

Adrenaline Enzyme Immunoassay

1. Pipette 25 µl of the freshly prepared Enzyme Solution into all wells.
2. Pipette 100 µl of the extracted standards, controls and patient samples to the appropriate wells.
3. Incubate for 30 minutes at room temperature on a shaker set at 400-500 rpm..
4. Pipette 50 µl of the Adrenaline Antiserum into all wells.
5. Incubate for 2 hours at room temperature on an orbital shaker (400-500 rpm).
6. Discard or aspirate the contents of the wells and wash thoroughly each with 300 µl diluted Washbuffer Concentrate. Repeat this washing procedure 2 times. Blot dry by inverting plate on absorbent material.
7. Pipette 100 µl of the Enzyme Conjugate into all wells.
8. Incubate for 30 minutes at room temperature on an orbital shaker (400-500 rpm).
9. Aspirate/discard and wash each well 3 times. Blot dry by inverting plate on absorbent material.
10. Pipette 100 µl of Substrate into all wells.
11. Incubate for 20 - 30 minutes at room temperature on an orbital shaker (400-500 rpm). *Avoid exposure to direct sun light!*
12. Add 100 µl of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
13. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm and a reference wavelength between 620 nm and 650 nm.

Noradrenaline Enzyme Immunoassay

1. Pipette 25 µl of the freshly prepared Enzyme Solution into all wells.
2. Pipette 20 µl of the extracted standards, controls and patient samples to the appropriate wells.
3. Incubate for 30 minutes at room temperature on a shaker set at 400-500 rpm.
4. Pipette 50 µl of the Noradrenaline Antiserum into all wells.
5. Incubate for 2 hours at room temperature on an orbital shaker (400-500 rpm).
6. Discard or aspirate the contents of the wells and wash thoroughly each with 300 µl diluted Washbuffer Concentrate. Repeat this washing procedure 2 times. Blot dry by inverting plate on absorbent material.
7. Pipette 100 µl of the Enzyme Conjugate into all wells.
8. Incubate for 30 minutes at room temperature on an orbital shaker (400-500 rpm).
9. Aspirate/discard and wash each well 3 times. Blot dry by inverting plate on absorbent material.
10. Pipette 100 µl of Substrate into all wells.
11. Incubate for 20 - 30 minutes at room temperature on an orbital shaker (400-500 rpm).
    Avoid exposure to direct sun light!
12. Add 100 µl of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
13. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm and a reference wavelength between 620 nm and 650 nm.

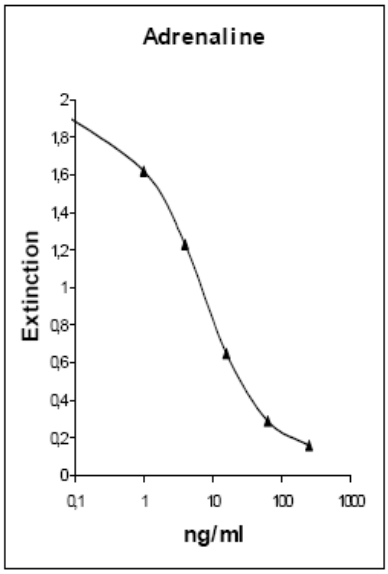
Calculation of results

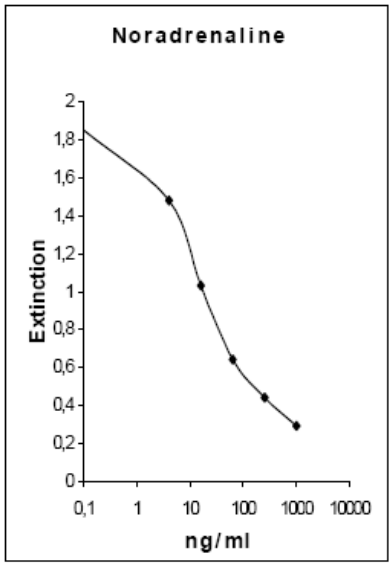
Concentration of the standards:
Adrenaline: A = 0 ng/ml / B = 1 ng/ml /C = 4 ng/ml / D = 16 ng/ml / E = 64 ng/ml / F = 256 ng/ml
Noradrenaline: A = 0 ng/ml / B = 4 ng/ml /C = 16 ng/ml / D = 64 ng/ml / E = 256 ng/ml / F = 1,024 ng/ml
Calculate the mean aborbance for each standard, control or unknown.
Plot the linear mean absorbance readings of the standards along the y-axis versus log of the standard concentrations in ng/ml along the x-axis, using a linear curve-fit.
Determine the analyte concentrations of the controls and unknowns from the standard curve by matching their mean absorbance readings with the corresponding analyte concentrations.

Urine samples and controls:
The concentrations of the **urine samples** and the **controls** can be read directly from the standard curve.

Plasma samples:
The read concentrations of the **plasma samples** have to be **divided by 30.**

Quantitative determination
The calibration curve from which the concentration of adrenaline and noradrenaline in the samples can be taken obtained by plotting the extinction values measured for the 6 standards (linear, y-axis) against the correspondir concentrations (logarithmic, x-axis). The results for unknowns can be calculated using one of the following curve-fittir techniques: spline fits, Akima or four-parameter logistic. The following plots are examples of typical calibration curve for the Adrenaline EIA and Noradrenaline EIA. Please do not use these curves for the determination of adrenaline ar noradrenaline concentrations in samples.





Glucose colorimetric assay (Quantichrom Glucose Assay Kit, Bio Assay Systems, Hayward, CA):

QuantiChrom™ Glucose Assay Kit (DIGL-200)

Quantitative Colorimetric Glucose Determination at 630nm

DESCRIPTION

Glucose ($C_6H_{12}O_6$) is a ubiquitous fuel molecule in biology. It is oxidized through a series of enzyme-catalyzed reactions to form carbon dioxide and water, yielding the universal energy molecule ATP. Due to its importance in metabolism, glucose level is a key diagnostic parameter for many metabolic disorders. Increased glucose levels have been associated with diabetes mellitus, hyperactivity of thyroid, pituitary and adrenal glands. Decreased levels are found in insulin secreting tumors, myxedema, hypopituitarism and hypoadrenalism.

Simple, direct and automation-ready procedures for measuring glucose concentrations find wide applications in research and drug discovery. BioAssay Systems' glucose assay kit is designed to measure glucose directly in serum or plasma without any pretreatment. The improved *o*-toluidine method utilizes a specific color reaction with glucose. The absorbance at 630nm is directly proportional to glucose concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 5 µL samples. Linear detection range 0.7 mg/dL (39 µM) to 300 mg/dL (16.6 mM) glucose in 96-well plate.
Simple and convenient. The procedure involves addition of a single working reagent and incubation for 8 min in a boiling water bath.
Improved reagent stability. The optimized formulation has greatly enhanced the reagent and signal stability.
Low interference in biological samples. No pretreatments are needed. Assays can be directly performed on serum and plasma samples.

APPLICATIONS:

Direct Assays: glucose in biological samples (e.g. serum and plasma).
Drug Discovery/Pharmacology: effects of drugs on glucose metabolism.
Food and Beverages: glucose in food, beverages etc.

KIT CONTENTS (200 tests in 96-well plates)

Reagent: 100 mL Standard: 1 mL 300 mg/dL glucose

Storage conditions. The reagent and standard should be stored at room temperature and -20°C, respectively. Shelf life: 12 months.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

IMPORTANT: THE REAGENT CONTAINS ACETIC ACID. THIS ASSAY IS PREFERABLY CARRIED OUT IN A CHEMICAL FUME HOOD.

Procedure using 96-well plate:

1. Dilute standard in distilled water as follows.

No	STD + H_2O	Vol (µL)	Glucose (mg/dL)
1	150µL + 0µL	150	300
2	100µL + 50µL	150	200
3	50µL + 100µL	150	100
4	25µL + 125µL	150	50
5	0µL + 150µL	150	0

Set up 1.5-mL centrifuge tubes. Transfer 5 µL diluted standards and samples to appropriately labeled tubes. Transfer 500 µL Reagent to each tube. Close the tubes tightly and mix. Store diluted standards at -20°C for future use.

