Factors affecting post-exercise glycaemia in individuals with type 1 diabetes.

West, Daniel J

FACTORS AFFECTING POST-EXERCISE GLYCAEMIA IN INDIVIDUALS WITH TYPE 1 DIABETES

DANIEL J. WEST

Submitted to Swansea University in fulfilment of the requirements for the Degree of Doctor of Philosophy

2011

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Nothing can stop the man with the right mental attitude

from achieving his goal

THOMAS JEFFERSON

(1743 – 1826)

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ACKNOWLEDGEMENTS

I wish to sincerely thank all of the following people:

The participants: Thanks for giving up so much of your time, without your commitment and dedication none of these investigations would have been possible.

Professor Steve Bain and Dr. Jeffrey Stephens: Thanks for your support with recruiting the participants and all the advice you provided during the publishing of the experimental chapters. Moreover, thanks for making the Diabetes UK APC such an enjoyable experience.

Dr. Liam Kilduff: Many thanks for all of the advice and support you have provided for me over the past 6 years. I really appreciate everything you have done for me.

Dr. Richard Bracken: Firstly, I wish to thank you for giving me the opportunity to study at doctorate level within such a good research team. However, most of all I would like to thank you for being a great supervisor; your enthusiasm, encouragement, support and meticulousness has not only been key in my learning and development, but also in the enjoyment I have had completing this body of work, and I am forever grateful.

SUMMARY

The overarching aim of this thesis was to examine factors that affect post-exercise glycaemia and contribute to minimising the risk of hypoglycaemia after exercise. An inability to regulate circulating insulin concentrations is considered the primary gluco-regulatory defect within T1DM. Therefore, the aim of chapter 3 was to examine the effects of pre-exercise rapid-acting insulin reductions on blood glucose responses before and after running in T1DM individuals, to test the hypothesis that reducing pre-exercise insulin dose may help preserve post-exercise glycaemia. The results demonstrate that a 75% reduction to pre-exercise rapid-acing insulin dose best preserved blood glucose before and after exercise, without increasing the risk of ketoacidosis, and reduced the risk of hypoglycaemia in free living conditions for 24 hours following running.

An important factor determining blood glucose concentrations and subsequent patterns of fuel oxidation is the rate of appearance of carbohydrate into the circulation. Potentially, low GI carbohydrates may raise blood glucose less and increase the percentage contribution of lipids as a fuel because of a slower digestion. Therefore, the aim of chapter 4 was to examine the metabolic and blood glucose responses to ingestion of a high or low GI carbohydrate, combined with a 75% reduced insulin dose, before, during and for 24 hours after running. The results demonstrate that compared to a high GI carbohydrate, the low GI carbohydrate increased blood glucose concentrations less before exercise and maintained blood glucose better for 24 hours after running, via lower carbohydrate and higher lipid oxidation rates during the latter stages of running.

After manipulating both the insulin dose and the pre-exercise carbohydrate GI, to improve post-exercise blood glucose concentrations, the timing of the ingestion of carbohydrate

(alongside a reduced insulin dose) before exercise is an important factor which may further refine these strategies. Therefore, chapter 5 examined the metabolic and blood glucose responses to alterations in the timing of carbohydrate feeding and insulin administration prior to running. Our results demonstrated that administration of both a reduced rapid-acting insulin dose and low GI carbohydrate 30 minutes before exercise improved glycaemia for 24 hours after running, by reductions in carbohydrate oxidation, leading to increased carbohydrate availability post-exercise.

LIST OF ABBREVIATIONS

Abbreviation	Meaning
ANOVA	Analysis of variance
BG	Blood glucose
β-OHB	β-hydroxybutyrate
BG_{AUC}	Blood glucose area under the curve
BMI	Body mass index
CHO	Carbohydrate
GI	Glycaemic index
H^+	Hydrogen ion
Hb	Haemoglobin
HbA_{1c}	Glycosylated Haemoglobin
Hct	Haematocrit
HGI	High glycaemic index
HR	Heart rate
HR_{peak}	Peak heart rate
IU	International unit
LGI	Low glycaemic index

LIST OF ABBREVIATIONS (Cont.)

NEFA	Non-esterified fatty acid
NPH	Neutral Protamine Hagedorn
rDNA	Recombinant DNA
T1DM	Type 1 Diabetes Mellitus
TG	Triglyceride
VO_{2peak}	Peak oxygen uptake

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PUBLICATIONS ARISING FROM THIS THESIS

Academic Journal Papers

West, D.J., Morton, R.D., Stephens, J.W., Bain, S.C., Kilduff, L.P., Luzio, S., Still, R. and Bracken, R.M. (2011). Isomaltulose improves post-exercise glycemia by reducing CHO oxidation in T1DM. *Medicine and Science in Sports and Exercise*, 43, 204-210.

West, D.J., Stephens, J.W., Bain, S.C., Kilduff, L.P., Luzio, S., Still, R. and Bracken, R.M. (2011). A combined insulin reduction and CHO feeding strategy 30 minutes before running best preserves blood glucose concentrations after exercise through improved fuel oxidation in T1DM. *Journal of Sports Sciences*, 29, 279-289.

Bracken, R., West, D.J., Stephens, J.W., Kilduff, L.P., Luzio, S. & Bain, S.C. (2011). Impact of pre-exercise rapid-acting insulin reductions on ketogenesis following running in Type 1 diabetes. *Diabetic Medicine*, 28, 218-212.

West, D.J., Morton, R., Stephens, J.W., Bain, S.C. & Bracken, R.M. (2010). Blood glucose responses to reductions in pre-exercise rap id-acting insulin for 24 h after running in individuals with type 1 diabetes. *Journal of Sports Sciences*, 28, 781-788.

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Chapter One

Review of literature

1.1 Introduction

Type 1 diabetes (T1DM) is characterised by an absolute or relative lack of insulin, due to autoimmune destruction of the insulin secreting β-cells within the pancreas. Ultimately, the loss of function of these cells results in the individual with T1DM being dependent upon exogenous insulin therapy. Within the UK, roughly 10% of all diabetes patients have T1DM, equalling roughly 230,000 people, at a cost to the National Health Service of approximately £1bn a year (Diabetes in the UK, 2008).

At present, the American College of Sports Medicine (ACSM; 2006) advocate T1DM individuals perform aerobic type exercise for various health benefits such as reduced glycosylated haemoglobin (HbA_{1c}; Mosher et al., 1998; Ramahlo et al., 2006; Salvatoni et al., 2005; Sideravičiūté et al., 2006), delayed micro- (The Diabetes Control and Complications Trial Research Group, 1993) and macro-vascular (UKPD Study Research Group, 1998) complications, increased insulin sensitivity (Lehmann et al., 1997), improved glucose clearance (Borghouts and Keizer, 2000) and ultimately, decreased mortality (Moy et al., 1993).

However, despite these benefits of exercise, inadequate glucose regulation means that engaging in exercise results in a heightened risk of developing hypoglycaemia, i.e. blood glucose concentrations falling below the normal physiological range of 3.5 – 7 $mmol.l^{-1}$, during and as long as 24 hours after exercise (MacDonald, 1987; Steppel and Horton, 2003; Tsalikian et al., 2005). Therefore, comprehensive strategies to help combat the increased risk of hypoglycaemia associated with exercise are required.

1.2 Blood glucose regulation in T1DM

At rest, exogenous insulin treatment reduces blood glucose concentrations, preventing hyperglycaemia and risk of ketosis (Cryer, 2001). However, circulating insulin concentrations are unregulated, and are the result of the passive absorption from the injection site of the previously administered insulin dose, and the particular insulin species' pharmacokinetics (Cryer, 2001). Therefore, during exercise, contracting skeletal tissue (Hayashi et al., 1997), increased insulin sensitivity (Rose et al., 2001) and an inability to regulate circulating insulin concentrations, (Grimm, 2005) results in an augmented rate of glucose uptake from circulation and reductions to blood glucose concentrations. Moreover, exercise may exacerbate the increase in appearance of exogenous insulin (Dandona et al., 1980), due to increased temperature (Koivisto et al., 1981) and blood flow (Lauritzen et al., 1980). Thus, unaltered and/or increasing insulin concentrations means that hepatic glucose output remains inhibited (Zinman et al., 1977), as does adipocyte lipolysis. Therefore, the primary mechanism involved in maintaining blood glucose concentrations during exercise is lost within T1DM (Figure 1.1, part A).

Glucagon, the secondary counter-regulatory response is also effected within T1DM (Figure 1.1, part A). Beta-cell death results in a progressive loss of α-cell function over time, potentially due to the loss of β-α cell signalling (Banarer et al., 2002), which results in the glucagon response to falling blood glucose becoming impaired or completely absent (Bolli et al., 1985; Gerich et al., 1973; Mokan et al., 1994). Consequently, the first (decreased insulin secretion) and second (increased glucagon secretion) defences against developing hypoglycaemia during exercise are defective in T1DM individuals. Failure of these defences results in individuals becoming reliant on the third counter-regulatory mechanism, adrenaline (Figure 1.1, part B). However, the adrenaline response to falling blood glucose concentrations

is typically attenuated (Amiel et al., 1988; Dagogo-Jack et al., 1993). Due to antecedent hypoglycaemia, and a decreased sympathetic neural system response (Steppel and Horton, 2003; Figure 1.1, part C), the adrenaline response to falling blood glucose concentrations is predominantly shifted to a lower threshold (Dagogo-Jack et al., 1993). A loss of the primary and secondary mechanisms and a defective tertiary mechanism (Figure 1.1, part A, B and C) means that blood glucose concentrations will fall and hypoglycaemia becomes a frequent and dangerous occurrence during exercise. The increased risk of developing hypoglycaemia during exercise is a major concern for T1DM individuals (Brazeau et al., 2008); as a result, the fear of experiencing a hypoglycaemic incident is a major barrier for T1DM individuals wishing to exercise (Brazeau et al., 2008).

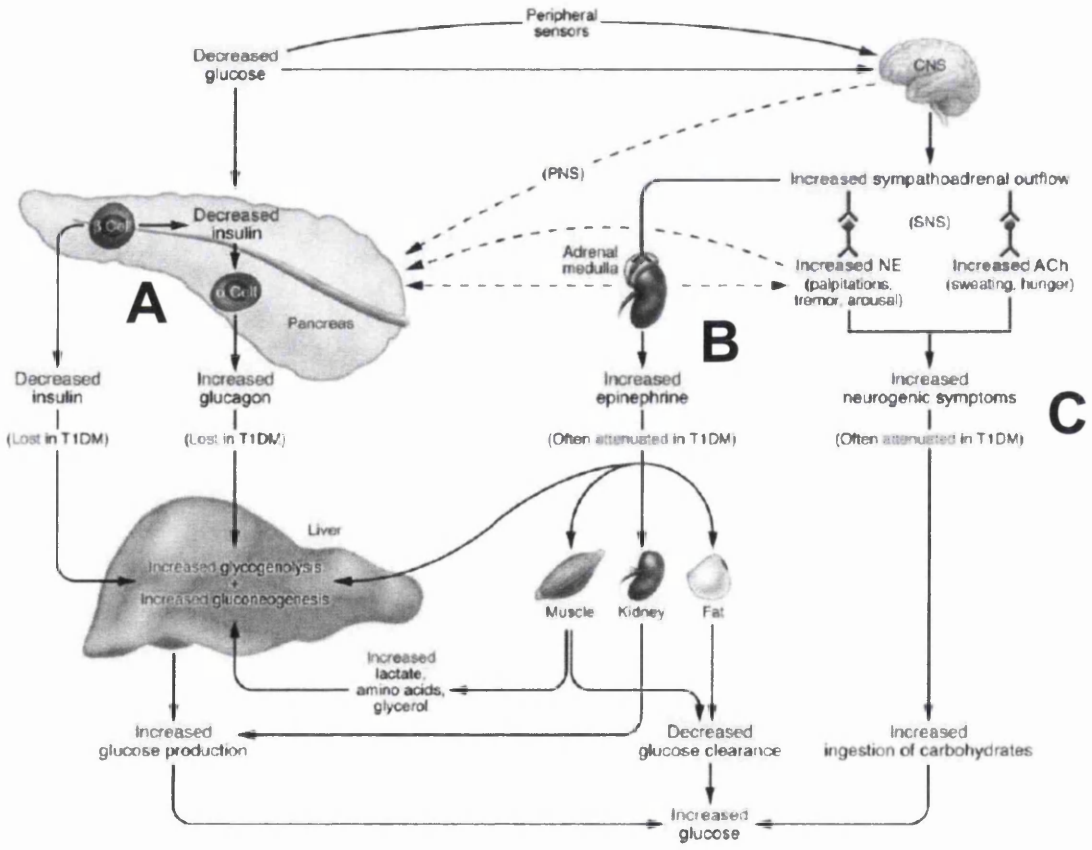


Figure 1.1: Blood glucose regulation in non-T1DM and T1DM individuals (adapted from Cryer, 2006).

1.3 Hypoglycaemia: variations in its form and time of onset

1.3.1 Iatrogenic hypoglycaemia

Unfortunately, due to intensive exogenous insulin therapy, hypoglycaemia is a fact of life for people with T1DM (Cryer, 2002). The primary aim of treating T1DM is to maintain blood glucose concentrations at or as close to euglycaemia as possible, however, with achieving this goal comes iatrogenic hypoglycaemia (Cryer et al., 2003), or treatment-induced hypoglycaemia. If too much insulin is administered in relation to dietary intake, the insulin mediated uptake of glucose, in addition to an inhibition of hepatic glucose production and compromised glucose-regulation (Cryer, 1994; 1997; 2001; Figure 1.1), causes falls in blood glucose below the normal physiological concentrations. These falls in blood glucose induce two forms of symptoms, neuroglycopaenic and neurogenic symptoms (Chiarelli et al., 1999). Neuroglycopaenic symptoms (e.g. confusion, unconsciousness, brain damage, death) are the result of a minimal glucose requirement by the brain (Becker and Ryan, 2000; Cryer et al., 2003). Neurogenic symptoms include physiological changes such as pallor, sweating, tachycardia and hunger (Chiarelli et al., 1999; Cryer et al., 2003). Type 1 diabetes individuals may experience 2 episodes of symptomatic hypoglycaemia per week, with thousands of incidences over a lifetime, and at least 1 severe or temporarily disabling hypoglycaemic incident per year (Macleod et al., 1993; Reichard et al., 1991; The Diabetes Control and Complications Research Group, 1993).

1.3.2 Exercise-induced hypoglycaemia

During Exercise

Insulin facilitates muscle glycogen synthesis through its action on both glucose transport and glycogen synthase activity (Wojtaszewski et al., 2002). Specifically, insulin stimulates translocation of glucose transporter proteins (GLUT-4) to the plasma membrane, thereby enhancing the glucose transport capacity (Simpson et al., 2001) of the cell. Moreover, insulin enhances the activity of glycogen synthase by decreasing phosphorylation of the enzyme (Cohen, 1993), contributing to the increased uptake of glucose and subsequent storage within the myocyte.

Keeping these mechanisms in mind, it has been demonstrated that muscle glucose uptake during exercise is increased and occurs by an insulin-independent mechanism (Nesher et al., 2001; Plough et al., 1984) and that insulin and contracting skeletal tissue have additive effects on glucose transport (Nesher et al., 1985; Plough et al., 1984). Moreover, during exercise the perfusion of the muscle is increased compared with rest, this increases the delivery of insulin to the active tissue (Wojtaszewski et al., 2002). Therefore, within the T1DM individual, contracting muscle and an inability to regulate circulating insulin concentrations, as these are the result of the passive absorption of the previously administered insulin dose, results in a synergistic uptake of blood glucose (DeFronzo et al., 1981). These factors, in combination with the suppressive effect of insulin on hepatic glucose output, and defective glucagon and adrenaline responses, results in a mismatch between glucose uptake and production and blood glucose concentrations will fall.

Post-exercise

The threat of developing hypoglycaemia is not only isolated to during exercise, it can also occur up to 24 hours after the cessation of exercise (MacDonald, 1987; Steppel and Horton, 2003; Tsalikian et al., 2005). In a study by MacDonald (1987), 300 regularly exercising individuals with T1DM were followed prospectively over 2 years. Sixteen percent of those participants regularly developed late-onset (6-15 hours after vigorous exercise) hypoglycaemia, with over 50% of the hypoglycaemic incidences resulting in a loss of consciousness (severe hypoglycaemia). Additionally, Tsalikian et al. (2005) found that hypoglycaemia developed overnight more frequently in T1DM children after performance of continuous exercise that day (four 15 min periods of walking at a heart rate of 140 bpm) compared to nights when daily exercise was not performed. Plasma glucose concentrations fell in almost all of the 50 subjects and 11 developed clinical hypoglycaemia.