2. Place the tubes in a tube holder and heat in a boiling water bath for 8 min. Cool down in cold water bath for 4 min.
3. Transfer 200 µL in duplicate into a clear bottom 96-well plate. Careful: avoid bubble formation. Read optical density at 620-650nm (peak absorbance at 630nm). Signal is stable for 60 min.

Procedure using cuvette:

1. Dilute standards and transfer 12 µL Standards and samples to appropriately labeled tubes. Transfer 1200 µL Reagent to each tube. Close the tubes tightly and mix.
2. Place the tubes in a tube holder and heat in a boiling water bath for 8 min. Cool down in cold water bath for 4 min.
3. Transfer 1000 µL into a clear bottom 96-well plate. Read optical density at 620-650nm (peak absorbance at 630nm). Signal is stable for 60 min.

Note: if the Sample OD is higher than the Standard OD at 300mg/dL, dilute the sample in double distilled water and repeat the assay.

CALCULATION

Subtract blank OD (water, #5) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The glucose concentration of Sample is calculated as

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{Slope} \quad (mg/dL)$$

OD_{SAMPLE} and OD_{BLANK} are optical density values of the sample and sample "Blank" (water or buffer in which the sample was diluted). Typical serum/plasma glucose values: 70 - 110 mg/dL.
Conversions: 1mg/dL glucose equals 55.5 µM, 0.001% or 10 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, boiling water bath, tube holder.

Procedure using 96-well plate:

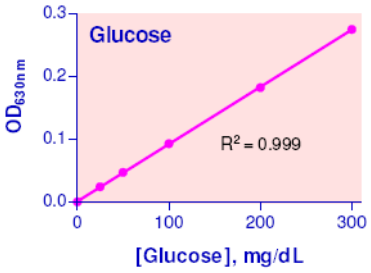
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Spectrophotometer and Cuvets for measuring OD at 620-650nm.

EXAMPLES:

Rat plasma, rat serum, goat serum and human plasma were assayed using the 96-well plate assay protocol. The glucose concentrations were 128 ± 2 (n = 4), 72.5 ± 0.8 (n = 4), 78.6 ± 0.6, 69.3 ± 0.7 mg/dL (n = 4), respectively. Coefficient of variance < 3%.



Calibration curve in 96-well plate

LITERATURE

1. Sacks DA, Greenspoon JS, Fotheringham N (1992). Could the fasting plasma glucose assay be used to screen for gestational diabetes? J Reprod Med. 37:907-909.

2. Northam BE, Smith JH, Fitzgerald MG, Nattrass M, Wright AD (1982). Value of serum glucose assay as part of the biochemical profile in screening for diabetes. Ann Clin Biochem. 19:412-415.

3. Giampietro O, Pilo A, Buzzigoli G, Boni C, Navalesi R (1982). Four methods for glucose assay compared for various glucose concentrations and under different clinical conditions. Clin Chem. 28:2405-2407.

Appendix VI – ANOVA tables and post-hoc tests for all dependent variables

All outputs are from Statistica version 7.0 (Statsoft Inc., Tulsa, OK)

Capillary blood glucose (mmol/l)

	SS	Degr. of Freedom	MS	F	p
Intercept	14746.00	1	14746.00	7614.633	0.000000
Error	23.24	12	1.94		
TIME	224.47	8	28.06	39.195	**0.000000**
Error	68.72	96	0.72		
MEAL	67.42	3	22.47	25.812	**0.000000**
Error	31.34	36	0.87		
TIME*MEAL	194.01	24	8.08	21.133	**0.000000**
Error	110.17	288	0.38		

*comparisons that are not of interest are shaded in grey

Capillary blood glucose post hoc meal x time

	TIME	MEAL	{1} 4.9154	{2} 5.0231	{3} 5.0385	{4} 5.0538
1	1	1		1.000000	1.000000	1.000000
2	1	2	1.000000		1.000000	1.000000
3	1	3	1.000000	1.000000		1.000000
4	1	4	1.000000	1.000000	1.000000	
5	2	1	1.000000	1.000000	1.000000	1.000000
6	2	2	0.564771	0.884803	0.913163	0.936403
7	2	3	0.000034	0.000034	0.000034	0.000034
8	2	4	0.000034	0.000034	0.000034	0.000034
9	3	1	1.000000	1.000000	1.000000	1.000000
10	3	2	0.769275	0.968992	0.979454	0.986898
11	3	3	0.000034	0.000034	0.000034	0.000034
12	3	4	0.000034	0.000034	0.000034	0.000034
13	4	1	1.000000	1.000000	1.000000	1.000000
14	4	2	0.974642	0.999514	0.999776	0.999904
15	4	3	0.000290	0.002340	0.003104	0.004103
16	4	4	0.000036	0.000071	0.000088	0.000111
17	5	1	1.000000	1.000000	1.000000	1.000000
18	5	2	1.000000	1.000000	1.000000	1.000000
19	5	3	1.000000	1.000000	1.000000	1.000000
20	5	4	0.936403	0.997377	0.998607	0.999299
21	6	1	1.000000	1.000000	1.000000	1.000000
22	6	2	0.999299	0.999999	1.000000	1.000000
23	6	3	1.000000	1.000000	1.000000	1.000000
24	6	4	1.000000	1.000000	1.000000	1.000000
25	7	1	0.998607	0.999997	0.999999	1.000000
26	7	2	0.537694	0.868663	0.899635	0.925407
27	7	3	1.000000	1.000000	1.000000	1.000000
28	7	4	1.000000	1.000000	1.000000	1.000000
29	8	1	1.000000	1.000000	1.000000	1.000000
30	8	2	1.000000	1.000000	1.000000	1.000000
31	8	3	1.000000	1.000000	1.000000	1.000000

32	8	4	1.000000	1.000000	1.000000	1.000000
33	9	1	0.979454	0.999668	0.999852	0.999938
34	9	2	0.040074	0.164607	0.195441	0.230159
35	9	3	0.357049	0.721948	0.769275	0.812580
36	9	4	0.084887	0.289499	0.333681	0.381185

Capillary blood glucose post hoc meal x time

	TIME	MEAL	{5} 4.9769	{6} 5.6154	{7} 8.5615	{8} 8.7846
1	1	1	1.000000	0.564771	0.000034	0.000034
2	1	2	1.000000	0.884803	0.000034	0.000034
3	1	3	1.000000	0.913163	0.000034	0.000034
4	1	4	1.000000	0.936403	0.000034	0.000034
5	2	1		0.769275	**0.000034**	**0.000034**
6	2	2	0.769275		0.000034	0.000034
7	2	3	0.000034	0.000034		1.000000
8	2	4	0.000034	0.000034	1.000000	
9	3	1	1.000000	0.997377	0.000034	0.000034
10	3	2	0.913163	1.000000	0.000034	0.000034
11	3	3	0.000034	0.000034	0.431493	0.031857
12	3	4	0.000034	0.000034	0.899635	0.230159
13	4	1	1.000000	0.746066	0.000034	0.000034
14	4	2	0.996469	1.000000	0.000034	0.000034
15	4	3	0.000959	0.974642	0.000034	0.000034
16	4	4	0.000046	0.510707	0.000034	0.000034
17	5	1	1.000000	0.483937	0.000034	0.000034
18	5	2	1.000000	0.832517	0.000034	0.000034
19	5	3	1.000000	0.248980	0.000034	0.000034
20	5	4	0.986898	1.000000	0.000034	0.000034
21	6	1	1.000000	0.968992	0.000034	0.000034
22	6	2	0.999976	1.000000	0.000034	0.000034
23	6	3	1.000000	0.381185	0.000034	0.000034
24	6	4	1.000000	0.406026	0.000034	0.000034
25	7	1	0.999938	1.000000	0.000034	0.000034
26	7	2	0.746066	1.000000	0.000034	0.000034
27	7	3	1.000000	0.962420	0.000034	0.000034
28	7	4	1.000000	0.999668	0.000034	0.000034
29	8	1	1.000000	0.993829	0.000034	0.000034
30	8	2	1.000000	0.999514	0.000034	0.000034
31	8	3	1.000000	0.791475	0.000034	0.000034
32	8	4	1.000000	0.868663	0.000034	0.000034
33	9	1	0.997377	1.000000	0.000034	0.000034
34	9	2	0.093835	1.000000	0.000034	0.000034
35	9	3	0.564771	1.000000	0.000034	0.000034
36	9	4	0.179544	1.000000	0.000034	0.000034

Capillary blood glucose post hoc meal x time

	TIME	MEAL	{9} 5.1615	{10} 5.5538	{11} 7.8231	{12} 7.9769
1	1	1	1.000000	0.769275	0.000034	0.000034
2	1	2	1.000000	0.968992	0.000034	0.000034
3	1	3	1.000000	0.979454	0.000034	0.000034
4	1	4	1.000000	0.986898	0.000034	0.000034

5	2	1	1.000000	0.913163	0.000034	0.000034
6	2	2	0.997377	1.000000	0.000034	0.000034
7	2	3	0.000034	0.000034	0.431493	0.899635
8	2	4	0.000034	0.000034	0.031857	0.230159
9	3	1		0.999852	**0.000034**	**0.000034**
10	3	2	0.999852		0.000034	0.000034
11	3	3	0.000034	0.000034		1.000000
12	3	4	0.000034	0.000034	1.000000	
13	4	1	1.000000	0.899635	0.000034	0.000034
14	4	2	1.000000	1.000000	0.000034	0.000034
15	4	3	0.025166	0.899635	0.000034	0.000034
16	4	4	0.000826	0.311150	0.000034	0.000034
17	5	1	1.000000	0.697030	0.000034	0.000034
18	5	2	1.000000	0.946196	0.000034	0.000034
19	5	3	0.999991	0.431493	0.000034	0.000034
20	5	4	0.999999	1.000000	0.000034	0.000034
21	6	1	1.000000	0.995304	0.000034	0.000034
22	6	2	1.000000	1.000000	0.000034	0.000034
23	6	3	1.000000	0.591814	0.000034	0.000034
24	6	4	1.000000	0.618692	0.000034	0.000034
25	7	1	1.000000	1.000000	0.000034	0.000034
26	7	2	0.996469	1.000000	0.000034	0.000034
27	7	3	1.000000	0.993829	0.000034	0.000034
28	7	4	1.000000	0.999991	0.000034	0.000034
29	8	1	1.000000	0.999514	0.000034	0.000034
30	8	2	1.000000	0.999986	0.000034	0.000034
31	8	3	1.000000	0.925407	0.000034	0.000034
32	8	4	1.000000	0.962420	0.000034	0.000034
33	9	1	1.000000	1.000000	0.000034	0.000034
34	9	2	0.564771	1.000000	0.000034	0.000034
35	9	3	0.979454	1.000000	0.000034	0.000034
36	9	4	0.746066	1.000000	0.000034	0.000034

Capillary blood glucose post hoc meal x time

	TIME	MEAL	{13} 4.9692	{14} 5.4385	{15} 6.1385	{16} 6.3308
1	1	1	1.000000	0.974642	0.000290	0.000036
2	1	2	1.000000	0.999514	0.002340	0.000071
3	1	3	1.000000	0.999776	0.003104	0.000088
4	1	4	1.000000	0.999904	0.004103	0.000111
5	2	1	1.000000	0.996469	0.000959	0.000046
6	2	2	0.746066	1.000000	0.974642	0.510707
7	2	3	0.000034	0.000034	0.000034	0.000034
8	2	4	0.000034	0.000034	0.000034	0.000034
9	3	1	1.000000	1.000000	0.025166	0.000826
10	3	2	0.899635	1.000000	0.899635	0.311150
11	3	3	0.000034	0.000034	0.000034	0.000034
12	3	4	0.000034	0.000034	0.000034	0.000034
13	4	1		0.995304	**0.000826**	**0.000044**
14	4	2	0.995304		0.564771	0.084887
15	4	3	0.000826	0.564771		1.000000
16	4	4	0.000044	0.084887	1.000000	

17	5	1	1.000000	0.954846	0.000188	0.000035
18	5	2	1.000000	0.998607	0.001498	0.000055
19	5	3	1.000000	0.812580	0.000060	0.000034
20	5	4	0.983511	1.000000	0.697030	0.137539
21	6	1	1.000000	0.999986	0.007076	0.000188
22	6	2	0.999961	1.000000	0.248980	0.019760
23	6	3	1.000000	0.913163	0.000111	0.000034
24	6	4	1.000000	0.925407	0.000126	0.000034
25	7	1	0.999904	1.000000	0.289499	0.025166
26	7	2	0.721948	1.000000	0.979454	0.537694
27	7	3	1.000000	0.999976	0.006186	0.000164
28	7	4	1.000000	1.000000	0.050090	0.002030
29	8	1	1.000000	1.000000	0.017470	0.000526
30	8	2	1.000000	1.000000	0.044847	0.001735
31	8	3	1.000000	0.997377	0.001114	0.000048
32	8	4	1.000000	0.999299	0.002030	0.000065
33	9	1	0.996469	1.000000	0.537694	0.076654
34	9	2	0.084887	0.999299	1.000000	0.995304
35	9	3	0.537694	1.000000	0.996469	0.721948
36	9	4	0.164607	0.999961	0.999997	0.974642

Capillary blood glucose post hoc meal x time

	TIME	MEAL	{17} 4.8923	{18} 5.0000	{19} 4.8154	{20} 5.4769
1	1	1	1.000000	1.000000	1.000000	0.936403
2	1	2	1.000000	1.000000	1.000000	0.997377
3	1	3	1.000000	1.000000	1.000000	0.998607
4	1	4	1.000000	1.000000	1.000000	0.999299
5	2	1	1.000000	1.000000	1.000000	0.986898
6	2	2	0.483937	0.832517	0.248980	1.000000
7	2	3	0.000034	0.000034	0.000034	0.000034
8	2	4	0.000034	0.000034	0.000034	0.000034
9	3	1	1.000000	1.000000	0.999991	0.999999
10	3	2	0.697030	0.946196	0.431493	1.000000
11	3	3	0.000034	0.000034	0.000034	0.000034
12	3	4	0.000034	0.000034	0.000034	0.000034
13	4	1	1.000000	1.000000	1.000000	0.983511
14	4	2	0.954846	0.998607	0.812580	1.000000
15	4	3	0.000188	0.001498	0.000060	0.697030
16	4	4	0.000035	0.000055	0.000034	0.137539
17	5	1		1.000000	1.000000	0.899635
18	5	2	1.000000		1.000000	0.993829
19	5	3	1.000000	1.000000		0.697030
20	5	4	0.899635	0.993829	0.697030	
21	6	1	1.000000	1.000000	1.000000	0.999852
22	6	2	0.998076	0.999995	0.974642	1.000000
23	6	3	1.000000	1.000000	1.000000	0.832517
24	6	4	1.000000	1.000000	1.000000	0.851225
25	7	1	0.996469	0.999986	0.962420	1.000000
26	7	2	0.457496	0.812580	0.230159	1.000000
27	7	3	1.000000	1.000000	1.000000	0.999776
28	7	4	0.999999	1.000000	0.999852	1.000000

29	8	1	1.000000	1.000000	0.999998	0.999995
30	8	2	1.000000	1.000000	0.999904	1.000000
31	8	3	1.000000	1.000000	1.000000	0.989696
32	8	4	1.000000	1.000000	1.000000	0.996469
33	9	1	0.962420	0.999005	0.832517	1.000000
34	9	2	0.028337	0.125357	0.008084	0.999904
35	9	3	0.289499	0.645276	0.125357	1.000000
36	9	4	0.062177	0.230159	0.019760	0.999997