During exercise, human skeletal muscle relies heavily on intra-muscular glycogen stores as a source of energy for contractile activity (Wojtaszewski et al., 2002) and in the post-exercise period resynthesis of glycogen is important and has a high metabolic priority (Wojtaszewski et al., 2002). To compensate for the glycogen depleted state, the activity of glycogen synthase has been demonstrated to increase after exercise (Nielsen et al., 2001), independent from activation by insulin (Bogardus et al., 1983; Munger et al., 1993; Zachwieja et al., 1991). In addition, cellular changes in the prior exercised muscle primes glucose transport and glycogen synthase activation when subsequently stimulated by insulin (Wojtaszewski et al., 2002). Therefore, enhanced insulin-stimulated recruitment of GLUT-4 transporters (Hansen et al., 1998), depleted muscle glycogen stores (Bogardus et al., 1983; Munger et al., 1993; Zachwieja et al., 1991), and an increased activity of glycogen synthase are mechanisms contributing to the increase insulin sensitivity after exercise (Wojtaszewski et al., 2002).

When considering these changes in insulin sensitivity, T1DM individuals are unable to compensate for the heightened sensitivity with a decrease in insulin concentrations, this results in and an augmented uptake of blood glucose from circulation (Bogardus et al., 1983). In addition, unaltered insulin concentrations means insulins suppressive effect at the hepatocyte and adipocyte remains. Moreover, glucagon is defective or absent (Mokan et al., 1994) and does not rise after exercise (Gallen, 2003); combining this with an attenuated adrenaline response means hepatic glucose production is unable to match the peripheral uptake of glucose by muscle, which is elevated to replenish glycogen stores, and concentrations will fall and individuals will develop hypoglycaemia.

1.3.3 Variation in the severity of hypoglycaemia

The severity of the hypoglycaemic incident can vary within an individual (Cryer, 2006). For example, a drop in blood glucose below the normal physiological range of ~3.5 - 4 $mmol.l^{-1}$ may induce symptomatic hypoglycaemia where the patient may become fatigued, develop parasthesia, pallor and have difficulty focusing (Cryer, 1997). However, defects in glucose sensing, primarily due to sympathoadrenal failure (Cryer, 2006), can result in typical symptoms of hypoglycaemia not materialising, and the individual becomes unaware of falling blood glucose (termed hypoglycaemia unawareness; Cryer 1999). Blood glucose concentrations may fall below 2.5 $mmol.l^{-1}$ and the individual is at risk of experiencing a severe hypoglycaemic incident that may result in a loss of consciousness, convulsions or even death (The Diabetes Control and Complications Research Group, 1993).

1.3.4 Defining hypoglycaemia

Based on a prior study examining exercise within T1DM individuals, a blood glucose concentration of ≤ 3.5 mmol.l^{-1} will be defined as 'hypoglycaemia' (Rabasa-Lhoret et al., 2001). Furthermore, as a drop in blood glucose below the normal physiological threshold of 4 – 7 mmol.l^{-1} (Saltiel and Kahn, 2001) can trigger symptoms of hypoglycaemia (Cryer, 1997), T1DM individuals are likely to correct their blood glucose concentrations before concentrations reach those that can be defined 'hypoglycaemic', therefore a blood glucose concentration of ≤ 4.0 mmol.l^{-1} will be defined as 'low blood glucose'.

1.4 Treating T1DM: exogenous insulin therapy

Under normal physiological conditions, insulin is stored in the β-cell in its hexamer form (6 monomers) due to a high tendency to self-associate, this is important as it facilitates proinsulin transportation as well as the conversion and intracellular storage of insulin microcrystals (Emdin et al., 1980). However, insulin is most readily absorbed through the capillary wall, and elicits its actions (binding to insulin receptors), in the monomeric form (Figure 1.2). This becomes a major obstacle for the administration of regular human insulin (e.g. Actrapid, Table 1.1) and animal preparations, such as bovine or porcine insulins, as these insulins self-associate (Bolli et al., 1999).

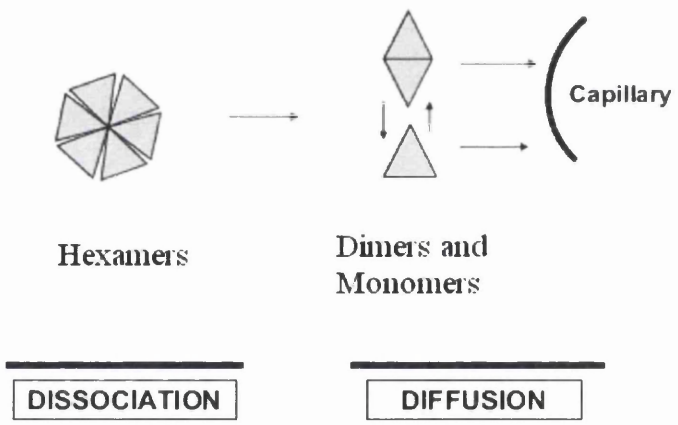


Figure 1.2: The dissociation of insulin hexamers (from injection site or pancreas) into dimers and monomers for diffusion across the capillary wall (adapted from Gin and Hanaire-Broutin, 2005).

The dissociation rate of these insulins into monomeric molecules is slow at the subcutaneous site of injection and consequently its absorption is slow (Figure 1.2). Therefore, the pre-prandial treatment with short-acting (Table 1.1) insulin preparations results in a less than optimal increase in insulin concentrations in the early phase of glucose absorption from the intestine. Consequently, blood glucose increases excessively 1-2 hours after meal ingestion (Dimitriadis and Gerich, 1983). Nonetheless, 4-5 hours after the subcutaneous insulin

injection, the continuing absorption from the injection site results in inappropriate hyperinsulinemia which increases the risk of hypoglycaemia as by that time meal absorption is nearly complete (Dimitriadis and Gerich, 1983). Using insulins with these absorption kinetics means tight glycaemic control is a difficult task for T1DM individuals. These reasons are the basis for the development of the modern insulin analogues.

According to Bolli et al. (1999) the combination of a prandial insulin peak with a flat, square-wave interprandial plasma insulin profile (Figure 1.3), would closely mimic the 24 hour plasma insulin pattern of non-T1DM individuals who exhibit very small blood glucose variations regardless of being in a fasted or fed state.

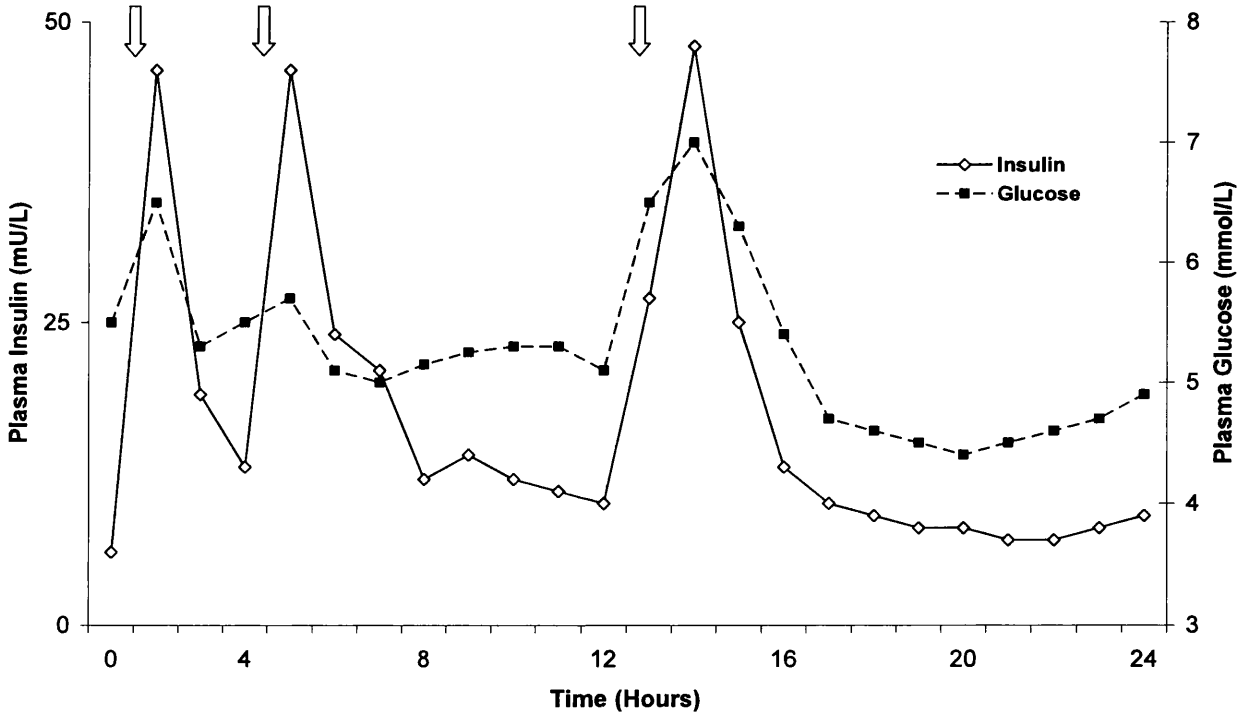


Figure 1.3: Twenty-four hour plasma insulin and glucose concentrations within a non-T1DM individual (adapted from Kruszynska, 2003).

Note: Descending arrows indicate feeding.

1.4.1 The development of the insulin analogues

Biotechnology research has shown that one amino acid modification can lead to changes in the tridimensional structure of the insulin molecule and to major alterations in its biological properties (Drejer et al., 1991). Dimarchi et al. (1994) studied the synthesis of Insulin-like growth factor-I (IGF-1), and identified that this hormone is highly homologous with insulin, particularly at the C-terminal of the B chain; however, this hormone does not self-associate into dimmers and hexamers. The normally occurring Pro-Lys sequence of insulin at positions B28 and B29 is reversed in IGF-1 (Brems et al., 1992). This gave premise for the hypothesis that the Lys-Pro sequence renders IGF-1 incapable of self-association and the reversal of this sequence in insulin (Figure 1.4) would lead to an insulin analogue incapable of self-association (Di Marchi et al., 1994), this theory was the basis for the development of the fast acting insulin analogue, lispro (Figure 1.4).

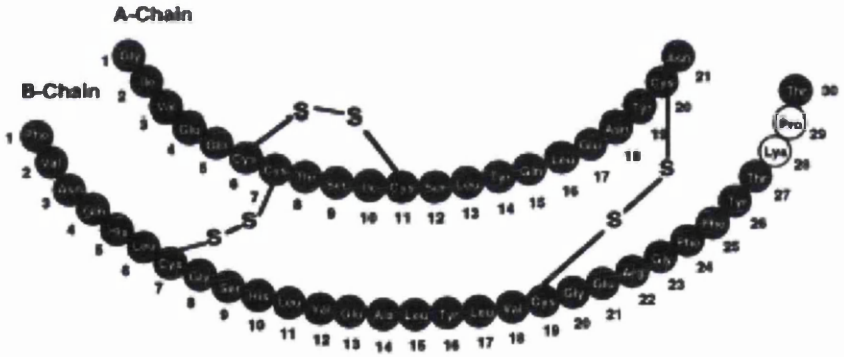


Figure 1.4: The amino acid structure of insulin lispro (Lys-Pro).

The reversal of the amino acids Pro B28 and Lys B29 resulted in the development of the widely used insulin lispro (Humalog®; Table 1.1); this modified analogue elicits an extremely fast rate of absorption from the site of injection as well as a rapid onset time (5-20 minutes) and a highly intense effect, this making insulin lispro very effective for prandial use (Howey et al., 1994). Manipulating amino acid sequences within the A and B chains of

insulin has led to the development of multiple forms of insulin, all of varying action times (Figure 1.5 and Table 1.1) e.g. insulin aspart and glargine.

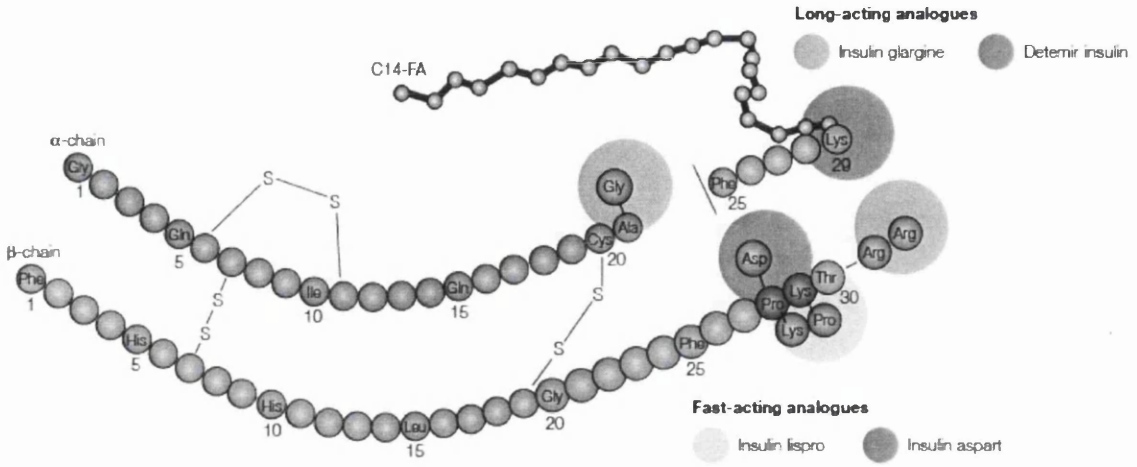


Figure 1.5: Alterations in the amino acid configuration of the A and B chain of insulin and the development of insulin analogues with varying action-time profiles (adapated from Owens, 2002).

The modification of the insulin structure allowed for T1DM individuals to be treated with a combination of slow and fasting acting insulins, providing a 24 hour insulin profile, similar to a non-T1DM individual, described by Bolli et al. (1999; Figure 1.3). This form of therapy is termed 'basal-bolus', where individuals administer once/bi-daily doses of a slow-acting insulin (e.g. insulin detemir or glargine) which provides a peak less low level of insulin for 24 hours, restraining hepatic glucose output and ketogenesis and maintains low rates of glucose uptake into insulin sensitive tissues. The fast/rapid acting insulins are administered at meal times and provide rapid-increases in insulin concentrations, which coincide with the digestion of the meal. Moreover, individuals may administer additional bolus insulin units in between meals to correct for high blood glucose concentrations.

Table 1.1: The types of insulin currently available to T1DM individuals.

Action	Insulin Type	Insulin Name	Manufacturer	Action Time (hours)			Reference
				Onset	Peak	Duration	
RAPID	Insulin Analogue	Humalog (Lispro)	Lilly	5 - 20 Min	45 - 60 mins	3 - 5 hours	Howey et al. (1994)
		Apidra (Glulisine)	Sanofi Aventis	10 mins	45 - 60 mins	3-5 hours	Danne et al. (2005)
		NovoRapid (Aspart)	Novo Nordisk	10 mins	45 - 60 mins	3-5 hours	Plank et al. (2002)
SHORT	Regular	Humulin R	Lilly	0.5 - 1 hour	2 - 4 hours	6 - 8 hours	Woodworth et al. (1994)
		Actrapid	Novo Nordisk	0.5 hours	2.5 - 5 hours	8 hours	
INTERMEDIATE	NPH	Humulin N	Lilly	1-2 hours	6-12 hours	18-24	Leopore et al. (2000)
		Novolin NPH	Novo Nordisk	1.5	04-12 hours	24	
	Lente	Humulin L	Lilly	1-3 hours	6-12 hours	18-24 hours	Diabetes Care Programme of Nova Scotia (2002)
		Novolin Lente	Novo Nordisk	2.5 hours	7-15 hours	22	
MIXEDPRE		Humalog Mix (25% lispro and 75% protamine suspension	Lilly	30 - 45 mins	0.75-2.5	18-24	
		Humulin 20/80 Humulin 30/70	Lilly	30 mins	2-12 hours	18-24	
LONG	Ultralente	Humulin U	Lilly	4-6 hours	8-20 hours	24-28 hours	Leopore et al. (2000)
		Novolin Ultralente	Novo Nordisk	4 hours	8-24 hours	28 hours	
	Insulin Analogue	Lantus (Glargine)	Lantus	1.5 hours	none	20-24 hours	Leopore et al. (2000)
	Insulin Analogue	Levemir (Detemir)	Novo Nordisk	2.5 hours	none	20-24 hours	Porcellati et al. (2007)

1.4.2 Calculating insulin dose: carbohydrate counting and rapid-acting insulin dose

Carbohydrate counting allows T1DM individuals to adjust their rapid-acting insulin units to meals with varying carbohydrate content (Kulkarni, 2005) allowing greater flexibility in their diets (Dias et al., 2010). Moreover, it has been shown to be important for glycaemic control, with reductions in HbA_{1c} of ~0.9% after just 3 months of carbohydrate counting (Dias et al., 2010). Within those individuals treated with the basal-bolus regimen it is important that they understand what their target blood glucose is, i.e. euglycaemia, how the different insulins they are treated with act (i.e. onset of action and duration), and it is vital that individuals record their blood glucose responses to different meals. Through trial and error the patient is able to calculate their insulin : carbohydrate ratio through blood glucose concentrations being higher or lower than the target concentration, post-meal (Kulkarni, 2003).