Capillary blood glucose post hoc meal x time

	TIME	MEAL	{21} 5.0846	{22} 5.3385	{23} 4.8615	{24} 4.8692
1	1	1	1.000000	0.999299	1.000000	1.000000
2	1	2	1.000000	0.999999	1.000000	1.000000
3	1	3	1.000000	1.000000	1.000000	1.000000
4	1	4	1.000000	1.000000	1.000000	1.000000
5	2	1	1.000000	0.999976	1.000000	1.000000
6	2	2	0.968992	1.000000	0.381185	0.406026
7	2	3	0.000034	0.000034	0.000034	0.000034
8	2	4	0.000034	0.000034	0.000034	0.000034
9	3	1	1.000000	1.000000	1.000000	1.000000
10	3	2	0.995304	1.000000	0.591814	0.618692
11	3	3	0.000034	0.000034	0.000034	0.000034
12	3	4	0.000034	0.000034	0.000034	0.000034
13	4	1	1.000000	0.999961	1.000000	1.000000
14	4	2	0.999986	1.000000	0.913163	0.925407
15	4	3	0.007076	0.248980	0.000111	0.000126
16	4	4	0.000188	0.019760	0.000034	0.000034
17	5	1	1.000000	0.998076	1.000000	1.000000
18	5	2	1.000000	0.999995	1.000000	1.000000
19	5	3	1.000000	0.974642	1.000000	1.000000
20	5	4	0.999852	1.000000	0.832517	0.851225
21	6	1		1.000000	1.000000	1.000000
22	6	2	1.000000		0.993829	0.995304
23	6	3	1.000000	0.993829		1.000000
24	6	4	1.000000	0.995304	1.000000	
25	7	1	1.000000	1.000000	0.989696	0.991982
26	7	2	0.962420	1.000000	0.357049	0.381185
27	7	3	1.000000	1.000000	1.000000	1.000000
28	7	4	1.000000	1.000000	0.999991	0.999995
29	8	1	1.000000	1.000000	1.000000	1.000000
30	8	2	1.000000	1.000000	0.999995	0.999997
31	8	3	1.000000	0.999986	1.000000	1.000000
32	8	4	1.000000	0.999998	1.000000	1.000000
33	9	1	0.999991	1.000000	0.925407	0.936403
34	9	2	0.311150	0.974642	0.017470	0.019760
35	9	3	0.884803	0.999995	0.212315	0.230159
36	9	4	0.483937	0.995304	0.040074	0.044847

Capillary blood glucose post hoc meal x time

	TIME	MEAL	{25} 5.3538	{26} 5.6231	{27} 5.0769	{28} 5.2077
1	1	1	0.998607	0.537694	1.000000	1.000000

2	1	2	0.999997	0.868663	1.000000	1.000000
3	1	3	0.999999	0.899635	1.000000	1.000000
4	1	4	1.000000	0.925407	1.000000	1.000000
5	2	1	0.999938	0.746066	1.000000	1.000000
6	2	2	1.000000	1.000000	0.962420	0.999668
7	2	3	0.000034	0.000034	0.000034	0.000034
8	2	4	0.000034	0.000034	0.000034	0.000034
9	3	1	1.000000	0.996469	1.000000	1.000000
10	3	2	1.000000	1.000000	0.993829	0.999991
11	3	3	0.000034	0.000034	0.000034	0.000034
12	3	4	0.000034	0.000034	0.000034	0.000034
13	4	1	0.999904	0.721948	1.000000	1.000000
14	4	2	1.000000	1.000000	0.999976	1.000000
15	4	3	0.289499	0.979454	0.006186	0.050090
16	4	4	0.025166	0.537694	0.000164	0.002030
17	5	1	0.996469	0.457496	1.000000	0.999999
18	5	2	0.999986	0.812580	1.000000	1.000000
19	5	3	0.962420	0.230159	1.000000	0.999852
20	5	4	1.000000	1.000000	0.999776	1.000000
21	6	1	1.000000	0.962420	1.000000	1.000000
22	6	2	1.000000	1.000000	1.000000	1.000000
23	6	3	0.989696	0.357049	1.000000	0.999991
24	6	4	0.991982	0.381185	1.000000	0.999995
25	7	1		1.000000	1.000000	1.000000
26	7	2	1.000000		0.954846	0.999514
27	7	3	1.000000	0.954846		1.000000
28	7	4	1.000000	0.999514	1.000000	
29	8	1	1.000000	0.991982	1.000000	1.000000
30	8	2	1.000000	0.999299	1.000000	1.000000
31	8	3	0.999961	0.769275	1.000000	1.000000
32	8	4	0.999995	0.851225	1.000000	1.000000
33	9	1	1.000000	1.000000	0.999986	1.000000
34	9	2	0.983511	1.000000	0.289499	0.721948
35	9	3	0.999998	1.000000	0.868663	0.995304
36	9	4	0.997377	1.000000	0.457496	0.868663

Capillary blood glucose post hoc meal x time

	TIME	MEAL	{29} 5.1385	{30} 5.2000	{31} 4.9846	{32} 5.0154
1	1	1	1.000000	1.000000	1.000000	1.000000
2	1	2	1.000000	1.000000	1.000000	1.000000
3	1	3	1.000000	1.000000	1.000000	1.000000
4	1	4	1.000000	1.000000	1.000000	1.000000
5	2	1	1.000000	1.000000	1.000000	1.000000
6	2	2	0.993829	0.999514	0.791475	0.868663
7	2	3	0.000034	0.000034	0.000034	0.000034
8	2	4	0.000034	0.000034	0.000034	0.000034
9	3	1	1.000000	1.000000	1.000000	1.000000
10	3	2	0.999514	0.999986	0.925407	0.962420
11	3	3	0.000034	0.000034	0.000034	0.000034
12	3	4	0.000034	0.000034	0.000034	0.000034
13	4	1	1.000000	1.000000	1.000000	1.000000

14	4	2	1.000000	1.000000	0.997377	0.999299
15	4	3	0.017470	0.044847	0.001114	0.002030
16	4	4	0.000526	0.001735	0.000048	0.000065
17	5	1	1.000000	1.000000	1.000000	1.000000
18	5	2	1.000000	1.000000	1.000000	1.000000
19	5	3	0.999998	0.999904	1.000000	1.000000
20	5	4	0.999995	1.000000	0.989696	0.996469
21	6	1	1.000000	1.000000	1.000000	1.000000
22	6	2	1.000000	1.000000	0.999986	0.999998
23	6	3	1.000000	0.999995	1.000000	1.000000
24	6	4	1.000000	0.999997	1.000000	1.000000
25	7	1	1.000000	1.000000	0.999961	0.999995
26	7	2	0.991982	0.999299	0.769275	0.851225
27	7	3	1.000000	1.000000	1.000000	1.000000
28	7	4	1.000000	1.000000	1.000000	1.000000
29	8	1		1.000000	1.000000	1.000000
30	8	2	1.000000		1.000000	1.000000
31	8	3	1.000000	1.000000		1.000000
32	8	4	1.000000	1.000000	1.000000	
33	9	1	1.000000	1.000000	0.998076	0.999514
34	9	2	0.483937	0.697030	0.103546	0.150613
35	9	3	0.962420	0.993829	0.591814	0.697030
36	9	4	0.671431	0.851225	0.195441	0.268766

Capillary blood glucose post hoc meal x time

	TIME	MEAL	{33} 5.4308	{34} 5.8615	{35} 5.6769	{36} 5.8077
1	1	1	0.979454	0.040074	0.357049	0.084887
2	1	2	0.999668	0.164607	0.721948	0.289499
3	1	3	0.999852	0.195441	0.769275	0.333681
4	1	4	0.999938	0.230159	0.812580	0.381185
5	2	1	0.997377	0.093835	0.564771	0.179544
6	2	2	1.000000	1.000000	1.000000	1.000000
7	2	3	0.000034	0.000034	0.000034	0.000034
8	2	4	0.000034	0.000034	0.000034	0.000034
9	3	1	1.000000	0.564771	0.979454	0.746066
10	3	2	1.000000	1.000000	1.000000	1.000000
11	3	3	0.000034	0.000034	0.000034	0.000034
12	3	4	0.000034	0.000034	0.000034	0.000034
13	4	1	0.996469	0.084887	0.537694	0.164607
14	4	2	1.000000	0.999299	1.000000	0.999961
15	4	3	0.537694	1.000000	0.996469	0.999997
16	4	4	0.076654	0.995304	0.721948	0.974642
17	5	1	0.962420	0.028337	0.289499	0.062177
18	5	2	0.999005	0.125357	0.645276	0.230159
19	5	3	0.832517	0.008084	0.125357	0.019760
20	5	4	1.000000	0.999904	1.000000	0.999997
21	6	1	0.999991	0.311150	0.884803	0.483937
22	6	2	1.000000	0.974642	0.999995	0.995304
23	6	3	0.925407	0.017470	0.212315	0.040074
24	6	4	0.936403	0.019760	0.230159	0.044847
25	7	1	1.000000	0.983511	0.999998	0.997377

26	7	2	1.000000	1.000000	1.000000	1.000000
27	7	3	0.999986	0.289499	0.868663	0.457496
28	7	4	1.000000	0.721948	0.995304	0.868663
29	8	1	1.000000	0.483937	0.962420	0.671431
30	8	2	1.000000	0.697030	0.993829	0.851225
31	8	3	0.998076	0.103546	0.591814	0.195441
32	8	4	0.999514	0.150613	0.697030	0.268766
33	9	1		0.999005	1.000000	0.999938
34	9	2	0.999005		1.000000	1.000000
35	9	3	1.000000	1.000000		1.000000
36	9	4	0.999938	1.000000	1.000000	

Blood lactate (mmol/l)

	SS	Degr. of Freedom	MS	F	p
Intercept	3055.933	1	3055.933	595.4811	0.000000
Error	61.582	12	5.132		
TIME	1281.608	8	160.201	49.1360	**0.000000**
Error	312.994	96	3.260		
MEAL	5.170	3	1.723	0.8705	0.465323
Error	71.264	36	1.980		
TIME*MEAL	19.978	24	0.832	0.5902	0.938331
Error	406.161	288	1.410		

Blood lactate post hoc for time

	{1} 1.6173	{2} 1.5500	{3} 1.6596	{4} 1.6885	{5} 1.7962	{6} 2.4827
1		1.000000	1.000000	1.000000	0.999891	0.273103
2	1.000000		0.999998	0.999985	0.998806	0.186780
3	1.000000	0.999998		1.000000	0.999986	0.338398
4	1.000000	0.999985	1.000000		0.999998	0.387302
5	0.999891	0.998806	0.999986	0.999998		0.589114
6	0.273103	0.186780	0.338398	0.387302	0.589114	
7	0.211527	0.140129	0.267578	0.310707	0.500340	1.000000
8	0.156446	0.100372	0.202279	0.238464	0.407690	0.999999
9	**0.000133**	**0.000133**	**0.000133**	**0.000133**	**0.000133**	**0.000133**

Blood lactate post hoc for time

	{7} 2.5288	{8} 2.5788	{9} 7.0962
1	0.211527	0.156446	**0.000133**
2	0.140129	0.100372	**0.000133**
3	0.267578	0.202279	**0.000133**
4	0.310707	0.238464	**0.000133**
5	0.500340	0.407690	**0.000133**
6	1.000000	0.999999	**0.000133**
7		1.000000	**0.000133**
8	1.000000		**0.000133**
9	**0.000133**	**0.000133**	

Ratings of perceived exertion (RPE)

	SS	Degr. of Freedom	MS	F	p
Intercept	56727.08	1	56727.08	1228.646	0.000000
Error	554.04	12	46.17		
TIME	1150.36	5	230.07	61.417	**0.000000**
Error	224.76	60	3.75		
MEAL	37.42	3	12.47	5.029	**0.005165**
Error	89.29	36	2.48		
TIME*MEAL	4.14	15	0.28	0.753	0.727294
Error	65.90	180	0.37		

RPE post-hoc meal

	MEAL	{1} 13.859	{2} 13.038	{3} 13.256	{4} 13.782
1	1		**0.012755**	0.097419	0.990047
2	2	**0.012755**		0.823168	**0.027393**
3	3	0.097419	0.823168		0.177660
4	4	0.990047	**0.027393**	0.177660	

RPE post hoc time

	TIME	{1} 11.365	{2} 12.154	{3} 12.865	{4} 13.212	{5} 13.923	{6} 17.385
1	1		0.313051	**0.002826**	**0.000242**	**0.000133**	**0.000133**
2	2	0.313051		0.427630	0.073701	**0.000371**	**0.000133**
3	3	**0.002826**	0.427630		0.942016	0.073701	**0.000133**
4	4	**0.000242**	0.073701	0.942016		0.427630	**0.000133**
5	5	**0.000133**	**0.000371**	0.073701	0.427630		**0.000133**
6	6	**0.000133**	**0.000133**	**0.000133**	**0.000133**	**0.000133**	

Respiratory Exchange Ratio RER

	SS	Degr. of Freedom	MS	F	p
Intercept	150.3938	1	150.3938	26979.05	0.000000
Error	0.0669	12	0.0056		
TIME	0.0508	2	0.0254	47.89	**0.000000**
Error	0.0127	24	0.0005		
MEAL	0.0110	3	0.0037	3.42	**0.027439**
Error	0.0388	36	0.0011		
TIME*MEAL	0.0010	6	0.0002	1.02	0.416628
Error	0.0117	72	0.0002		

RER post hoc Meal

MEAL	{1} .97066	{2} .97938	{3} .98334	{4} .99408

1	1		0.646830	0.335288	**0.016549**
2	2	0.646830		0.950653	0.215336
3	3	0.335288	0.950653		0.480461
4	4	**0.016549**	0.215336	0.480461	

RER post hoc time

	TIME	{1} 1.0060	{2} .97694	{3} .96265
1	1		0.000131	0.000129
2	2	0.000131		0.011369
3	3	0.000129	0.011369	

Carbohydrate oxidation (g/min)

	SS	Degr. of Freedom	MS	F	p
Intercept	1372.159	1	1372.159	773.2152	0.000000
Error	21.295	12	1.775		
TIME	4.216	2	2.108	24.7334	**0.000001**
Error	2.046	24	0.085		
MEAL	1.620	3	0.540	3.7717	**0.018812**
Error	5.154	36	0.143		
TIME*MEAL	0.110	6	0.018	0.7598	0.603865
Error	1.742	72	0.024		

Carbohydrate oxidation post hoc meal

	{1} 2.8323	{2} 2.9146	{3} 3.0132	{4} 3.1031
1		0.772790	0.169157	**0.016200**
2	0.772790		0.661133	0.142611
3	0.169157	0.661133		0.721891
4	**0.016200**	0.142611	0.721891	

Carbohydrate oxidation post hoc time

	TIME	{1} 3.1863	{2} 2.9195	{3} 2.7917
1	1		**0.000393**	**0.000130**
2	2	**0.000393**		0.086039
3	3	**0.000130**	0.086039	

Fat oxidation (g/min)

	SS	Degr. of Freedom	MS	F	p
Intercept	1.621952	1	1.621952	37.01831	0.000055
Error	0.525778	12	0.043815		
TIME	0.326903	2	0.163451	33.87404	**0.000000**
Error	0.115806	24	0.004825		
MEAL	0.125815	3	0.041938	3.33805	**0.029905**
Error	0.452295	36	0.012564		
TIME*MEAL	0.023771	6	0.003962	1.68480	0.137094
Error	0.169308	72	0.002352		

Fat oxidation post hoc meal

	{1} .14235	{2} .10862	{3} .09337	{4} .06353
1		0.551292	0.234065	**0.018617**
2	0.551292		0.931180	0.301111
3	0.234065	0.931180		0.646003
4	**0.018617**	0.301111	0.646003	

Fat oxidation post hoc time

	TIME	{1} .04402	{2} .10594	{3} .15594
1	1		**0.000484**	**0.000129**
2	2	**0.000484**		**0.003429**
3	3	**0.000129**	**0.003429**	

Total Carbohydrate oxidation (g)

	SS	Degr. of Freedom	MS	F	p
Intercept	3717507	1	3717507	734.1424	0.000000
Error	60765	12	5064		
MEAL	4188	3	1396	3.7351	**0.019557**
Error	13455	36	374		

Total Carbohydrate oxidation post hoc meal

	MEAL	{1} 254.91	{2} 264.14	{3} 271.19	{4} 279.28
1	C chog		0.620385	0.158095	**0.014129**
2	L chog	0.620385		0.789258	0.208110
3	PE chog	0.158095	0.789258		0.711379
4	P chog	**0.014129**	0.208110	0.711379	

Total fat oxidation (g)

	SS	Degr. of Freedom	MS	F	p
Intercept	4340.691	1	4340.691	36.09333	0.000061
Error	1443.156	12	120.263		
MEAL	337.434	3	112.478	3.30519	**0.030985**
Error	1225.105	36	34.031		

Total fat oxidation post hoc meal

	MEAL	{1} 12.811	{2} 9.6138	{3} 8.4031	{4} 5.7178
1	C fatg		0.509229	0.235303	**0.018849**
2	L fatg	0.509229		0.951511	0.337279
3	PE fatg	0.235303	0.951511		0.647155
4	P fatg	**0.018849**	0.337279	0.647155	