Individuals new to the basal-bolus regimen may start with a ratio of 0.5 - 1 insulin unit per 10 or 15 g of carbohydrates, and through carefully recording their blood glucose responses they are able to self-adjust this ratio until optimal. For example, a T1DM individual elicited euglycaemic blood glucose concentrations after administering 5 insulin units with a meal containing 75 g of carbohydrates. For this individual, the insulin : carbohydrate ratio would be 1 IU per 15 g, or 1:15 (Kulkarni, 2005). This ratio may change over the course of the day and individuals may need to alter this ratio to account for physical activity and/or planned or unplanned exercise (Kulkarni, 2005).

1.4.3 Calculating basal insulin dose

In contrast to rapid-acting insulin, there is no method to calculate basal dose other than trial and error. The clinician or diabetes specialist nurse may choose to increase or decrease the basal dose depending on HbA_{1c}, daily mean blood glucose concentrations, fasted blood glucose concentrations, between meal glucose excursions and the incidence of hypoglycaemia. Due to the importance of identifying an optimal basal dose for glycaemic control and restraining both hepatic glucose output and ketogenesis, it is doubtful that the individual will alter this dose in anticipation of exercise. Moreover, altering basal insulin units will have an influence on metabolism for as long as 24 hours after administration. Conversely, rapid-acting insulin alterations have a far more short-term effect, and it is for this reason that rapid-acting insulin is adjusted to changes in diet and exercise.

1.5 The benefits of exercise for T1DM individuals

Regular exercise can reduce the risk of chronic disease and premature death within both T1DM (Moy et al., 1993) and non-T1DM individuals (Warburton et al., 2006). Regular exercise within T1DM and non-T1DM has been shown to improve body composition, i.e. reduce abdominal adipocity and improved weight control (Lehmann et al., 1997; Seidell et al., 1991; Slattery et al., 1992; Warburton et al., 2001), enhance lipid lipoprotein profiles, i.e. increase high density lipoproteins and reduce low density lipoproteins (Berg et al., 1997; Durant et al., 1993; Lehmann et al., 1997; Tell and Vellar, 1988), improve glucose homeostasis and insulin sensitivity (Kelley and Goodpaster, 1999; Lehmann et al., 1997; Wallberg-Henriksson et al., 1998; Warburton et al., 2001; Young, 1995), reduce blood pressure (Lehmann et al., 1997; Whelton et al., 2002), improve autonomic tone (Tiukinhoy et al., 2003), reduce systemic inflammation (Adamopoulos et al., 2001), decrease blood coagulation (Physical Activity and Cardiovascular Health, 1996), improve coronary blood flow (Hambrecht et al., 2000), augment cardiac function (Gokce et al., 2002; Warburton et al., 1999) and enhance endothelial function (Fuchsjäger-Mayrl et al., 2002; Gokce et al., 2002; Kobayashi et al., 2003). There are additional benefits of exercise which are specific to T1DM individuals, exercise training has been demonstrated to reduce daily insulin dose (Ramalho et al., 2006) and improve long-term glycaemic control, through reductions in HbA_{1c} (Campaigne et al., 1984; Mosher et al., 1998; Salvatoni et al., 2005; Sideravičiūté et al., 2006), and reduce the incidences of hypoglycaemia (Lehmann et al., 1997). However, the improvements in glycaemic control demonstrated within the existing literature have been induced by different exercise regimens (e.g. Mosher et al., 1998; Ramalho et al., 2006; Sideravičiūté et al., 2006; Table 1.2). Moreover, research has demonstrated beneficial changes in the T1DM individual's condition independent of changes in HbA_{1c} (Fuchsjäger-Lehmann et al., 1997; Mayrl et al., 2002; Table 1.3).

Table 1.2: Research examining the effects of different exercise regimens on anthropometric and metabolic parameters in T1DM individuals.

Reference	Participants	Type	Frequency and Duration	Exercise	Findings
Mosher et al. (1998)	10 T1DM males	Mixed, Aerobic Circuits	45 minutes, 3 x a week for 12 weeks.	Running/cycling/rowing combined with body weight circuits (e.g. lunges, skipping, press ups)	↑ CV fitness, ↑ lean mass, ↑ strength, ↓ HbA_{1c} (~0.96%) and ↓ LDL
Ramalho et al. (2006)	16 T1DM	Aerobic vs. Resistance	Aerobic – 40 min at ACSM guidelines for diabetes, 1 x per week. Resistance – 3 x 40 min per week for 12 weeks	Aerobic – Running/walking. Resistance – 9 exercises, 3 sets of 8-12 reps on each exercise	Aerobic ↑ HbA_{1c}, while the resistance group elicited small, non-significant ↓. Both groups elicited ↔ in blood lipid profile. Both groups ↓ total daily insulin units.
Sideravičiūtė et al. (2006)	19 T1DM Females	Aerobic	45 minutes, 2 x per week for 14 weeks	Pulse rate controlled swimming	↓ in HbA_{1c} of ~ 0.74%

Table 1.3: Research demonstrating beneficial effects of different exercise regimens on anthropometric and metabolic parameters, independent of changes in glycaemic control, within T1DM individuals.

Reference	Participants	Type	Frequency and Duration	Exercise	Findings
Lehmann et al. (1997)	20 T1DM	Mixed, Aerobic Activity	At least 135 min per week	Running, Cycling, Hiking	↔ HbA_{1c}, ↑ CV fitness, ↑ lean mass, ↑ strength, (~0.96%) and ↓% BF, ↓LDL, ↑HDL, ↑ insulin sensitivity
Fuchsjäger-Mayrl et al. (2002)	18 T1DM (11 males, 7 females)	Aerobic	3 x 1 hour per week	Cycling	↔ HbA_{1c} ,↑CV fitness, ↑ endothelial function, ↓ insulin dose, ↔ HDL, LDL, BP, body mass

1.6 Exercise modality, intensity and duration

At present the American College of Sports Medicine (2006) suggest T1DM individuals perform aerobic type exercise, for 20 – 60 minutes, 3 – 4 times per week at an intensity of 50 – 80% of HR reserve or VO_2 reserve. However, in terms of avoiding hypoglycaemia during and after the exercise bout, the most appropriate exercise modality, i.e. intermittent or continuous exercise, remains questionable. According to Guelfi et al. (2005b) continuous exercise is associated with a greater risk of hypoglycaemia than intermittent type exercise.

Research has demonstrated that the performance of intermittent high-intensity sprints (11 x 4 s cycle sprints, every 2 mins for 20 minutes) does not increase the risk of developing hypoglycaemia during and for 60 minutes post-exercise, when compared to a resting control trial (Guelfi et al., 2005a). Moreover, intermittent (INT) exercise has been shown to preserve blood glucose concentrations more so than continuous (CON) exercise, and reduce the risk of hypoglycaemia during and after exercise (Guelfi et al., 2005b; Maran et al., 2010). In a study by Guelfi et al. (2005b), blood glucose responses to both continuous and intermittent exercise were compared. Participants performed 30 minutes of cycling at 40% VO_{2peak} (CON) or 30 minutes of cycling at 40% VO_{2peak}, interspersed with 4 s maximal sprints every 2 minutes (INT). Blood glucose responses revealed a lesser decline during exercise under INT, despite performing more work. Moreover, concentrations remained stable for 60 minutes post-exercise, whereas they continued to decline under CON. The preserved blood glucose concentrations were suggested to be related to large increases in catecholamines and growth hormone. According to Bussau et al. (2006) the drop in blood glucose that occurs post-exercise after continuous exercise, as within the research of Guelfi et al. (2005b), can be prevented by the addition of a 10 s maximal sprint at the end of the exercise bout (20 minutes of cycling at 40% VO_{2peak}). However, it is important to note that none of the aforementioned

research employed pre-exercise insulin reductions. If pre-exercise insulin reductions were employed, such large differences in blood glucose between continuous and intermittent exercise may not occur.

Research has demonstrated no difference in blood glucose responses to continuous or intermittent exercise within 9 T1DM individuals (Bracken et al., 2008). Participants performed 45 minutes of continuous running and 45 minutes of intermittent running (designed to simulate intermittent team game play). Participants reduced their rapid-acting insulin dose by 50% with the meal prior to exercise and blood glucose responses were monitored during and for 24 hours after exercise using a continuous glucose monitoring system. Blood glucose responses revealed both conditions to induce a decline in blood glucose, however there were no between condition-differences in blood glucose responses. Comparing the research of Bracken and colleagues with Guelfi et al. (2005b), the heavy reduction to pre-exercise insulin may be a contributing factor to the lack of difference between conditions. Moreover, there is potential that if insulin reductions were employed before high-intensity interval exercise, the exercise induced increases in counter-regulatory hormones could create a milieu that promotes post-exercise hyperglycaemia.

From a practical application point of view, the work of Guelfi et al. (2005b) was designed to simulate team game demands, however, only lasted 30 minutes and was performed on a cycle ergometer. Potentially, performing maximal sprints on a cycle ergometer may be more simple than during other types of exercise, e.g. treadmill running. Moreover, within the research of Guelfi et al. (2005b) and Bussau et al. (2006) participant mean age was just 22 and 21 years old, respectively. Potentially, it is unlikely that more elderly T1DM individuals will be inclined to perform high-intensity intermittent exercise as a method to preserve blood glucose

during after exercise, as the risk of injury may be higher (Wenger and Bell, 1986). Additionally, although high-intensity (90-100% VO_{2max}) exercise will increase cardiovascular fitness more so than lower intensity exercise (Tabata et al., 1996; Wenger and Bell, 1986), research suggests that when lower intensity exercise exceeds 35 minutes, there are similar gains in cardiovascular fitness, when compared with short-duration high-intensity training (Wenger and Bell, 1986). In light of this, methods to help preserve blood glucose concentrations during sub-maximal continuous exercise should be investigated.

At present, current literature examining different factors (e.g. exercise mode, insulin and carbohydrate administration before exercise) affecting blood glucose responses within T1DM individuals has been predominantly during and after cycling (Bussau et al., 2006; Campaigne et al., 1987; Chokkalingham et al., 2007; Dandona et al., 1980; Dubé et al., 2005; Guelfi et al., 2005a; 2005b; Hernandez et al., 2000; Jenni et al., 2008; Mauvais-Jarvis et al., 2003 Perrone et al., 2005; Peter et al., 2005; Rabasa-Lhoret et al., 2001; Touminen et al., 1995), with little research examining blood glucose responses to running (Bracken et al., 2008). Cycling is a primarily concentric form of exercise i.e. the muscle shortens as it contracts. However, in many daily activity patterns including body weight supporting exercises, such as jogging or running, there is a significant proportion of eccentric muscle action, where the muscle lengthens in the performance of the movement. Moreover, eccentric muscle actions have been demonstrated to hinder insulin action and glucose uptake for many hours following exercise (Asp et al., 1995; Asp et al., 1996). Additionally, running which supports body mass, would also act as a greater metabolic stress than cycling, which could have the potential to influence blood glucose responses not just during exercise, but also potentially in the hours after.

1.7 Strategies for preventing and/or minimising post-exercise hypoglycaemia

In light of the aforementioned defects in glucose regulation and the high risk of hypoglycaemia associated with exercise, strategies that help combat hypoglycaemia have received considerable attention within the literature (Campaigne et al., 1987; De Feo et al., 2006; Dubé et al., 2005; Grimm, 2005; Guelfi et al., 2005b; Hernandez et al., 2000; Iafusco et a;., 2006; Muvais-Jarvis et al., 2003; Rabasa-Lhoret et al., 2001). An important aspect of the research focuses on reducing the pre-exercise insulin dose (Campaigne et al., 1987; Muvais-Jarvis et al., 2003; Rabasa-Lhoret et al., 2001).

1.7.1 The importance of insulin dose on post-exercise glycaemia

Reducing the dose of insulin administered with a carbohydrate meal would result in a reduction in the insulin to carbohydrate ratio, resulting in a reduced clearance of blood glucose after digestion of the carbohydrate meal. This would result in a preservation of blood glucose above normal concentrations before exercise (Rabasa-Lhoret et al., 2001). During exercise the reduced insulin concentrations would aid in promoting a pattern of substrate oxidation and glucose regulation similar to a non-T1DM individual (Chokkalingam et al., 2007), i.e. reduced uptake of glucose and a subsequent reduction in carbohydrate oxidation with a concomitant increased contribution to energy turnover from NEFA's (Chokkalingam et al., 2007; Figure 1.6). The lower circulating insulin concentrations would have a lesser restraining influence over hepatic glucose production, and the provision of NEFA, from the adipose tissue, for the active musculature (Figure 1.6). Moreover, a reduction in insulin mediated glucose uptake, in combination with elevations in the catecholamines would reduce the uptake and subsequent combustion of blood glucose and preserve blood glucose concentrations (Cryer, 2003). In the post-exercise period, the greater blood glucose concentrations would help compensate for the heightened uptake of glucose by the exercised

tissue (Steppel and Horton, 2003), which is increased to replenish muscle glycogen stores. Moreover, the lower insulin concentrations would provide a milieu where the actions of the gluco-regulatory hormones are not inhibited and insulin's suppressive effect at the hepatocyte and adipocyte is lessened, such that substrates can be provided for both hepatic glucose production and replenishment of muscle glycogen stores (Steppel and Horton, 2003). Therefore, the greater pre-exercise blood glucose concentrations and an improved hormonal milieu, i.e. lower circulating insulin concentrations, during exercise would aid in preserving blood glucose concentrations during exercise such that post-exercise concentrations can be better maintained and ultimately lessen the risk of hypoglycaemia.

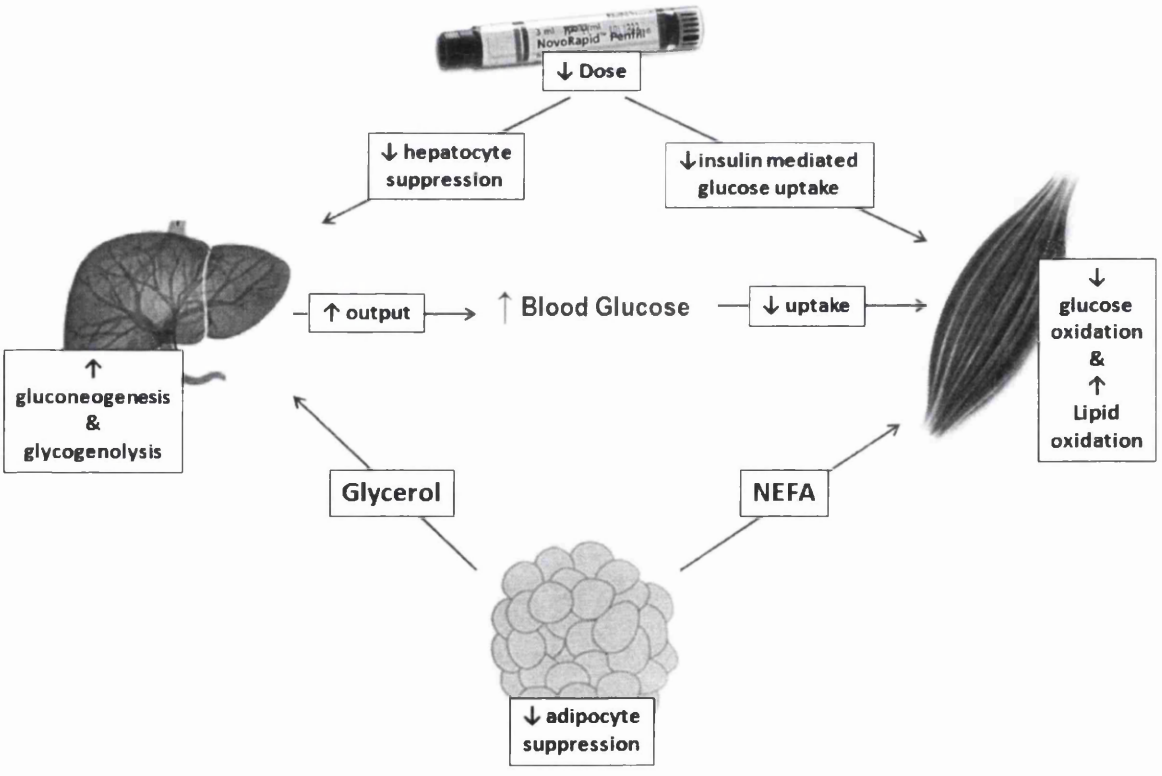


Figure 1.6: The theoretical effects of reductions in insulin dose on blood glucose regulation and fuel metabolism during exercise.

Within the existing literature examining pre-exercise insulin reductions, recommendations have varied from >50% (Campaigne et al., 1987), 10-40% (De Feo et al., 2006), 10-50% (Grimm, 2005), 50-90% (Mauvais-Jarvis et al., 2003) and 50-75% (Rabasa-Lhoret et al., 2001; Table 1.4). Some of the variation in the recommended reduction can be accounted for by differences in the insulin species used by participants and the exercise model employed within the respective studies (Table 1.4).

Table 1.4: Summary of current literature investigating the effects of reducing pre-exercise insulin dose on the maintenance of glycaemia.

Reference	Participants	Participant Insulin Regimen	Insulin reduction	Exercise	Findings
Campaigne et al. (1987)	9 T1DM males	2 daily - premixed (NPH & Soluble)	A - 50% ↓ of intermediate insulin B - 50% ↓ of soluble insulin C - No change	45 Minutes, continuous cycling at 60% VO_{2max}	Hypoglycaemia occurred despite reductions. ↑ nocturnal hypoglycaemia under C. Hypoglycaemia occurred despite insulin reductions.
Rabasa-Lhoret et al. (2001)	8 T1DM males	Basal Ultralente with prandial Lispro	No change, 50% or 75% ↓ in all exercise protocols	Cycling at - A - 25% VO_{2max} for 1 h B - 50% VO_{2max} for 30 and 60 mins C - 75% VO_{2max} for 30 mins	No insulin reduction ↑ chance of hypo at all intensities. Appropriate adjustments maintain glycaemia during and after exercise
Mauvais-Jarvis et al. (2003)	12 T1DM males	NPH and Regular insulin twice (n = 6) or 3 times (n = 6) daily	50% ↓ for subjects on twice daily regimen, 90% ↓ for 3 times daily	1 h, continuous cycling at 70% $V0_{2max}$	No reduction ↑ chance of hypoglycaemia. 50-90% reductions depending on insulin regimen can maintain glycaemia during and after exercise

An early study by Campaigne et al. (1987; Table 1.3) was one of the first to research pre-exercise insulin reductions, examining blood glucose responses during and after 45 minutes of cycling, within 9 T1DM males who were treated with a bi-daily, intermediate/short acting insulin mix. The authors demonstrated that despite 50% reductions in the intermediate or the soluble insulin prior to exercise, hypoglycaemia still occurred in 6 of the 9 subjects at some point during or after exercise, predominantly the night (self-reported) of the trial day. Additionally, Mauvais-Jarvis et al. (2003; Table 1.3) examined pre-exercise insulin reductions during and for 2 hours after exercise, within 12 T1DM individuals. Six of the participants were treated with regular insulin in the morning and at noon and NPH before bed, while the other 6 participants were treated with bi-daily mixed insulin regimen of 30% regular insulin combined with 70% NPH insulin. Participants performed 60 minutes of cycling at 70% VO_{2max}, 90 minutes after a set meal where participants administered an unaltered insulin dose or a 90% insulin reduction (participants on 3 daily injections) / 50% insulin reduction (bi-daily mixed regimen). The plasma glucose responses within the study of Mauvais-Jarvis et al. (2003) are presented in Figure 1.7.

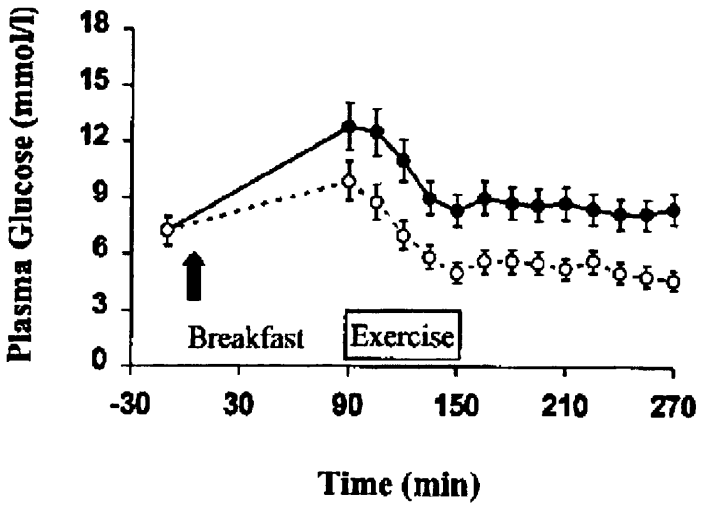


Figure 1.7: Changes in plasma glucose levels during exercise and recovery performed with (■) and without (□) an insulin reduction. Adapted from Mauvais-Jarvis et al. (2003).

Eight participants had to receive an oral glucose solution during the condition without an insulin reduction due to rapidly falling plasma glucose concentrations. Plasma glucose levels were consistently higher during and for 2 hours after exercise within the insulin reduction trial (Figure 1.7). It was concluded that a 50-90% reduction in insulin dose, depending on their insulin regimen, can allow T1DM individuals to engage in intensive exercise without causing hypoglycaemia and worsening glycaemic control (Mauvais-Jarvis et al., 2003)

The research of Campaigne et al. (1987) and Mauvais-Jarvis et al. (2003) lacked specific guidelines for pre-exercise insulin reductions, i.e. dose adjustments relating to exercise intensity and duration. Moreover, with the increased prescription of the basal-bolus regimen to treat T1DM individuals, dose adjustments specific to this kind of treatment, as opposed to the mixed insulins of Campaigne et al. (1987) and Mauvais-Jarvis et al. (2003), needed investigating. In addition, the time post-exercise where the potential for monitoring blood glucose was just 2-12 hours (Campaigne et al., 1987; Mauvais-Jarvis et al., 2003), and as previously demonstrated hypoglycaemia may develop up to 24 hours after exercise (MacDonald, 1987; Tsalikian et al., 2005), so this window of examination needs to be greater to determine the effectiveness of the insulin reduction.

Research by Rabasa-Lhoret et al. (2001; Table 1.4) furthered the area, addressing the issues of exercise intensity and duration within individuals treated with the more preferentially prescribed, basal-bolus regimen (Ultralente with prandial insulin lispro). Participants performed 60 minutes at 25% VO_{2max}, 30 and 60 minutes at 50% VO_{2max}, and 30 minutes at 75% VO_{2max}, with blood glucose concentrations monitored throughout exercise and for an hour post-exercise. All trials were performed after administration of a full insulin dose (**Full**), a 50% reduction (**50%**) and after a 75% reduction (**25%**). Rabasa-Lhoret and colleagues

demonstrated that the drop in blood glucose that occurs with exercise at 25% VO_{2max} for 60 minutes did not differ between **Full** and **50%**, however, greater pre-exercise concentrations result in a safer glycaemic profile after exercise, i.e. a greater preservation of blood glucose. Plasma glucose at the end of exercise was Δ-2.9 ± 1.1 $mmol.l^{-1}$ below baseline after **Full**, compared with Δ-0.6 ± 0.9 $mmol.l^{-1}$ after **50%**. This trend followed during exercise at 50% VO_{2max} for 30 minutes; the decrease in plasma glucose, relative to rest, at the end of exercise was less under **50%** (Δ-0.4 ± 1.3 $mmol.l^{-1}$) compared with **Full** (Δ-2.1 ± 0.7 $mmol.l^{-1}$) and resulted in greater plasma glucose concentrations during and for 1 hour after exercise. Plasma glucose responses revealed that the greatest preservation of post-exercise glycaemia occurred after a 75% reduction, when exercising at 50% VO_{2max} for an hour and 75% VO_{2max} for 30 minutes. The 75% reduction trial resulted in a better maintenance of glycaemia during and after exercise (~7 – 10 $mmol.l^{-1}$), with less chance of developing hypoglycaemia, compared to just a 50% reduced dose, which elicited post-exercise concentrations of ~4.5 – 7 $mmol.l^{-1}$. Based on plasma glucose responses during exercise, the optimal pre-exercise insulin reductions recommended by Rabsa-Lhoret et al. (2001) are presented in Table 1.5.

Table 1.5: Guidelines for the reduction in pre-exercise bolus insulin dose recommended by Rabasa-Lhoret et al. (2001).

	% Dose Reduction	
Exercise Intensity (% VO_{2max})	30 min of exercise	60 min of exercise
25	25	50
50	50	75
75	75	-

When comparing blood glucose responses across studies it is evident that the choice of insulin species is an important factor. Currently, T1DM individuals are predominantly treated with the modern insulin analogues (Table 1.1) in a basal-bolus regimen; these rDNA insulins (e.g. insulin glargine/detemir and aspart/lispro) offer very different, more favourable, action-time profiles and less variability than longer established insulins, such as regular human insulin and NPH insulin (Brange and Vølund, 1999; Leopore et al., 2000; Tuominen et al., 1995). Moreover, research has demonstrated that the uptake kinetics of modern basal analogues are not affected by exercise (Peter et al., 2005), however, the bolus analogues are (Touminen et al., 1995). With this in mind, there is no literature that has examined pre-exercise insulin reductions using these kinds of insulins. Moreover, with the potential for these insulins to affect metabolism and blood glucose concentrations from 5-24 hours after administration (Leopore et al., 2000; Plank et al., 2002), and the potential for developing hypoglycaemia increased for a similar time frame after exercise (MacDonald, 1987; Tsalikian et al., 2005), as well as current literature only investigating blood glucose responses for just 1 - 12 hours post-exercise (Campaigne et al., 1987; Mauvais-Jarvis et al., 2003; Rabasa-Lhoret et al., 2001), a more detailed examination of the effects of pre-exercise insulin reductions after exercise is required.

1.7.2 The safety of using pre-exercise insulin reductions

Exogenous insulin treatment reduces blood glucose, which prevents hyperglycaemia and risk of ketosis (Cryer, 2001). Under normal physiological conditions ketones (acetoacetate, β-hydroxybutyrate and acetone) are produced through hepatic fatty acid metabolism during periods of low carbohydrate conditions. Ketogenesis allows fat-derived energy to be generated in the liver and used by other organs, such as the brain, heart, kidney cortex and skeletal muscle (Laffel, 1999). However, reduction or omission in insulin dose is a significant

factor in the development of diabetic ketoacidosis accounting for 13-45% of reported DKA cases (Wallace and Matthews, 2004). The formation of ketone bodies above non-physiological levels (>1 $mmol.l^{-1}$) has been proven to increase oxygen radical formation and cause lipid peroxidation (Jain et al., 1998; Jain et al., 1999a; 1999b) as well as induce metabolic acidosis (Laffel, 1999). Diabetic ketoacidosis is characterised by an absolute or relative deficiency of circulating insulin and combined increases in counter-regulatory hormones (catecholamines, glucagon, cortisol, growth hormone), particularly glucagon and adrenaline, hyperglycaemia and metabolic acidosis (Wallace and Matthews, 2004). Moreover, physical exercise also increases ketone body formation (Koeslag et al., 1980; Horowitz et al., 2000), alters acid-base balance and increases counter-regulatory hormones (Figure 1.8). Therefore, the potential for a combined effect of a pre-exercise insulin reduction strategy and performance of exercise might exacerbate ketogenesis and result in hyperketonaemia (>1.0 $mmol.l^{-1}$) or development of ketoacidosis (>3.0 $mmol.l^{-1}$) (Laffel et al., 1999; Figure 1.8). However, there is limited data available to refute this hypothesis.

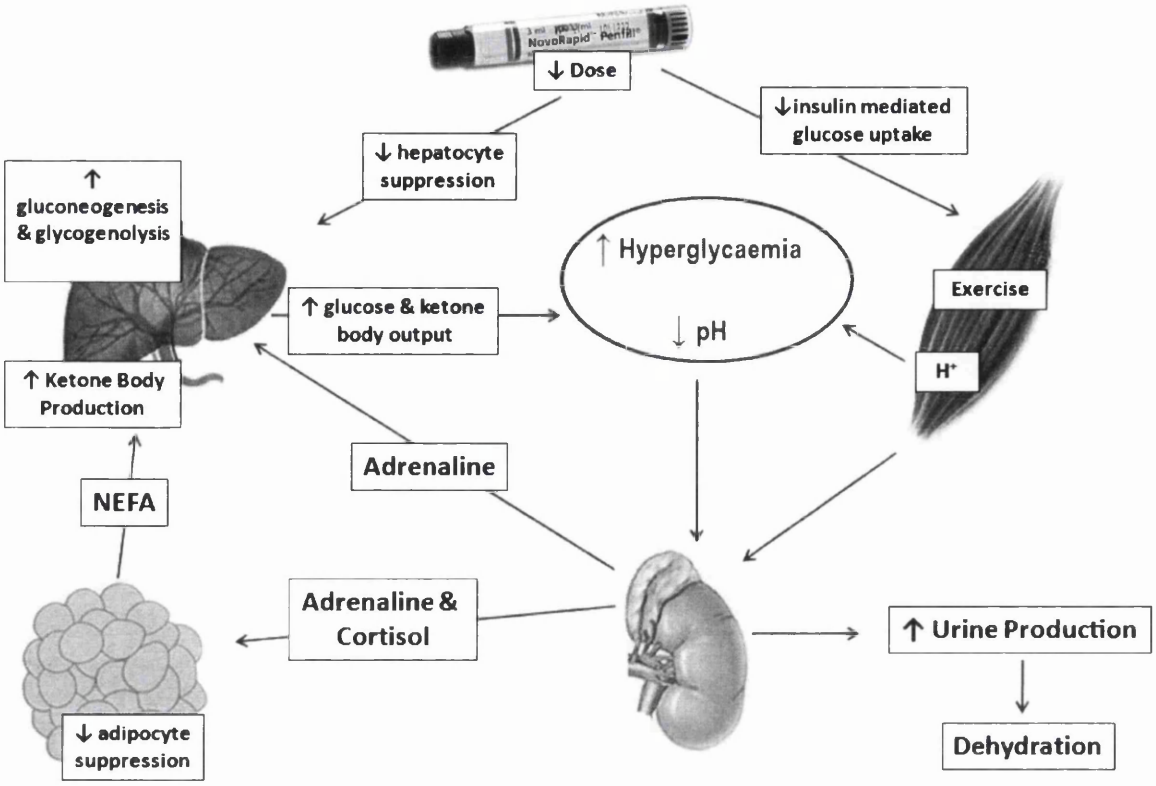


Figure 1.8: Theoretical effects of exercising under a reduced insulin dose on ketogenesis.

1.8 Current recommendations for carbohydrate intake and exercise in T1DM

The literature concerning the amount of carbohydrate to consume before, during and after exercise to prevent hypoglycaemia within T1DM individuals has produced diverse findings. For example, Hernandez et al. (2000; Table 1.6) suggested that 60 - 120 g of carbohydrates should be consumed, in equal bolus', before, during and immediately after exercise to prevent late-onset hypoglycaemia. The range in recommended carbohydrate intake is likely due to differences in glycogen depletion across participants (Perry and Gallen, 2009). Whereas Iafusco (2006) recommends consumption of 15 g of simple carbohydrates immediately before exercise and consumption of a hypotonic sports drink (e.g. Gatorade®, 4% sucrose, 2% fructose) during exercise. Conversely, Dubé et al. (2005; Table 1.6) suggests just a 35 g bolus of dextrose is needed, immediately before exercise, to combat hypoglycaemia. Dubé and colleagues investigated pre-exercise glucose requirements in order to preserve blood glucose concentrations during exercise. Three hours after consuming a standardised breakfast, with their usual insulin dose, participants consumed a 0, 15 or 30 g of bolus of glucose 15 minutes prior to 60 minutes of cycling at 50% VO_{2max}. The drop in blood glucose during exercise was similar across trials, however, the addition of 15 or 30 g of glucose prolonged the delay before glucose infusion was required (0 g ~32; 15 g ~51; 30 g ~56 min). During the exercise bout, 7 of 9 individuals required glucose infusion under the 0 g trial, 4 of 9 required glucose infusion under the 15 g trial, and just 3 under the 30 g condition. Based on the glucose infusion requirements, the authors estimated that a beverage containing 35 g of glucose should be consumed immediately before exercise to maintain blood glucose during 60 minutes of moderate intensity exercise.

In addition to the recommendations of Iafusco (2006) and the research of Dubé et al. (2005), Perrone et al. (2005; Table 1.6) addressed the concentration of the carbohydrate beverage

consumed immediately before and throughout the exercise bout. Participants cycled at 55-60% VO_{2max} for 60 minutes, consuming either an 8% or a 10% carbohydrate solution before and during exercise. Throughout the duration of the trial, blood glucose concentrations were lower under the 8% solution; moreover, 4 individuals experienced hypoglycaemia under this condition. Furthermore, blood glucose concentrations dropped ~1.8 $mmol.l^{-1}$ in the hour post-exercise, whereas concentrations remained stable under the 10% condition. Based on the blood glucose responses and hypoglycaemic incidences the authors recommended T1DM individuals consume a 10% carbohydrate solution before and during exercise to maintain glycaemia. Additionally, to quickly correct falling blood glucose during exercise, Gallen (2005) recommends consumption of a 15% carbohydrate solution.