Repeated Sprint Test (distance in metres)

	SS	Degr. of Freedom	MS	F	p
Intercept	1696154	1	1696154	972.1122	0.000000
Error	20938	12	1745		
SPRINT	301	4	75	6.8583	**0.000189**

Error	527	48	11		
MEAL	279	3	93	5.3427	**0.003786**
Error	627	36	17		
SPRINT*MEAL	125	12	10	1.7979	**0.053568**
Error	835	144	6		

Repeated Sprint Test post hoc meal x sprint

	SPRINT	MEAL	{1} 79.769	{2} 83.923	{3} 82.615	{4} 82.385
1	1	1		**0.001841**	0.223739	0.376803
2	1	2	0.001841		0.998221	0.987078
3	1	3	0.223739	0.998221		1.000000
4	1	4	0.376803	0.987078	1.000000	
5	2	1	0.999989	0.000054	0.010011	0.024940
6	2	2	0.320798	0.992855	1.000000	1.000000
7	2	3	0.043741	0.999999	1.000000	0.999999
8	2	4	0.748058	0.847996	1.000000	1.000000
9	3	1	0.999883	0.000047	0.005204	0.013692
10	3	2	0.887734	0.689751	0.999989	1.000000
11	3	3	1.000000	0.033198	0.748058	0.887734
12	3	4	0.999883	0.118902	0.945546	0.987078
13	4	1	0.999680	0.000045	0.003708	0.010011
14	4	2	0.999211	0.000044	0.002621	0.007259
15	4	3	0.999997	0.000061	0.013692	0.033198
16	4	4	1.000000	0.007259	0.436787	0.627772
17	5	1	1.000000	0.000089	0.024940	0.057061
18	5	2	0.998221	0.223739	0.987078	0.998221
19	5	3	1.000000	0.013692	0.563811	0.748058
20	5	4	0.627772	0.920171	1.000000	1.000000

Repeated Sprint Test post hoc meal x sprint

	SPRINT	MEAL	{5} 78.846	{6} 82.462	{7} 83.154	{8} 81.923
1	1	1	0.999989	0.320798	0.043741	0.748058
2	1	2	0.000054	0.992855	0.999999	0.847996
3	1	3	0.010011	1.000000	1.000000	1.000000
4	1	4	0.024940	1.000000	0.999999	1.000000
5	2	1		**0.018564**	**0.000893**	0.118902
6	2	2	0.018564		1.000000	1.000000
7	2	3	0.000893	1.000000		0.999211
8	2	4	0.118902	1.000000	0.999211	
9	3	1	1.000000	0.010011	0.000431	0.073671
10	3	2	0.223739	0.999999	0.992855	1.000000
11	3	3	0.977990	0.847996	0.320798	0.992855
12	3	4	0.847996	0.977990	0.627772	0.999883
13	4	1	1.000000	0.007259	0.000301	0.057061
14	4	2	1.000000	0.005204	0.000213	0.043741
15	4	3	1.000000	0.024940	0.001285	0.148531
16	4	4	0.999211	0.563811	0.118902	0.920171
17	5	1	1.000000	0.043741	0.002621	0.223739
18	5	2	0.689751	0.996302	0.801194	0.999997
19	5	3	0.996302	0.689751	0.183400	0.964507

20	5	4	0.073671	1.000000	0.999883	1.000000

Repeated Sprint Test post hoc meal x sprint

	SPRINT	MEAL	{9} 78.692	{10} 81.692	{11} 80.462	{12} 80.846
1	1	1	0.999883	0.887734	1.000000	0.999883
2	1	2	0.000047	0.689751	0.033198	0.118902
3	1	3	0.005204	0.999989	0.748058	0.945546
4	1	4	0.013692	1.000000	0.887734	0.987078
5	2	1	1.000000	0.223739	0.977990	0.847996
6	2	2	0.010011	0.999999	0.847996	0.977990
7	2	3	0.000431	0.992855	0.320798	0.627772
8	2	4	0.073671	1.000000	0.992855	0.999883
9	3	1		0.148531	0.945546	0.748058
10	3	2	0.148531		0.999211	0.999997
11	3	3	0.945546	0.999211		1.000000
12	3	4	0.748058	0.999997	1.000000	
13	4	1	1.000000	0.118902	0.920171	0.689751
14	4	2	1.000000	0.094114	0.887734	0.627772
15	4	3	1.000000	0.269608	0.987078	0.887734
16	4	4	0.996302	0.977990	1.000000	0.999999
17	5	1	1.000000	0.376803	0.996302	0.945546
18	5	2	0.563811	1.000000	1.000000	1.000000
19	5	3	0.987078	0.992855	1.000000	1.000000
20	5	4	0.043741	1.000000	0.977990	0.999211

Repeated Sprint Test post hoc meal x sprint

	SPRINT	MEAL	{13} 78.615	{14} 78.538	{15} 78.923	{16} 80.077
1	1	1	0.999680	0.999211	0.999997	1.000000
2	1	2	0.000045	0.000044	0.000061	0.007259
3	1	3	0.003708	0.002621	0.013692	0.436787
4	1	4	0.010011	0.007259	0.033198	0.627772
5	2	1	1.000000	1.000000	1.000000	0.999211
6	2	2	0.007259	0.005204	0.024940	0.563811
7	2	3	0.000301	0.000213	0.001285	0.118902
8	2	4	0.057061	0.043741	0.148531	0.920171
9	3	1	1.000000	1.000000	1.000000	0.996302
10	3	2	0.118902	0.094114	0.269608	0.977990
11	3	3	0.920171	0.887734	0.987078	1.000000
12	3	4	0.689751	0.627772	0.887734	0.999999
13	4	1		1.000000	1.000000	0.992855
14	4	2	1.000000		1.000000	0.987078
15	4	3	1.000000	1.000000		0.999680
16	4	4	0.992855	0.987078	0.999680	
17	5	1	1.000000	1.000000	1.000000	0.999962
18	5	2	0.499601	0.436787	0.748058	0.999962
19	5	3	0.977990	0.964507	0.998221	1.000000
20	5	4	0.033198	0.024940	0.094114	0.847996

Repeated Sprint Test post hoc meal x sprint

	SPRINT	MEAL	{17} 79.077	{18} 81.077	{19} 80.231	{20} 82.077
1	1	1	1.000000	0.998221	1.000000	0.627772

2	1	2	0.000089	0.223739	0.013692	0.920171
3	1	3	0.024940	0.987078	0.563811	1.000000
4	1	4	0.057061	0.998221	0.748058	1.000000
5	2	1	1.000000	0.689751	0.996302	0.073671
6	2	2	0.043741	0.996302	0.689751	1.000000
7	2	3	0.002621	0.801194	0.183400	0.999883
8	2	4	0.223739	0.999997	0.964507	1.000000
9	3	1	1.000000	0.563811	0.987078	0.043741
10	3	2	0.376803	1.000000	0.992855	1.000000
11	3	3	0.996302	1.000000	1.000000	0.977990
12	3	4	0.945546	1.000000	1.000000	0.999211
13	4	1	1.000000	0.499601	0.977990	0.033198
14	4	2	1.000000	0.436787	0.964507	0.024940
15	4	3	1.000000	0.748058	0.998221	0.094114
16	4	4	0.999962	0.999962	1.000000	0.847996
17	5	1		0.847996	0.999680	0.148531
18	5	2	0.847996		0.999997	0.999962
19	5	3	0.999680	0.999997		0.920171
20	5	4	0.148531	0.999962	0.920171	

Serum Glucose

	SS	Degr. of Freedom	MS	F	p
Intercept	4800.593	1	4800.593	1190.442	0.000000
Error	40.326	10	4.033		
TIME	29.514	3	9.838	15.828	**0.000002**
Error	18.646	30	0.622		
MEAL	1.896	3	0.632	1.779	0.172449
Error	10.657	30	0.355		
TIME*MEAL	6.367	9	0.707	1.676	0.106468
Error	37.996	90	0.422		

Serum Glucose post-hoc for time

	TIME	{1} 4.5538	{2} 5.2232	{3} 5.5618	{4} 5.5519
1	1		**0.002256**	**0.000165**	**0.000166**
2	2	**0.002256**		0.205293	0.227392
3	3	**0.000165**	0.205293		0.999932
4	4	**0.000166**	0.227392	0.999932	