This is interesting, as carbohydrate concentration is an important factor in post-ingestion blood glucose responses, with research demonstrating reduced gastric emptying rates with concentrations ranging from 6-20% (Davis et al., 1990; Jeukendrup et al., 2007; Maughan and Leiper, 1999; Murray et al., 1997). However, this research was carried out within non-T1DM individuals. Low blood glucose concentrations have been demonstrated to increase gastric emptying rates within non-T1DM individuals (Schvarcz et al., 1993), thus potentially explaining the greater concentrations, recommended by Peronne et al. (2005) and Gallen (2005). Moreover, it should be noted that gastric emptying rates are also affected by elevated blood glucose (Schvarcz et al., 1997). Schvarcz and colleagues demonstrated a slowing of gastric emptying when blood glucose concentrations were clamped at just 8 $mmol.l^{-1}$, in comparison with 4 $mmol.l^{-1}$, within T1DM individuals. In addition, gastric emptying rates are also influenced by long term glycaemic control (Jing et al., 2009). Therefore, blood glucose concentrations and long term glycaemic control may be important factors that contribute to the optimal concentration of carbohydrates to be administered, moreover may be contributing

factors to inter-individual variability in blood glucose responses that may exist after administration of carbohydrate.

Based on the current literature it seems 15 – 35 g of simple carbohydrates should be consumed immediately prior to exercise. Moreover, a carbohydrate solution of 10% should be consumed during the exercise bout. However, the existing literature did not examine the influence of carbohydrate feeding on blood glucose responses in the more long term period after exercise. Moreover, it is important to note that none of the aforementioned research employed a pre-exercise insulin reduction. Potentially, employing an insulin reduction strategy could have resulted in very different carbohydrate requirements. Moreover, an optimal reduction to the insulin dose administered with the meal before exercise may even negate the need to consume any additional carbohydrates before, during and immediately after the exercise bout.

Table 1.6: Summary of current literature examining carbohydrate consumption in order to prevent hypoglycaemia during and after exercise.

Reference	Participants	Participant Insulin Regimen	Protocol	Exercise	Findings
Hernandez et al. (2000)	7 T1DM (6 males, 1 female)	Bovine/porcine ultralente with regular human insulin	Water (0 g CHO), Whole Milk (40 g), Skim Milk (66 g), Sports Drink A (121 g) and Sports Drink B (74 g) consumed in thirds immediately before, during, and after exercise. BG monitored 12 hours post-exercise.	60 minutes cycling at 60% VO_{2max}. After 30 min a 10 min rest period was carried out for fluid ingestion.	No trial completely prevented hypoglycaemia. Milk trials had ↓ pre-bed BG concentrations. During milk trials - no early morning incidents' of hypoglycaemia, there was 1 incident under Sports Drink B. Authors conclude CHO beverage must be consumed before, during and after exercise. Amount may depend on level of glycogen depletion across participants.
Dubé et al. (2005)	9 T1DM (6 males, 3 females)	Bi-daily NPH and prandial insulin lispro	3 hours after a standardised breakfast (8 $kcal.kg^{-1}$) participants consumed either 0 g, 15 g or 30 g of glucose immediately before exercise.	60 minutes, continuous cycling at 50% VO_{2max}	30 g delayed the time before glucose needed to be infused more than 15 g. 7 of 9 needed glucose infusion under 0 g, 4 of 9 under 15 g and 3 under 30 g. Authors estimate a beverage of 35 g of glucose is required to maintain BG for 60 min of moderate exercise.
Perrone et al. (2005)	16 T1DM (10 males, 6 females)	Intermediate or ultra-long insulin	Participants consumed an 8% (5.4 g glucose; 2.6 g fructose per 100 ml) or 10% (6.7 g glucose; 3.3 g fructose per 100 ml) before and during exercise	60 minutes, continuous cycling at 55-60% VO_{2max}	4 incidents of hypoglycaemia during exercise under 8% and none under 10%. 60 min post-exercise BG ↓ under 8%, but ↔ under 10%. Authors recommend a 10% carbohydrate solution to avoid hypoglycaemia during exercise.

1.8.1 Carbohydrate type: the glycaemic index

The glycaemic index (GI; Figure 1.9) is a method of clinically classifying foods/meals containing carbohydrates according to glycaemic responses after ingestion (Wolever et al., 1991). For example, carbohydrates with a high GI, such as white bread, will induce a rapid increase in blood glucose concentrations after ingestion (Foster-Powell et al., 2002). Conversely, carbohydrates with a low GI, such as peaches, will induce more gradual increases, and lesser peaks, in blood glucose (Foster-Powell et al., 2002).

From a diabetes management perspective, this classification of foodstuffs is useful as both clinicians and patients can adjust their diets to include low GI carbohydrates due to benefits such as a greater feeling of satiety (Foster-Powell et al., 2002), improved insulin sensitivity and blood lipid profiles (Jenkins et al., 1985), lower daily mean blood glucose concentrations (Nansel et al., 2008), reduced incidence of hypoglycaemia and reductions in HbA_{1c} (Brand et al., 1991; Gilbertson et al., 2001; Thomas et al., 2007). Within the study of Nansel et al. (2008), consumption of low GI foodstuffs, such as peaches, kidney beans or brown rice, resulted in glucose concentrations (assessed using a continuous glucose monitor) being within a target range of 3.9 – 9.9 $mmol.l^{-1}$ significantly more of the time than under the HGI trial (67 vs. 47 %). Moreover, the participants elicited a lower mean blood glucose concentration (LGI 7.6 ± 2.0 vs. HGI 10.1 ± 2.6 $mmol.l^{-1}$) and required less bolus insulin per 10 g of CHO.

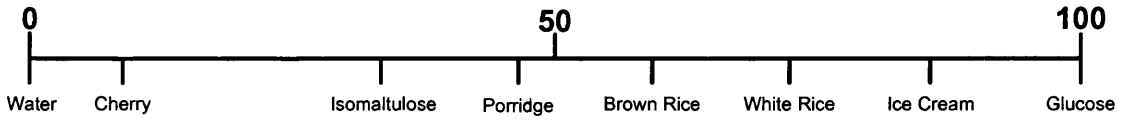


Figure 1.9: The glycaemic index.

With regards exercise, research in non-T1DM demonstrates less change in blood glucose concentration during exercise after low GI carbohydrates (Demarco et al., 1999; Achten et al., 2007; Table 1.7). Demarco et al. (1999; Table 1.6) examined 10 trained cyclists during 2 hours of cycling at 70% VO_{2max}, followed immediately by a time trial to exhaustion at 100% VO_{2max}, having consumed either a high or low GI meal before exercise. Blood glucose responses revealed a more gradual rise and fall in concentrations over the 120 minute exercise period under LGI, with concentrations at 120 minutes not different to immediate pre-exercise values. However, concentrations under HGI had significantly dropped over the 2 hour period. During the 120 minutes of cycling there was a greater lipid oxidation rate under LGI, in comparison with HGI. Moreover, despite a 60% increase in time to exhaustion, blood glucose concentrations were greater at the cessation of exercise under LGI. Achten et al. (2007) investigated plasma glucose responses to 150 minutes of cycling at ~ 60 % VO_{2max} after ingesting isomaltulose or sucrose with results demonstrating little change in plasma glucose over the duration of the protocol after consumption of isomaltulose (GI 32), compared to a ~1.2 $mmol.l^{-1}$ greater plasma glucose concentration 15 min after sucrose consumption. Moreover, consumption of low GI carbohydrates alters exercising fuel metabolism (Achten et al., 2007; Demarco et al., 1999; Stevenson et al., 2006). Lipid utilisation increases and carbohydrate oxidation rates decrease during a bout of isocaloric exercise, in non-T1DM individuals, following consumption of a low GI meal (Achten et al., 2007; Demarco et al., 1999; Stevenson et al., 2006). Stevenson et al. (2006) demonstrated a 56 % greater lipid oxidation rate and 31 % lower carbohydrate oxidation during a one hour treadmill run at 65 % VO_{2max} 3 hours following consumption of a low (139 g CHO; GI 44) or high GI (139 g CHO; GI 78) meal in eight female participants. Similarly, ingestion of a 50 g bolus of isomaltulose (GI 32) increased lipid oxidation rate more than sucrose and reduced

carbohydrate oxidation rate ~0.2 $g.min^{-1}$ during 150 minutes of cycling at 60 % VO_{2max} (Achten et al., 2007).

Table 1.7: Summary of current literature investigating the metabolic and glycaemic effects of altering the GI of the pre-exercise meal.

Reference	Participants	Participant Insulin Regimen	Pre-exercise meal	Exercise	Findings
Demarco et al. (1999)	10 males	Non-T1DM participants	Low GI (GI 36) or high GI (GI 70) meal 30 minutes prior to exercise.	120 minutes, continuous cycling at 70% VO_{2max}, followed by time to exhaustion at 100% VO_{2max}.	More gradual rise and fall in BG under LGI; at 120 min BG was stable under LGI, however had ↓ under HGI. There was an ↑ lipid oxidation during exercise under LGI. Moreover, ↑ time to exhaustion under LGI with higher post-exercise BG, when compared with HGI.
Stevenson et al. (2006)	8 females	Non-T1DM participants	High GI: 3.1 MJ, 139 g CHO, 10 g fat, 10 g protein, GI = 78 Or Low GI: 3.1 MJ, 139 g CHO, 9 g fat, 23 g protein, GI = 44	60 minutes, continuous running at 65% VO_{2max}	Lower plasma glucose and insulin at rest under low GI trial. Lower CHO (71 vs 101 g) and greater lipid (8 vs. 19 g) utilised under LGI.
Achten et al. (2007)	10 males	Non-T1DM participants	50 g isomaltulose (ISO) or sucrose (SUC)	150 minutes, continuous cycling at 60% $V0_{2max}$	1.2 $mmol.l^{-1}$ lower peak in BG after ISO. Lower peak in insulin after ISO. Less energy derived from CHO under ISO in comparison with SUC (13 vs. 25%) and a ↑ contribution from lipids.

Based on the existing literature, it seems consumption of low GI carbohydrates has the potential to induce less change in blood glucose before and during exercise. Moreover, low GI carbohydrates may suppress fat oxidation less during exercise, which may subsequently spare both endogenous and exogenous carbohydrate use, resulting in better preservation of blood glucose during and in the post-exercise period. However, there is scant research that has examined blood glucose and metabolic responses to exercise after different GI carbohydrates within T1DM. Moreover, none of the existing literature examining carbohydrate requirements within T1DM implemented a pre-exercise insulin reduction, which raises the possibility that if insulin dose is sufficiently reduced before exercise, additional carbohydrates may not be required before and during exercise.

1.8.2 Exogenous carbohydrate oxidation and glycaemia

As demonstrated, carbohydrate consumption prior to or during exercise is important for the exercising T1DM individual (Dubé et al., 2005; Hernandez et al., 2000; Iafusco, 2006). However, it has been suggested that a greater contribution to energy turnover from exogenous carbohydrate sources would spare endogenous carbohydrate reserves (liver and possibly muscle glycogen; Jeukendrup, 2004). Keeping in mind an increased glucose uptake to replenish muscle glycogen stores after exercise (Wojtaszewski et al., 2002), a sparing of endogenous carbohydrate stores would have implications for post-exercise blood glucose concentrations, and potentially the development of hypoglycaemia.

According to Jeukendrup (2004) exogenous carbohydrate oxidation is influenced by the type and amount of carbohydrate ingested and the exercise intensity, however is seemingly unaffected by the timing of consumption, gastric emptying (Rehrer et al., 1992; Saris et al., 1993) or solution osmalality (Shi et al., 1995). Shi and colleagues suggested that different

forms of carbohydrate in solution (glucose, fructose, sucrose) increased carbohydrate absorption despite an increase in osmalality. The authors attributed the increased uptake to different transporters used in the uptake process, i.e. glucose is transported across the luminal membrane by a sodium-glucose transporter (SGLT1) and fructose is transported by GLUT-5, therefore there was a reduced competition for transporters and an increase in total carbohydrate absorption. This was confirmed by Jentjens et al. (2003) where glucose ingestion alone resulted in maximal exogenous carbohydrate oxidation rates, during exercise, of 0.83 g/min, whereas a glucose and fructose mix increased oxidation rates by ~56% (~1.26 g/min). These factors may be important for the exercising T1DM individual; blood glucose concentrations are the primary concern for avoiding hypoglycaemia during exercise, however, if exogenous carbohydrate oxidation rates can be increased, through ingestion of different forms of carbohydrates, in addition to a greater contribution from lipids, a preservation of endogenous carbohydrate reserves may take place which may subsequently maintain blood glucose concentrations post-exercise. However, there is limited data to refute this hypothesis.

1.9 Pre-exercise timing of carbohydrate consumption and insulin administration

The timing of pre-exercise carbohydrate consumption and insulin administration is largely dependent upon the insulin species (Table 1.1) and the altered uptake kinetics that are associated with exercise (Dandona et al., 1980; Fernqvist et al., 1986; Touminen et al., 1995). Research has demonstrated that exercise can result in greater peaks in insulin as well as increasing absorption rates and ultimately increasing the risk of hypoglycaemia (Dandona et al., 1980; Touminen et al., 1995). The altered rates of absorption are likely due to a combination of increases in blood flow (Lauritzen et al., 1980; Linde and Gunnarsson, 1985; Vora et al., 1993) and temperature (Koivisto et al., 1980; 1981) that are associated with exercise.

Animal preparations and regular human insulin interact differently with exercise (Fernqvist et al., 1986; Table 1.8). Fernqvist and colleagues demonstrated that the exercise induced peak in insulin concentrations was less with regular human insulin as apposed to porcine insulin. Moreover, Touminen et al. (1995) demonstrated that the rDNA insulins, human insulin and the analogue insulin lispro, also interact differently with exercise, moreover the pre-exercise timing is particularly important in subsequent insulin and blood glucose responses (Table 1.8). Touminen and colleagues identified that when exercise was performed 40 minutes after administration, insulin lispro induced an earlier and 56% greater peak in insulin concentrations, consequently resulting in a greater drop in blood glucose with exercise, when compared to regular human insulin. Moreover, when exercising this close to administration, the exercise bout was associated with a 2.2 fold greater risk of hypoglycaemia. However, when exercising 180 minutes post-administration, the drop in blood glucose was less under insulin lispro, and the risk of hypoglycaemia was reduced by

46%, when compared to regular human insulin. This research highlights pre-exercise timing as an important factor to consider, as the intense rise and peak in insulin that is elicited soon after administration of rapid-acting insulin, results in marked increases in the risk of hypoglycaemia during exercise.

Table 1.8: Summary of current literature examining the interaction between insulin, and different insulin species, and exercise.

Reference	Participants	Participant Insulin Regimen	Protocol	Exercise	Findings
Dandona et al. (1980)	5 T1DM	Not Specified	10 IU regular human insulin administered before resting or before exercise.	60 minutes of intermittent moderate intensity cycling	Exercise induced a doubling in peak insulin concentrations, which occurred 40 minutes earlier, when compared with rest.
Fernqvist et al. (1986)	9 T1DM	NPH with regular human insulin	10 IU of regular human insulin or porcine insulin 30 minutes before exercise.	40 minutes continuous cycling, moderate intensity	Exercise increase absorption of both insulin's, the increase was ↓ under human. Insulin AUC was similar between the two insulins. Blood glucose dropped similarly between the two insulin species.
Touminen et al. (1995)	10 T1DM (7 males, 3 females)	NPH with Prandial regular human insulin	Exercise was performed early (40 min post-administration) and late (180 min post-administration) after administration of lispro or regular insulin (6.3 IU).	40 minutes, continuous cycling, moderate intensity	Lispro peaked earlier and 56% ↑ concentrations. Lispro associated with a 2.2 fold ↑ risk of hypoglycaemia during early exercise but 46% ↓ during late exercise. Drop in BG with exercise ↑ under lispro, soon after administration, but ↓ during late exercise.

Note: AUC = area under the curve

The lower variability and more favourable action-time profiles of the rapid-acting insulins (Brange and Vølund , 1999; Tuominen et al., 1995) make these analogues of insulin ideal for prandial use, and have been shown to significantly improve glycaemic control within T1DM individuals, without increasing the risk of hypoglycaemia (Tamás et al., 2001). However, the intense and rapid rise in insulin concentrations (peaking 45 – 60 minutes after administration, Plank et al., 2002) means that it is currently recommended to avoid administration of rapid-acting insulin within 90-120 minutes of exercise due to the risk of over-insulinisation of the active musculature during exercise (De Feo et al., 2006; Perry and Gallen, 2009), as demonstrated by the early work of Touminen et al. (1995).

At present it is currently recommended that insulin dose should be reduced before performing exercise, regardless of the insulin species or time before exercise (Campaigne et al., 1987; Muvais-Jarvis et al., 2003; Rabasa-Lhoret et al., 2001). However, to date there is no literature that examined pre-exercise timing as a factor to consider in subsequent blood glucose responses. Specifically, there is limited data available on the absorption kinetics of the insulin analogues when administered in reduced doses with the carbohydrate meal at different times prior to exercise. Within the study of Touminen et al. (1995) participants administered ~6.3 IU of insulin, however, if employing a heavy insulin reduction, as recommended by Rabsa-Lhoret et al. (2001), pre-exercise insulin dose could be as little as ~3 IU. Therefore, there is potential that administering such small doses of insulin closer to the exercise bout may in fact not increase the risk of hypoglycaemia. However, there is limited literature available to confirm or refute this hypothesis.

1.10 Hormonal factors contributing to inter-individual differences in blood glucose responses to exercise

When examining blood glucose responses to exercise, an important factor to consider is the duration of T1DM. As the disease progresses with time, T1DM individuals endure a gradual decline in key gluco-regulatory hormone responses to falling blood glucose (Amiel et al., 1988; Banarer et al., 2002; Dagogo-Jack et al., 1993; Mokan et al., 1994). Therefore, the duration of the disease could be a contributing factor to large inter-individual differences in blood glucose responses when exposed to identical stimuli (e.g. exercise, diet, and insulin dose).

1.10.1 Glucagon responsiveness

Glucagon is a key hormone in glucose regulation during exercise however there may be large inter-individual variability in the glucagon response to both exercise and falling blood glucose. The glucagon response can be normal, impaired or totally absent (Taborsky et al., 1998). Early research by Bolli et al. (1983) suggested that the variance in glucagon responsiveness between individuals could be due to the duration of the disease; newly diagnosed patients were found to have a normal α-cell function and those of 1-5 years clearly having a blunted response. Furthermore, Gerich et al. (1973) demonstrated that the glucagon response was absent in patients who had T1DM for over a decade. The mechanism behind the loss of α-cell function with time is due to progressive loss of residual β-cell function (Fukuda et al., 1988) and a reduced autonomic input through progressive denervation of the α-cell (Tominaga et al., 1987). Therefore, residual α-cell function may be a potential explanatory mechanism behind between-individual differences in the blood glucose response to exercise.

1.10.2 The sympathoadrenal influence on blood glucose

With progressive loss of the primary mechanism against falling blood glucose, glucagon, the adrenaline response also becomes attenuated with time (Cryer, 2002). A single hypoglycaemic episode can result in a reduced sympatho-adrenal input, with individuals not experiencing symptoms of hypoglycaemia and are unaware of dangerously low blood glucose concentrations. Moreover, the reduced autonomic input results in an attenuated adrenaline secretion (Cryer, 2002) to falling blood glucose. Antecedent hypoglycaemia has been shown to shift the glycaemic threshold for stimulating adrenaline release, symptomatic and cognitive dysfunction responses, to lower blood glucose concentrations (Dagogo-Jack et al., 1993; Fanelli et al., 1998). Moreover, reduced adrenomedullary stores of adrenaline have been demonstrated within T1DM individuals with defective adrenal responses to falling blood glucose (De Galan et al., 2004). Therefore, keeping in mind the importance of adrenaline on maintaining blood glucose concentatrations during exercise, i.e. reducing glucose clearance (Howlett et al., 1999), increasing breakdown of intramuscular triglycerides (Howlett et al., 2000) and glycogen (Watt et al., 2001), and stimulating hepatic glucose output (Howlett et al., 1999), a reduced adrenaline secretion would have large metabolic consequences, in particular for the development of hypoglycaemia. Based on these data, the oxidation of blood glucose as a fuel would not decrease, and the promotion of intramuscular fuel sources would be lessened, as well as a reduced stimulation of hepatic glucose production; combining these factors promotes a fall in blood glucose, ultimately leading to hypoglycaemia. Therefore, inter-individual differences in the adrenaline response to falling blood glucose may be a contributing factor in the preservation/lack of preservation of blood glucose between individuals when exposed to the same protocol.

1.11 Summary of thesis and experimental chapter aims

The main aim of this thesis is to examine factors that affect post-exercise glycaemia within T1DM individuals. This thesis will specifically examine the influence of pre-exercise insulin dose, the glycaemic index of the carbohydrates ingested before exercise, and the timing of the administration of both insulin and carbohydrate prior to exercise, on post-exercise glycaemia. Moreover, this thesis will examine if altering these factors is a safe strategy for the exercising T1DM individual to engage in.

The experimental chapters will examine:

1: The metabolic and glycaemic effects of pre-exercise reductions in rapid-acting insulin, before, during and for 24 hours after a single bout of running exercise.

2: The metabolic and glycaemic effects of alterations to the glycaemic index of the pre-exercise carbohydrate, before, during and for 24 hours after a single bout of running exercise.

3: The metabolic and glycaemic effects of alterations in the timing of pre-exercise carbohydrate consumption and insulin administration, before, during and for 24 hours after a single bout of running exercise.

Chapter 2

Methodology

2.1 Ethics

Ethical approval for carrying out these studies was attained from the Local Research Ethics Committee (LREC) of the Abertawe Bro Morgannwg NHS trust (Appendix A1).

2.2 Type 1 Diabetes participants

2.2.1 Recruitment of participants

Type 1 diabetes volunteers were sought from advertisements placed on the Swansea university home page and local and national newspapers. Potential participants were also recruited from within the Diabetes clinics in the Swansea area (Morriston and Singleton Hospital Diabetes Clinics, Abertawe Bro Morgannwg NHS trust). A database was created of all those patients interested in taking part in the research. Initial contact was made via telephone or through email, following which volunteers were sent a study information pack (Appendix A2 - A4), which included a reply form (Appendix B). Those who were willing to take part were screened against an inclusion/exclusion criteria (Table 2.1) and were invited to visit the exercise physiology laboratories.

Table 2.1: Inclusion/exclusion criteria for participants across all experimental chapters.

Aged 18 to 55 years
HbA1c < 10%
Using a Basal-Bolus Regimen of modern insulin analogues - excluding insulin glulisine (Apidra®)
No diabetic complications other than retinopathy
No medication other than insulin
Regularly exercising and able to continuously run for 45 minutes
No muscular-skeletal problems

2.2.2 Insulin regimen of participants

Those volunteers selected to partake in these studies were using a basal-bolus regimen and were taking a set number of basal units for a period of at least 3 months. Participants selected were being treated with once daily insulin glargine (Lantus®, Sanofi Aventis, France) or bi-daily insulin detemir (Levemir®, NovoNordisk, Denmark) as the basal component of their insulin regimen. These two basal insulin analogues are promoted as equivalent, in terms of a peak less 24 hour insulin profile (Gulve, 2008). Furthermore, participants were using a prandial rapid-acting insulin of either insulin lispro (Humalog®, Lilly, USA) or aspart (Novorapid®, NovoNordisk, Denmark). Research has demonstrated no differences in the pharmacokinetic or pharmacodynamic profiles (Plank et al., 2002), or metabolic effects (Homko et al., 2003) of these insulin analogues. The injection site and the timing of administration of the basal insulin were not homogenous within the subject group; however, this remained unaltered between the experimental trials. Bolus insulin dose was calculated via the carbohydrate counting method, i.e. number of rapid-acting insulin units per 10 g of carbohydrate consumed (Tables 2.2 to 2.4).

Table 2.2: Chapter 3 participant insulin regimen details.

	Participant ID								
Insulin	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Mean ± SEM
Basal	28^{G}	22^{G}	44^{G}	42^{G}	27^{G}	66^{D}	30^{G}	22^{G}	35 ± 2
Bolus (per 10 g CHO)	1^{A}	1^{L}	2^{A}	1.5^{A}	1.5^{L}	1^{A}	1^{A}	1^{A}	1.3 ± 0.1

Note: **G** = glargine, **D** = detemir, **A** = aspart, **L** = lispro

Table 2.3: Chapter 4 participant insulin regimen details.

	Participant ID								
Insulin	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Mean ± SEM
Basal	28^{G}	22^{G}	39^{D}	46^{G}	29^{D}	53^{G}	30^{D}	32^{G}	35 ± 2
Bolus (per 10 g CHO)	1^{A}	1^{A}	1.5^{A}	1^{L}	1^{L}	1^{A}	0.5^{A}	2^{A}	1.1 ± 0.1

Note: **G** = glargine, **D** = detemir, **A** = aspart, **L** = lispro

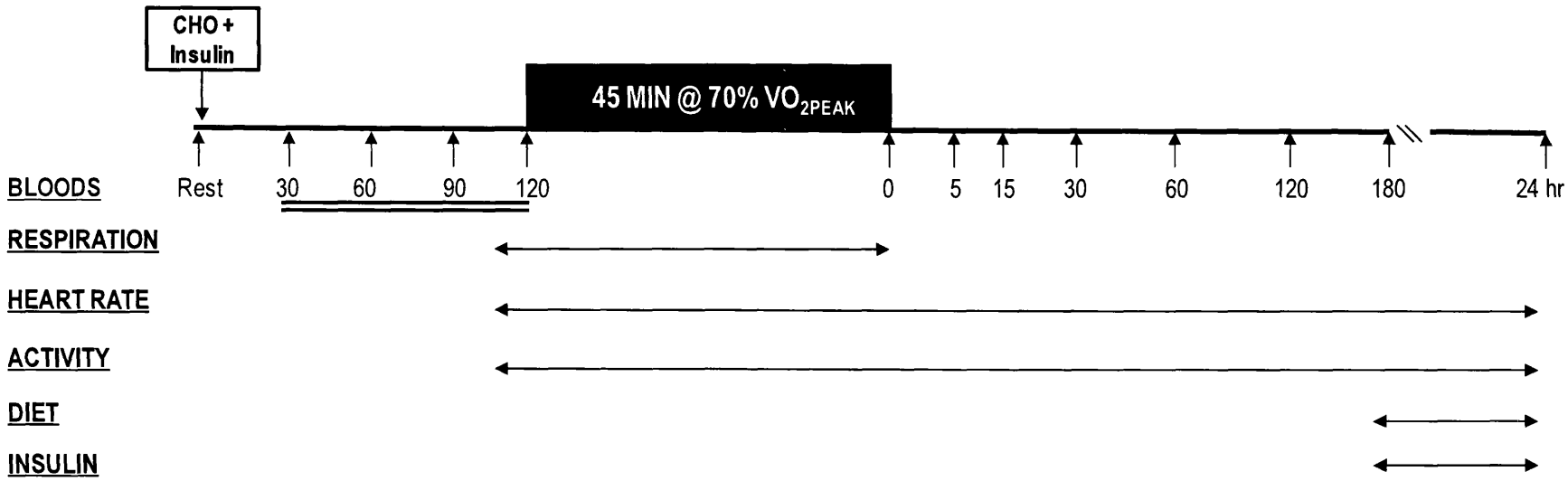
Table 2.4: Chapter 5 participant insulin regimen details.

	Participant ID								
Insulin	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Mean ± SEM
Basal	30^{G}	26^{G}	64^{G}	44^{G}	30^{G}	52^{G}	40^{G}	-	41 ± 2
Bolus (per 10 g CHO)	0.5^{A}	1^{L}	2^{L}	1^{A}	1^{A}	1^{A}	1^{A}	-	1.1 ± 0.1

Note: **G** = glargine, **D** = detemir, **A** = aspart, **L** = lispro

2.3 Experimental design chapters 3 to 5

Figure 2.1: Schematic layout of experimental design of chapters 3 to 5.



Note: Double line indicates alterations in the length of the pre-exercise period within chapter 5.

2.3.1 Health screening

Upon each visit to the laboratory, participants completed informed consent (Appendix D - G) and a physical activity and readiness questionnaire (PAR-Q; ACSM, 2006; Appendix C). Moreover, all procedures for the trial were clarified with every participant.

2.3.2 General study protocol

After initially attending the laboratory for prelimary testing for the quantification of VO_{2peak} and HR_{peak}, participants arrived at the exercise physiology laboratory between 6 and 8 am after an overnight fast, and having consumed similar evening meals between trials. After the collection and processing of resting blood samples, participants were given a carbohydrate-based meal and instructed to administer their rapid-acting (pre-determined dose dependent on trial) insulin (insulin lispro or aspart) into the abdomen, once fully administered, a stop-clock was started.

Blood samples were taken every thirty minutes, for 120 minutes post meal (in chapters 3 and 4; the time between carbohydrate administration and exercise ranged between 30-120 minutes within chapter 5). After the carbohydrate meal was administered, participants anthropometric measures, height (Holtain Stadiometer, Holtain Ltd, UK) and mass (Seca Digital Scales, Seca Ltd, UK), were recorded. Fifteen minutes prior to exercise the metamax-3b (Metamax 3b, Cortex Biophysik, Germany) was placed on the participants, along with a heart rate monitor (RS-400, Polar, Finland), and activity monitor (Sensewear Pro; Bodymedia, PA, USA) for the collection of a 15 minute resting cardio-respiratory sample. After the final pre-exercise blood sample was taken (Figure 2.2), participants subsequently performed 45 min of steady state treadmill (Woodyway, Germany) exercise at ~70 %

VO_{2peak}. Throughout exercise respiratory parameters were collected and participants were closely monitored during exercise for any symptoms of hypoglycaemia.

At 45 minutes an immediate post-exercise sample was taken (Figure 2.3), the stop clock was then started for the 3 hour post-exercise period. Additional samples were taken at 5, 15, 30, 60, 120 and 180 min post exercise. Participants remained at rest for the entire post-exercise period, drinking water *ad libitum*.

Figure 2.2: A T1DM individual wearing the metamax-3b at rest and during 45 minutes of running ~70% VO_{2peak}.

Figure 2.3: Immediate post-exercise blood sample.

2.3.3 Preliminary Testing

2.3.3.1 Anthropometric measurements

Bioelectrical impedance analysis (BIA)

Prior to starting the procedure, the BIA (Bodystat Quadscan 4000, Bodystat Ltd, USA) unit was tested for accuracy by running a test against a metal of a fixed resistance (500 Ohms). After quantification of height (Holtain Stadiometer, Holtain Ltd, UK) and mass (Seca Digital Scales, Seca Ltd, UK) participants were required to remain supine for 15 minutes. Participants were positioned so both legs and arms were adducted at 35 - 45° angle from the trunk. Alcohol wipes (70 % Alcotip Swabs, Uhs, UK) were used to clean electrode sites on the hands and feet before two injector electrodes (red) were attached to the dorsal surface of the right hand and the right foot and detector electrodes (black) were placed on the ankle of the right foot and just below the radioulnar joint on the right hand. After 15 minutes of laying supine had elapsed, and participant details were entered into the BIA device, the procedure was started. Coefficient of variation, over 4 trials, for % body fat (BF), fat free mass (FFM) and total body water was 2.0 ± 0.2, 3.1 ± 0.9 and 0.7 ± 0.1 %, respectively. Coefficient of variation for measurements of mass between trials within chapter 3 was 0.5 ± 0.03 %, chapter 4 was 0.7 ± 0.1 %, and chapter 5 was 0.6 ± 0.1 %,

Table 2.5: Participant anthropometric characteristics across chapters 3 to 5.

	Chapter 3	Chapter 4	Chapter 5
Height (m)	1.76 ± 0.01	1.80 ± 0.01	1.80 ± 0.01
Mass (kg)	84.1 ± 2.0	84.0 ± 1.9	84.3 ± 1.3
BMI (kg/m^2)	27.0 ± 0.4	26.1 ± 0.3	26.3 ± 0.3
% BF	22.1 ± 0.6	21.4 ± 0.5	--
% FFM	78.0 ± 0.6	78.2 ± 0.5	--

Note: Data presented as mean ± SEM.

2.3.3.2 Quantification of peak cardio-respiratory characteristics

Participants performed a continuous maximal incremental treadmill protocol where heart rate and breath-by-breath respiratory data were collected for the determination of peak rate of oxygen uptake (VO_{2peak}) with subsequent peak heart rate (HR_{peak}).

Equipment Calibration

Respiratory parameters were collected via a portable gas analyser system (Metamax 3b, Cortex Biophysik, Liepzig); the Metamax 3b main principles of operation are through the generation of an electrical current, via an oxygen cell, which is directly proportional to the partial pressure of oxygen (PO_2), and the quantification of PCO_2 through absorption of infrared radiation by the carbon dioxide present in the expired air (McFarlane, 2001). Prior to use a two point gas calibration was performed. Ambient air was used to give a maximal O_2 value, and a baseline CO_2 value. A gas of known concentration (16 % O_2, 5 % CO_2, Brin's Oxygen Company Ltd) was used to set a baseline O_2 value and maximal CO_2 value. The volume transducer was calibrated with a 3 – litre syringe (Hans Rudolf) and the pressure against the ambient barometric pressure (230 series, NovaLynx, USA).