Serum free fatty acid

	SS	Degr. of Freedom	MS	F	p
Intercept	34.00404	1	34.00404	698.3730	0.000000
Error	0.48690	10	0.04869		
TIME	2.11990	3	0.70663	16.3086	**0.000002**
Error	1.29987	30	0.04333		
MEAL	1.58859	3	0.52953	11.2593	**0.000041**

Error	1.41092	30	0.04703		
TIME*MEAL	0.12348	9	0.01372	1.2187	0.293617
Error	1.01321	90	0.01126		

Serum free fatty acid meal post hoc

	MEAL	{1} .60404	{2} .38308	{3} .38907	{4} .38202
1	1		**0.000377**	**0.000483**	**0.000363**
2	2	**0.000377**		0.999276	0.999996
3	3	**0.000483**	0.999276		0.998781
4	4	**0.000363**	0.999996	0.998781	

Serum free fatty acid time post hoc

	TIME	{1} .30312	{2} .36914	{3} .50285	{4} .58309
1	1		0.457276	**0.000653**	**0.000162**
2	2	0.457276		**0.025513**	**0.000352**
3	3	**0.000653**	**0.025513**		0.289590
4	4	**0.000162**	**0.000352**	0.289590	

Serum insulin

	SS	Degr. of Freedom	MS	F	p
Intercept	18537.76	1	18537.76	77.70339	0.000005
Error	2385.71	10	238.57		
TIME	440.68	3	146.89	2.82989	0.055140
Error	1557.22	30	51.91		
MEAL	1516.64	3	505.55	9.08361	**0.000197**
Error	1669.65	30	55.65		
TIME*MEAL	1055.86	9	117.32	3.75891	**0.000475**
Error	2808.95	90	31.21		

Serum insulin post hoc time x meal

	TIME	MEAL	{1} 5.5555	{2} 10.057	{3} 12.901	{4} 23.495
1	1	1		0.873628	0.157467	**0.000151**
2	1	2	0.873628		0.998025	**0.000167**
3	1	3	0.157467	0.998025		**0.002557**
4	1	4	**0.000151**	**0.000167**	**0.002557**	
5	2	1	1.000000	0.897838	0.179674	**0.000151**
6	2	2	0.625064	1.000000	0.999988	**0.000253**
7	2	3	0.951073	1.000000	0.987840	**0.000156**
8	2	4	0.676755	1.000000	0.999963	**0.000225**
9	3	1	1.000000	0.987258	0.397628	**0.000151**
10	3	2	0.299180	0.999939	1.000000	**0.000907**
11	3	3	0.989664	1.000000	0.945191	**0.000152**
12	3	4	0.888213	1.000000	0.997298	**0.000165**
13	4	1	1.000000	0.762060	0.094657	**0.000151**

14	4	2	0.693941	1.000000	0.999946	**0.000217**
15	4	3	0.855483	1.000000	0.998632	**0.000170**
16	4	4	0.517252	1.000000	0.999999	**0.000349**

Serum insulin post hoc time x meal

	TIME	MEAL	{5} 5.6928	{6} 11.033	{7} 9.5158	{8} 10.858
1	1	1	1.000000	0.625064	0.951073	0.676755
2	1	2	0.897838	1.000000	1.000000	1.000000
3	1	3	0.179674	0.999988	0.987840	0.999963
4	1	4	**0.000151**	**0.000253**	**0.000156**	**0.000225**
5	2	1		0.665793	0.963454	0.715912
6	2	2	0.665793		0.999999	1.000000
7	2	3	0.963454	0.999999		1.000000
8	2	4	0.715912	1.000000	1.000000	
9	3	1	1.000000	0.895676	0.997896	0.922328
10	3	2	0.333085	1.000000	0.999053	1.000000
11	3	3	0.993205	0.999929	1.000000	0.999976
12	3	4	0.910566	1.000000	1.000000	1.000000
13	4	1	1.000000	0.476434	0.882531	0.528802
14	4	2	0.732377	1.000000	1.000000	1.000000
15	4	3	0.881816	1.000000	1.000000	1.000000
16	4	4	0.558785	1.000000	0.999988	1.000000

Serum insulin post hoc time x meal

	TIME	MEAL	{9} 6.6558	{10} 12.180	{11} 8.8841	{12} 9.9768
1	1	1	1.000000	0.299180	0.989664	0.888213
2	1	2	0.987258	0.999939	1.000000	1.000000
3	1	3	0.397628	1.000000	0.945191	0.997298
4	1	4	**0.000151**	**0.000907**	**0.000152**	**0.000165**
5	2	1	1.000000	0.333085	0.993205	0.910566
6	2	2	0.895676	1.000000	0.999929	1.000000
7	2	3	0.997896	0.999053	1.000000	1.000000
8	2	4	0.922328	1.000000	0.999976	1.000000
9	3	1		0.611143	0.999888	0.989892
10	3	2	0.611143		0.990620	0.999903
11	3	3	0.999888	0.990620		1.000000
12	3	4	0.989892	0.999903	1.000000	
13	4	1	0.999999	0.195468	0.963548	0.782666
14	4	2	0.930250	1.000000	0.999984	1.000000
15	4	3	0.983560	0.999965	1.000000	1.000000
16	4	4	0.825629	1.000000	0.999539	1.000000

Serum insulin post hoc time x meal

	TIME	MEAL	{13} 5.0624	{14} 10.799	{15} 10.150	{16} 11.390
1	1	1	1.000000	0.693941	0.855483	0.517252
2	1	2	0.762060	1.000000	1.000000	1.000000
3	1	3	0.094657	0.999946	0.998632	0.999999
4	1	4	**0.000151**	**0.000217**	**0.000170**	**0.000349**
5	2	1	1.000000	0.732377	0.881816	0.558785
6	2	2	0.476434	1.000000	1.000000	1.000000
7	2	3	0.882531	1.000000	1.000000	0.999988

8	2	4	0.528802	1.000000	1.000000	1.000000
9	3	1	0.999999	0.930250	0.983560	0.825629
10	3	2	0.195468	1.000000	0.999965	1.000000
11	3	3	0.963548	0.999984	1.000000	0.999539
12	3	4	0.782666	1.000000	1.000000	1.000000
13	4	1		0.546830	0.737327	0.374978
14	4	2	0.546830		1.000000	1.000000
15	4	3	0.737327	1.000000		1.000000
16	4	4	0.374978	1.000000	1.000000	

Serum epinephrine plus norepinephrine

	SS	Degr. of Freedom	MS	F	p
Intercept	16711.75	1	16711.75	61.31230	0.000014
Error	2725.68	10	272.57		
TIME	1486.22	3	495.41	17.60645	**0.000001**
Error	844.13	30	28.14		
MEAL	83.55	3	27.85	2.10218	0.120883
Error	397.44	30	13.25		
TIME*MEAL	203.91	9	22.66	3.51651	**0.000905**
Error	579.86	90	6.44		

Serum epinephrine plus norepinephrine post hoc meal x time

	TIME	MEAL	{1} 6.6310	{2} 6.1269	{3} 6.7705	{4} 6.3222
1	1	1		1.000000	1.000000	1.000000
2	1	2	1.000000		1.000000	1.000000
3	1	3	1.000000	1.000000		1.000000
4	1	4	1.000000	1.000000	1.000000	
5	2	1	0.693710	0.367930	0.776911	0.490446
6	2	2	0.823925	0.512186	0.885852	0.641946
7	2	3	0.881532	0.597755	0.929090	0.723419
8	2	4	0.915761	0.660501	0.952864	0.779099
9	3	1	0.910462	0.649970	0.949294	0.770006
10	3	2	0.297940	0.105279	0.376267	0.163050
11	3	3	0.874823	0.586705	0.924254	0.713258
12	3	4	**0.047514**	**0.011338**	0.068074	**0.020185**
13	4	1	**0.001038**	**0.000281**	**0.001607**	**0.000434**
14	4	2	**0.000184**	**0.000154**	**0.000210**	**0.000160**
15	4	3	**0.000151**	**0.000151**	**0.000151**	**0.000151**
16	4	4	**0.000151**	**0.000151**	**0.000151**	**0.000151**

Serum epinephrine plus norepinephrine post hoc meal x time

	TIME	MEAL	{5} 9.0137	{6} 8.7854	{7} 8.6569	{8} 8.5615
1	1	1	0.693710	0.823925	0.881532	0.915761
2	1	2	0.367930	0.512186	0.597755	0.660501
3	1	3	0.776911	0.885852	0.929090	0.952864
4	1	4	0.490446	0.641946	0.723419	0.779099
5	2	1		1.000000	1.000000	1.000000
6	2	2	1.000000		1.000000	1.000000
7	2	3	1.000000	1.000000		1.000000

8	2	4	1.000000	1.000000	1.000000	
9	3	1	1.000000	1.000000	1.000000	1.000000
10	3	2	1.000000	0.999986	0.999919	0.999749
11	3	3	1.000000	1.000000	1.000000	1.000000
12	3	4	0.993080	0.972457	0.948832	0.923754
13	4	1	0.475516	0.335770	0.267968	0.223570
14	4	2	0.096340	0.054586	**0.038792**	**0.029853**
15	4	3	**0.000153**	**0.000151**	**0.000151**	**0.000151**
16	4	4	**0.000151**	**0.000151**	**0.000151**	**0.000151**

Serum epinephrine plus norepinephrine post hoc meal x time

	TIME	MEAL	{9} 8.5778	{10} 9.6432	{11} 8.6735	{12} 10.466
1	1	1	0.910462	0.297940	0.874823	**0.047514**
2	1	2	0.649970	0.105279	0.586705	**0.011338**
3	1	3	0.949294	0.376267	0.924254	0.068074
4	1	4	0.770006	0.163050	0.713258	**0.020185**
5	2	1	1.000000	1.000000	1.000000	0.993080
6	2	2	1.000000	0.999986	1.000000	0.972457
7	2	3	1.000000	0.999919	1.000000	0.948832
8	2	4	1.000000	0.999749	1.000000	0.923754
9	3	1		0.999791	1.000000	0.928505
10	3	2	0.999791		0.999935	0.999992
11	3	3	1.000000	0.999935		0.952505
12	3	4	0.928505	0.999992	0.952505	
13	4	1	0.230728	0.856719	0.276209	0.998478
14	4	2	**0.031228**	0.346097	**0.040589**	0.849258
15	4	3	**0.000151**	**0.000181**	**0.000151**	**0.000914**
16	4	4	**0.000151**	**0.000151**	**0.000151**	**0.000151**

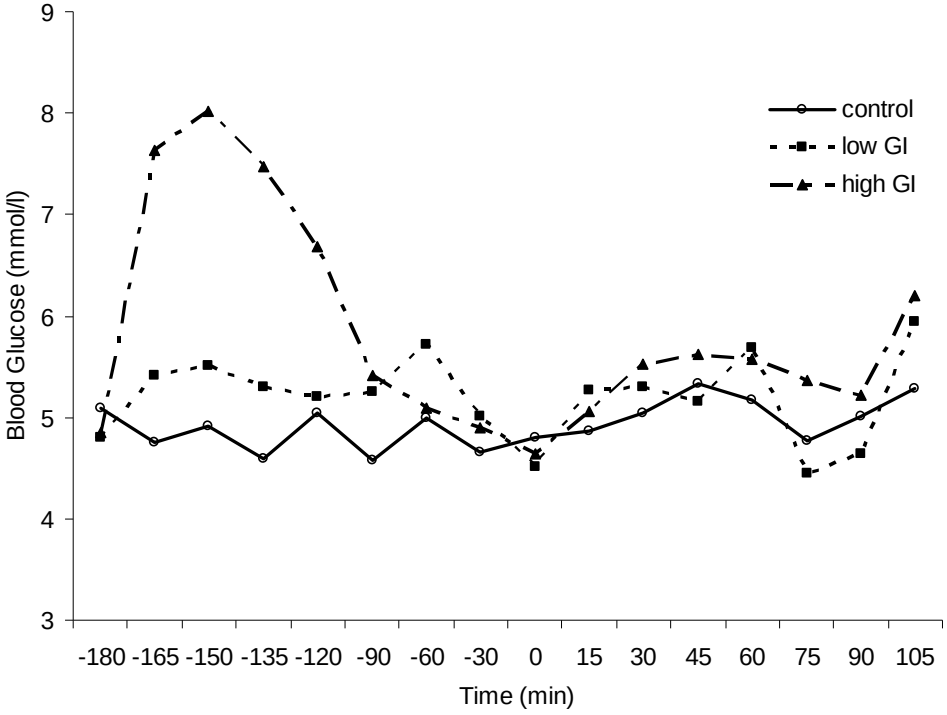
Serum epinephrine plus norepinephrine post hoc meal x time

	TIME	MEAL	{13} 11.728	{14} 12.568	{15} 15.605	{16} 17.781
1	1	1	**0.001038**	**0.000184**	**0.000151**	**0.000151**
2	1	2	**0.000281**	**0.000154**	**0.000151**	**0.000151**
3	1	3	**0.001607**	**0.000210**	**0.000151**	**0.000151**
4	1	4	**0.000434**	**0.000160**	**0.000151**	**0.000151**
5	2	1	0.475516	0.096340	**0.000153**	**0.000151**
6	2	2	0.335770	0.054586	**0.000151**	**0.000151**
7	2	3	0.267968	**0.038792**	**0.000151**	**0.000151**
8	2	4	0.223570	**0.029853**	**0.000151**	**0.000151**
9	3	1	0.230728	**0.031228**	**0.000151**	**0.000151**
10	3	2	0.856719	0.346097	**0.000181**	**0.000151**
11	3	3	0.276209	**0.040589**	**0.000151**	**0.000151**
12	3	4	0.998478	0.849258	**0.000914**	**0.000151**
13	4	1		0.999990	**0.042509**	**0.000171**
14	4	2	0.999990		0.284931	**0.000732**
15	4	3	**0.042509**	0.284931		0.813106
16	4	4	**0.000171**	**0.000732**	0.813106	

Appendix VII – Selected figures from pilot testing

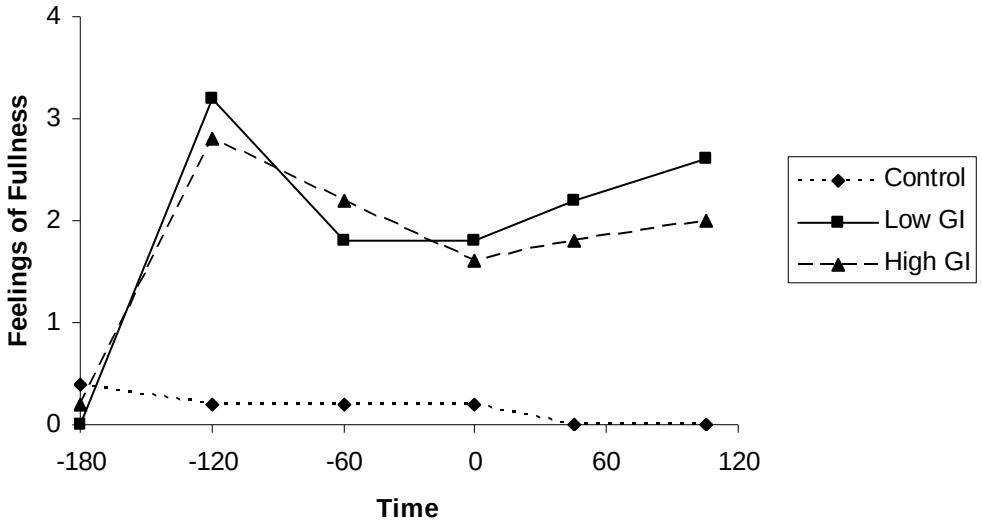
Selected figures from pilot testing of the pre-exercise meals

A



A) Blood glucose response to the low GI (lentils) and high GI (potato + egg whites) meals used in the pilot study. Blood glucose concentration returned to baseline 2 hours after the high GI meal (i.e., at time -60 minutes) and was not different between conditions at any time point after.

B



B) Feelings of fullness after consuming the low and high GI meals in the pilot study (on a scale from 0 [no feelings at all] to 4 [extreme fullness]). Feelings of fullness had subsided 2 hours after the meal (-60 minutes) and remained relatively constant from this point on.