Maximal incremental treadmill assessment

Participants arrived at the laboratory in a fed and hydrated state, with at least 2 hours after administering a bolus insulin dose. After self-checking blood glucose, anthropometric measures (height and mass) were taken, a heart rate monitor (RS-400, Polar, Finland) was then placed across the chest. Participants did not start the protocol if blood glucose was less than 6.5 $mmol.l^{-1}$ (De Feo et al., 2006). Those subjects with a blood glucose concentration < 6.5 $mmol.l^{-1}$ were given 117 ml of an hypertonic drink (20 g CHO; Lucozade®, GlaxoSmithKline, UK) immediately prior to exercise (De Feo et al., 2006). After a 2-point

gas calibration, the metamax-3b was placed on each participant (Figure 2.4), and breath-by-breath data was subsequently collected and transferred wirelessly to the laboratory computer (Figure 2.6).

Figure 2.4: A T1DM individual wearing the Metamax-3b.

Participants then completed a standardised warm-up (3 minutes at 6 $km.hr^{-1}$) immediately before performing a graded treadmill exercise test; this protocol involved participants running in 3 minute blocks of steady state exercise at a treadmill velocity of 8 $km.hr^{-1}$, a velocity which was increased by 1 $km.hr^{-1}$ every 3 minutes (Jones and Doust, 1996; Figure 2.5). Participants were instructed to continue exercising until volitional exhaustion. The peak rate of O_2 consumption (VO_{2peak}) was reached when RER was greater than 1.15, heart rate (HR) of 220-age (bpm), RPE of 18 and/or a distinct plateau in oxygen consumption, the velocity at which this point occurred was defined as V_{max}. VO_{2peak} was calculated as the average VO_2 over the final minute of the protocol. Throughout the protocol participants were closely monitored for signs of hypoglycaemia, through frequent questioning of how they were feeling, and observing for any symptoms such as confusion and pallor. All participants were given lots of verbal encouragement during exercise.

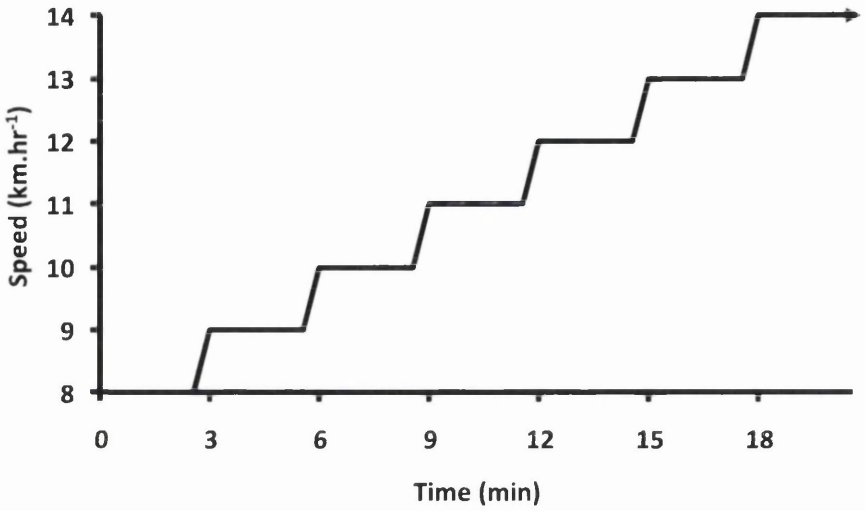


Figure 2.5: Graphical representation of the continuous incremental treadmill test used to establish VO_{2peak} and HR_{peak}.

Figure 2.6: A typical screen shot of breath-by-breath data from the metamax-3b.

Table 2.6: Peak cardio-respiratory characteristics of participants across chapters 3 to 5.

	Chapter 3	Chapter 4	Chapter 5
VO_{2peak} ($l.min^{-1}$)	3.5 ± 0.1	3.7 ± 0.1	4.3 ± 0.1
VO_{2peak} ($ml.kg.min^{-1}$)	43.8 ± 1.0	44.9 ± 0.7	50.9 ± 0.5
HR_{peak} (bpm)	190 ± 2	190 ± 1	192 ± 2
V_{max} ($km.hr^{-1}$)	12.0 ± 0.2	11.9 ± 0.2	12.5 ± 0.1

Note: Data presented as mean ± SEM.

Using V_{max} and VO_{2peak}, the velocity that elicits 70% VO_{2peak} could be calculated, as well as the corresponding VO_2:

i.e. V_{max}*0.7 = trial velocity ($km.hr^{-1}$), VO_{2peak}*0.7 = 70% VO_{2peak} ($l.min^{-1}$)

This intensity and duration was chosen because it falls within the current ACSM (2006) exercise guidelines for T1DM individuals. In all chapters, exercise was performed below ventilatory threshold (VT) velocity (Table 2.7).

Table 2.7: Summary of the velocities at which participants reached ventilatory threshold, during the maximal incremental treadmill assessment, and experimental trials' treadmill velocity.

Chapter	VT ($km.hr^{-1}$)	Trial Velocity ($km.hr^{-1}$)
3	10.1 ± 0.2	8.5 ± 0.1
4	10.3 ± 0.1	8.3 ± 0.1
5	10.4 ± 0.1	8.5 ± 0.1

Note: Data presented as mean ± SEM

2.3.4 Experimental procedures

2.3.4.1 Catheterisation

Within each study, participants attended the exercise physiology laboratory at the same time (between 6 and 8 am) and 7 days apart. Prior to arrival at the laboratory all participants were instructed to consume 1 pint (568 ml) of water. Upon arrival to the laboratory, participants were seated while a 20-gauge catheter (Venflon, Becton Dickinson, Helsingborg, Sweden) was inserted into the ante-cubital vein, of the non-dominant arm, and secured with a Veca-C dressing (Venflon, Becton Dickinson, Helsingborg, Sweden). A 10 cm extension with 3-way stop cock was used to allow easy access for samples (Connect, Becton Dickinson, Helsingborg, Sweden; Figure 2.8). Saline (Sodium Chloride BP, 0.9% w/v, Braun, UK) was infused periodically to keep the catheter patent.

2.3.4.2 Blood sampling

On the resting sample for each participant's first trial, 15 ml of blood was collected. Two 10 ml syringes (BD 10 ml syringe, Luer-Lok™ Tip, BD USA) and a 1 ml Ca^{2+}-heparinised syringe (23 IU heparin, Rapidlyte, Bayer, USA) were filled with whole blood (Figure 2.7). One of the 10 ml syringes was filled with 4 ml of blood only. Thereafter, on each sample 11 ml of blood was collected (one x 10 ml and one x 1 ml syringe). On the resting sample the syringe containing 4 ml of whole blood was dispensed into an K^{+}-EDTA tube (Vacuette, Greiner Bio-One, GmBH, Austria) which was sent to Morriston Hospital Biochemistry Laboratories for the analysis of glycosylated Haemoglobin (HbA_{1c}) by HPLC with cation exchange (G7, Tosoh, UK; Table 2.7). The 10 ml syringe was dispensed evenly into a serum separation tube (SST; Vacuette, Greiner Bio-One, GmBG, Austria) and Lithium-Heparin tube (Vacuette, Greiner Bio-One, GmBH, Austria).

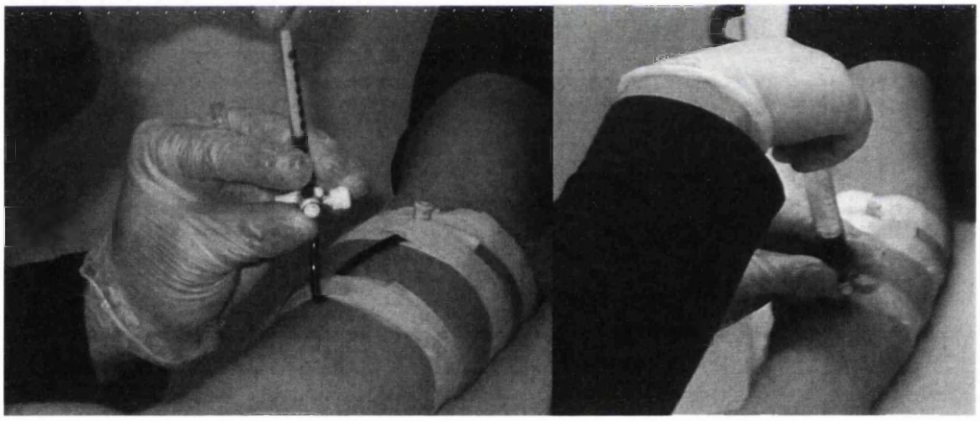


Figure 2.7: Withdrawal of whole blood using a 1 ml Ca^{2+}-heparinised syringe (left) and a 10 ml syringe (right).

Two hundred µl of 0.1 $mol \cdot l^{-1}$ of both ethylene glycol bis-(β-aminoethyl ether)-N',N',N',N'-tetraacetic acid (EGTA; SIGMA, USA), as anticoagulant and glutathione (SIGMA, USA) as antioxidant (Appendix H), were added to the Lithium-Heparin tube before being immediately

placed in the centrifuge (Heraeus Labofuge 400R, Kendro Laboratory Products, Germany) for 10 minutes at 3000 rev·min^{-1}. The sample in the SST was left to clot, on an orbital shaker (VSR 23, Grant, UK), for 30 minutes before also being centrifuged for 10 min at 3000 rev·min^{-1}; the resultant serum and plasma were extracted and placed into 5 ml aliquot tubes (Sarstedt, Germany) and then immediately stored at -80 °C (Innova U101, New Brunswick Scientific, UK; Figure 2.8).

The 1 ml Ca^{2+}-heparinised syringe was immediately analysed for blood pH, Hct, glucose and lactate (GEM 3000, Instrumentation Laboratories, Warrington, UK; Appendix I) and haemoglobin (Hemocue AB, Sweden; Figure 2.7) and then placed on ice, in case a repeat sample was required. Pre and post-exercise quantification of Hb and Hct allowed plasma volume shifts to be calculated via the method of Dill and Costill (1974; Appendix J).

Table 2.8: Glycosylated haemoglobin values (HbA_{1c} %) across chapters 3 to 5.

	Chapter		
Participant	3	4	5
1	8.4	9.8	8.5
2	8.9	-	-
3	7.5	7.3	-
4	8.4	-	-
5	8.2	-	-
6	8.3	9.9	7.6
7	7.7	8.0	9.8
8	9.2	9.3	-
9	-	7.8	8.8
10	-	6.1	-
11	-	5.9	-
12	-	-	8.9
13	-	-	8.1
14	-	-	6.7
Mean ± SEM	8.3 ± 0.01	8.0 ± 0.2	8.3 ± 0.1

GEM 3000 Reliability

The GEM 3000 was tested for reliability on whole blood, which was placed on ice between samples, ranging from very low (<3 $mmol.l^{-1}$) to very high (>20 $mmol.l^{-1}$) concentrations (Table 2.9). The GEM system was considered reliable with a coefficient of variation of < 10 % at all blood glucose concentrations.

Table 2.9: GEM 3000 coefficient of variation for 3 consecutive blood glucose samples at concentrations ranging from hypoglycaemic to hyperglycaemic.

Blood Glucose Sample ($mmol.l^{-1}$)					
1	2	3	Mean BG ($mmol.l^{-1}$)	SD	COV (%)
2.6	2.4	2.4	2.5	0.1	4.7
3.3	3.3	3.2	3.3	0.1	1.8
4.9	4.7	4.6	4.7	0.2	3.2
7.2	7.1	7	7.1	0.1	1.4
9.3	8.3	8	8.5	0.7	8.0
11.4	11.2	10.9	11.2	0.3	2.3
15.2	14.5	14.1	14.6	0.6	3.8
16.1	16.2	15.8	16.0	0.2	1.3
23.1	22.9	22.6	22.9	0.3	1.1

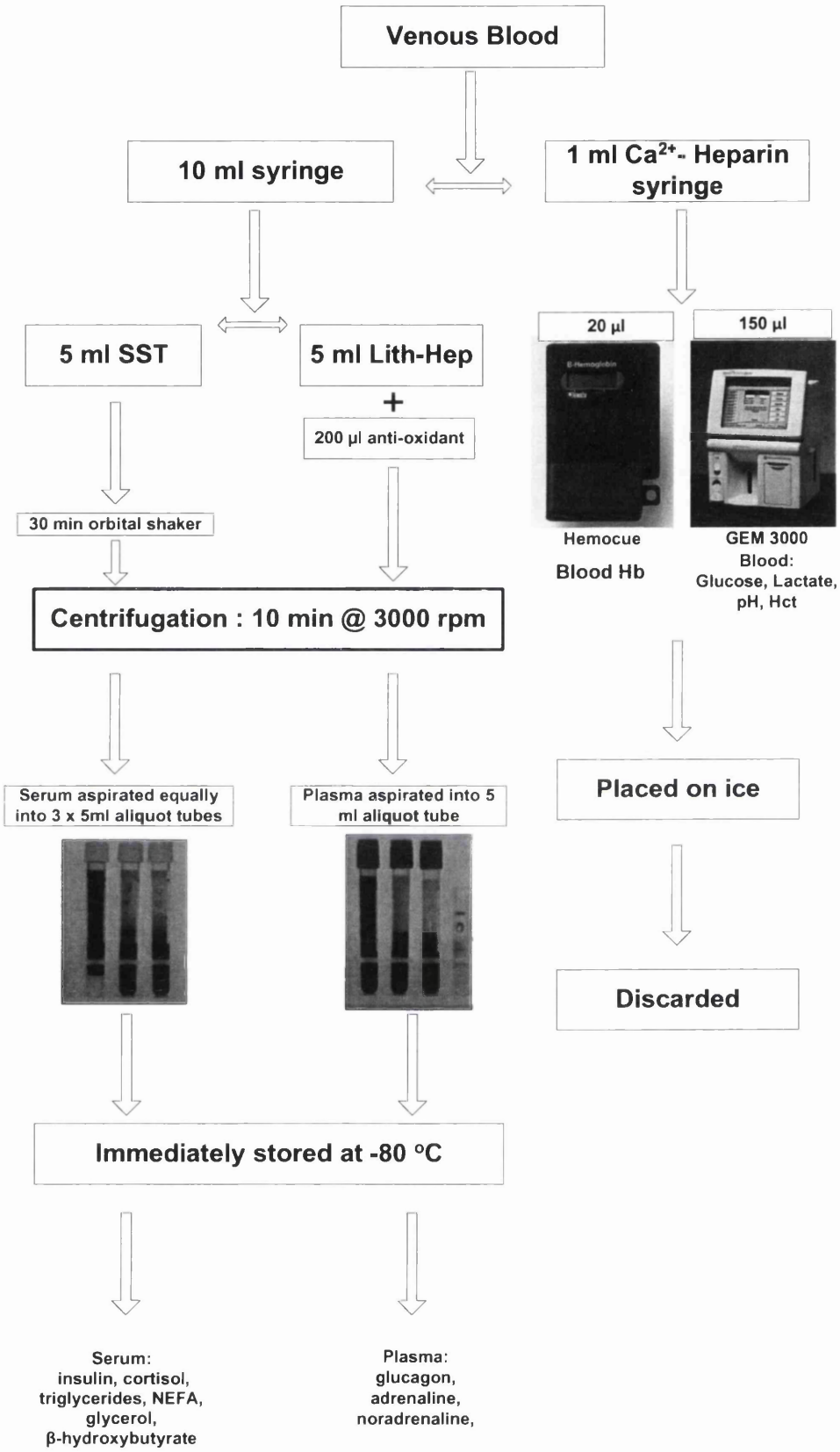


Figure 2.8: Step by step processing of venous blood samples.

2.3.5 Quantification of blood, serum and plasma analytes

Table 2.10 provides a summary of the analysis of blood across chapters 3 to 5.

Table 2.10: Summary of blood analysis across chapters 3 to 5.

	Blood Analytes		
Variable	**Chapter 3**	**Chapter 4**	**Chapter 5**
Glycaemic	Glucose	Glucose	Glucose
	HbA_{1c}	HbA_{1c}	HbA_{1c}
Acid-Base	Lactate	Lactate	Lactate
	pH	pH	pH
Plasma Volume	Hct	Hct	Hct
	Hb	Hb	Hb
Gluco-regulatory	Insulin	-	Insulin
	Glucagon	Glucagon	-
	Adrenaline	Adrenaline	Adrenaline
	Noradrenaline	Noradrenaline	Noradrenaline
	Cortisol	Cortisol	Cortisol
Lipids	-	NEFA	NEFA
	-	Glycerol	-
	-	Triglyceride	Triglyceride
Ketoacid	β-hydroxybutyrate	β-hydroxybutyrate	β-hydroxybutyrate

2.3.5.1 Blood glucose and lactate

Blood glucose and lactate were analysed using the GEM 3000. The glucose and lactate sensors are amperometric electrodes consisting of a platinum electrode poised at a positive potential with respect to the card reference electrode. Glucose or lactate determination is accomplished by enzymatic reaction of glucose or lactate with oxygen in the presence of glucose oxidase or lactate oxidase and the detection of the resulting hydrogen peroxide with

the platinum electrode. The current flow between the platinum electrode and the ground electrode is proportional to the rate at which hydrogen peroxide molecules diffuse to the platinum and are oxidised, which in turn is directly proportional to the metabolic (glucose or lactate) concentration:

$$I = (S \times \text{metabolite}) + IZ$$

Where I is the electrode current, S is the sensitivity, and IZ is the zero current. The value S and IZ can be calculated from the calibration data for the sensor. The equation can be solved for the metabolite concentration, where I becomes the electrode current produced by the blood sample. Figure 2.9 illustrates the configuration of the glucose/lactate sensor. The sensor is constructed of a 3 layer composite membrane consisting of an inner layer for screening out the interferences, the enzyme for oxidation reaction, and the outer layer for controlling the metabolite diffusion in the enzyme layer.

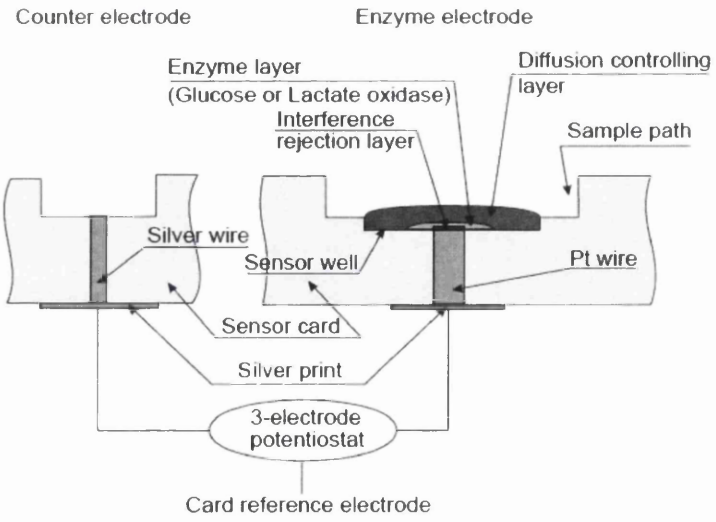


Figure 2.9: Internal components of the glucose and lactate sensor within the GEM 3000.

2.3.5.2 Serum and plasma analytes

Samples were left to thaw to room temperature and were placed on a vortex (IKA Vortex, Fisher Scientific, UK) before analysis. Detailed descriptions of the methods performed for each analyte are presented in Table 2.11 and described below.

Table 2.11: Summary of assays used for the quantification of gluco-regulatory hormones across chapters 3 to 5.

Analyte	Collection	Method	Kit Number	Kit Name	Manufacturer	Sensitivity	Intra-assay reliability (%)
Insulin	Serum	Sandwich Immunochemiluminometrtic assay	IV2-001/101	Invitron insulin assay	Invitron, UK	0.25 mU/l	4.7
Glucagon	Plasma	Competitive immunoassay	48-GLUCA-90	Glucagon EIA	Alpco Diagnostics, USA	50 pg/ml	3.3 - 5.1
Adrenaline	Plasma	Sandwich ELISA	RE59242	CAT-COMBI	Immunobiological laboratories, Hamburg	10 pg/ml	7.1
Noradrenaline	Plasma	Sandwich ELISA	RE59242	CAT-COMBI	Immunobiological laboratories, Hamburg	20 pg/ml	7.4
Cortisol	Serum	Competitive Electrochemiluminescence immunoassay	118875116 122	Elecsys cortisol assay	Cobas-Roche, UK	0.5 nmol.l^{-1}	3.8-5.6

Note: Sensitivity and intra-assay reliability data are manufacturer derived values.

2.3.5.2.1 Insulin

The assay is a two-site sandwich immunoassay, with a solid phase antibody specific to insulin immobilised onto microtitre wells and a soluble antibody labelled with an acridinium ester. The serum samples are incubated at 37 °C along with standards, quality control samples and the labelled antibody in the antibody coated wells. This causes the insulin to become 'sandwiched' between the 2 types of antibody molecules. The excess labelled antibody is then removed in a wash step before reading in a plate reader with *in situ* reagent addition capability to quantify the bound luminesence. The acridinium ester is a stable compound, which when oxidised under alkaline conditions, will generate light, the brightness of which is linked to the amount of label, and hence insulin, present.

Summary of Procedures

Labelled insulin antibody was pipetted into all wells followed by standard, quality control (Lyphocheck, Biorad, UK) or serum samples. The plate was then covered with an adhesive plate sealer and incubated for 2 hours at 37 °C. Once incubation was complete, the plate was washed 3 times with an automatic plate washer (Wellwash 4, Denley Ltd, Sussex, UK.), before being read in a plate luminometer (Microplate luminometer LB 96P, EG&G Berthold, Germany). From the resultant counts, a calibration curve was set up to calculate the concentration of the unknown samples (Multicalc, Pharmacia Wallac, Milton Keynes, UK).

Cross-reactivity – Influence of the insulin analogues

The Invitron assay is 100 % cross reactive with human insulin, however as T1DM individuals were examined, the influence of any residual β-cell function was considered negligible. Additionally, this assay is 100 % cross reactive with insulins lispro, aspart and glargine. Therefore, as insulin glargine has been demonstrated to elicit a peak less, steady 24 hour

insulin concentration (Gulve, 2008) and is not effected by exercise (Peter et al., 2005), any changes in insulin concentrations detected by this assay were considered to be due to changes in the appearance/disappearance of insulin lispro or aspart. The Invitron assay is 300 % cross-reactive with insulin detemir. Therefore, within chapters 3 and 5, only T1DM individuals using insulin glargine could be examined/recruited.

2.3.5.2.2 Glucagon

The glucagon EIA for the determination of plasma pancreatic glucagon within plasma is based on competitive enzyme immunoassay. The 96 well plate is coated with rabbit anti-glucagon antibodies. Glucagon standards, samples and labelled antigen are added to the wells for a competitive immunoreaction. After incubation and plate washing, Horse radish peroxidase (HRP) labelled streptavidin (SA) is added to form HRP labelled SA-biotinylated pancreatic glucagon antibody complexes on the wells surface. The HRP activity is determined by the addition of O'phenylenediamine dihydrochloride (OPD), imitating a colour reaction, and pancreatic glucagon can subsequently be determined.

General Procedure

Glucagon standards or plasma samples were pippetted into the wells and incubated at 4 °C for 24 hours. After incubation, the wells were manually washed 3 times and the SA-HRP solution was added to the wells before being incubated for 1 hour at room temperature on a microtiter plate shaker (Microplate Orbital Shaker 115 Vac - 60 Hz, Cole-Parmer, UK). After incubation the wells were washed a further three times before adding OPD to the 96 wells. The plate was left at room temperature for 20 minutes for colour reaction, before the addition of stop solution. The plate was immediately read at an optical absorbance of 490 nm (Fluostar - Omega Plate Reader, BMG Labtech; UK) with a calibration curve created to calculate the concentrations of the unknown samples.

2.3.5.2.3 Catecholamines (Adrenaline and Noradrenaline)

The CatCombi, a sandwich ELISA, was used for the determination of plasma concentrations of adrenaline and noradrenaline. The plate wells are coated with goat anti-rabbit antibody. The addition of the control and plasma samples results in the epitope of adrenaline or noradrenaline, within the sample, binding to the plate wells during the incubation period. The addition of a second antibody, which binds to a different region of the adrenaline or noradrenaline molecule, and substrate solution induces a colour reaction; the density of which is directly proportional to the amount of adrenaline or noradrenaline within the sample.

General Procedure

Phase 1- Extraction

Note: Incubation includes the plate being placed on an orbital shaker at room temperature. The methods describe the process for three separate plates (extraction plate, and plates for the separate quantification of adrenaline and noradrenaline).

Standards, controls and plasma samples were pippetted into the wells along with extraction buffer and incubated for 30 minutes. After removal of residual fluid, bi-distilled water was added to each well and the plate was incubated for 5 minutes. After removing all fluid from the plate wells, extraction buffer and acylation reagent was added before a 20 minute incubation period. After this, release buffer was added to all wells before being incubated for a further 30 minutes.

Phase 2

Freshly prepared catechol-O-methyl transferase solution was added to each well along with the extracted standard, control and plasma samples. Next adrenaline and noradrenaline antiserum was added to each plate, respectively, before being incubated for 2 hours.

Phase 3 – ELISA

After incubation, the plate was washed 3 times before enzyme conjugate was added to each well. The plates were incubated for a further 60 minutes. Following this, the plates were manually washed a further three times before substrate solution was added to each well and the plates were incubated for 40 minutes. After incubation *para*-Nitrophenylphosphate stop solution was added to each well the plate was subsequently measured at an optical density of 405 nm. From this, a calibration curve was created to calculate the concentrations of the unknown samples.

2.3.5.2.3 Cortisol

Serum cortisol was measured by routine hospital procedures using a competitive electrochemiluminescence immunoassay (Cortisol, Cobas-Roche, UK) on an automated system (Roche/Hitachi Modular P800 analyser, Roche Diagnostics, Germany).

Assay Principle

This assay makes use of the competition test principle using a polyclonal antibody which is specifically directed against cortisol. Endogenous cortisol in the sample which has been liberated from binding protein, with danazol, competes with exogenous cortisol derivative, which has been labelled with ruthenium complex, for the binding sites on the biotinylated antibody.

2.3.5.2.4 Non-esterified fatty acids (NEFA)

Serum non-esterified fatty acids were analysed by routine hospital procedures using an enzymatic colourimetric assay (NEFA-HR(2), Wako Chemicals, Germany) on an automated system (Roche/Hitachi Modular P800 analyser, Roche Diagnostics, Germany).

Assay Principle

NEFA within the serum sample is converted to Acyl-CoA, AMP and pyrophosphoric acid (PPi) by the action of Acyl-CoA synthetase (ACS), under coexistence with coenzyme A (CoA) and adenosine 5-triphosphate disodium salt (ATP). Obtained Acyl-CoA is oxidised and yields 2,3-trans-Enoyl-CoA and hydrogen peroxide by the action of Acyl-CoA oxidase (ACOD). In the presence of peroxidase (POD), the hydrogen peroxide formed yields a blue purple pigment by quantitative oxidation condensation with 3-Methyl-N-Ethyl-N-(β-Hydroxyethyl)-Aniline (MEHA) and 4-amino-antipyrine (4-AA). NEFA concentration is obtained by measuring absorbance of the blue purple pigment.

2.3.5.2.5 Triglycerides

Serum triglycerides were analysed by routine hospital procedures using an enzymatic colourimetric assay (Triglycerides GPO-PAP, Cobas-Roche, UK) on an automated system (Roche/Hitachi Modular P800 analyser, Roche Diagnostics, Germany).

Assay Principle

This assay uses a lipoprotein lipase from micro-organisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder

endpoint reaction). The colour intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.

2.3.5.2.6 Glycerol

Glycerol was analysed using an enzymatic colourimetric assay (Caymans Glycerol Assay, Cayman Chemical, USA).

Assay Principle

Glycerol is phosphorylated by glycerol kinase to produce glycerol-3-phophate and adenosine-5'-diphophate. The glycerol-3-phosphate is oxidised by glycerol phosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Peroxidase catalyses the redox-coupled reaction of H_2O_2 with 4-aminoantipyrine (4-AAP) and N-ethyl-N-(3-sulfopropyl)-m-anisidine (ESPA), producing a purple product, with an absorbance maximum at 540 nm. The intensity of the purple colouring is proportional to the concentration of glycerol.

2.3.5.2.7 β-Hydroxybutyrate

Assay Principle

β-hydroxybutyrate was analysed using an ELISA assay (Ranbut, Randox Laboratories, UK). The assay principle relies on the production of acetoacetate and H^+ after β-hydroxybutyrate has been oxidised by hydroxybutyrate dehydrogenase.

β-hydroxybutyrate + NAD^+ -- 3-hydroxybutyrate dehydrogenase -- Acetoacetate + H^+ NADH.

The change in absorbance at 340 nm can be directly correlated with the β-hydroxybutyrate concentration. This assay is sensitive to 0.1 $mmol.l^{-1}$ and has an intra assay reliability of 3.7%.

2.4 Hypoglycaemia

Throughout the post-exercise period participants were constantly monitored for signs of hypoglycaemia. While in a rested position, research staff constantly engaged the participants in conversation, while looking for signs of hypoglycaemia such as confusion and pallor, moreover the participants were frequently asked how they were feeling and if they were experiencing any typical symptoms of hypoglycaemia (e.g. paraesthesia and difficulty focusing). If at any point in the post-exercise period blood glucose dropped below 3.5 $mmol.l^{-1}$ (Rabasa-Lhoret et al., 2001), 117 ml of a sports drink (20g CHO; Lucozade®, GlaxoSmithKline, UK) was administered.

2.5 Pre-exercise meal

Chapter 3

Prior to testing, local and national T1DM individuals were sent questionnaires, about their insulin regimen and exercising habits, by email, mail and over the phone (e.g. Appendix K). From this information (n = 20) it seemed a typical pre-exercise meal consisted of largely fruit and wheat based cereals with around 60 - 80 g of CHO. In light of this, and multiple pilot 'tasting' sessions, a meal of peaches and wheat was the most palatable and easy to store combination and caused no GI discomfort. Participants consumed a 1.12 MJ carbohydrate based meal (60 g CHO, 2 g Protein, 2 g Fat) of a medium glycaemic index (GI 46 - 50; 40 g peaches, 20 g wheat) which was blended and mixed with 50 ml of distilled water.

Chapter 4

Participants consumed 75 g of either a high GI (dextrose; My Protein, UK; GI 96) or low GI (Isomaltulose; Beneo Group, Germany; GI 32) carbohydrate, mixed with 750 ml of water in a 10% solution (Peronne et al., 2005). Seventy-five g of carbohydrates were administered based on resting carbohydrate oxidation rates of ~0.25 $g.min^{-1}$ and maximal rates of exogenous carbohydrate oxidation during exercise of ~1 $g.min^{-1}$ (Jeukendrup, 2004), i.e. rest period: 0.25 g x 120 = 30 g, exercise: 1 x 45 = 45 g. Dextrose and isomaltulose were chosen as these two carbohydrate forms represent the highest and lowest GI carbohydrates currently available in powdered supplement form. This made the formulation of two carbohydrate only solutions, at extremes of the GI scale, possible.

Chapter 5

Based on the recommended strategy of chapter 4, participants consumed 75 g of isomaltulose as the pre-exercise meal.

2.6 Data collection following the laboratory testing

On each occasion, heart rate (60 s sample rate; RS-400, Polar, Finland) and the number of steps taken (60 s sample rate; Sensewear Pro Armband^{TM}; Bodymedia, PA, USA) were recorded for 21 hours after leaving the laboratory to quantify post-exercise activity. Participants were given blank diary sheets to record blood glucose from their own glucose meters, food intake and the number of units of insulin administered over the 21 hour post-laboratory period (e.g. Appendix L). These variables allowed for calculation of glucose area under the curve (calculated using the method of Wolever and Jenkins (1986) and time averaged for the 21 hour post-exercise period), energy and carbohydrate intake (Compeat Pro, Nutrition Systems, UK), insulin administration and the frequency of hypoglycaemic ($\leq$ 3.5 mmol.l^{-1}) and low blood glucose ($\leq$ 4 mmol.l^{-1}) incidents encountered.

2.6.1 Self-recorded blood glucose

Within in chapters 3 and 4, the participants' own glucose meters were used to measure post-laboratory blood glucose responses. Each monitor was tested for reliability, using 5 samples, against a 6.3 (CV 0.6 – 2.4 %) and 12.5 (CV 0.8 – 2.6 %) mmol.l^{-1} glucose standard, all monitors demonstrated adequate limits of reliability. Within chapter 5, participants were provided with a glucose monitor (Freestyle Lite, Abbott, UK) which was tested for reliability against whole blood, using 3 consecutive samples, at concentrations ranging from hypoglycaemic to hyperglycaemic (Table 2.12). Moreover, the device was in agreement with the GEM 3000 (Figure 2.10). Throughout the post-laboratory period blood glucose was self-recorded every 2 hours until going to sleep and immediately upon awaking. Blood glucose was recorded 6 times on each occasion; two hour intervals were based upon data provided by 20 T1DM individuals who were questioned as to how often they record their blood glucose after performing their typical activity session.

Within the laboratory, participants received carbohydrate supplementation if blood glucose fell below 3.5 $mmol.l^{-1}$. Therefore, hypoglycaemia was defined as a blood glucose concentration <3.5 $mmol.l^{-1}$ within and post-laboratory (Rabasa-Lhoret et al., 2001). Due to inter-individual differences in blood glucose sensing, and participants regularly checking their blood glucose concentrations, falling blood glucose may have been corrected before leading to hypoglycaemia. Therefore, a blood glucose threshold of ≤4 $mmol.l^{-1}$ was defined as 'low blood glucose'.

Table 2.12: Freestyle lite® coefficient of variation for 3 consecutive blood glucose samples at concentrations ranging from hypoglycaemic to hyperglycaemic.

Blood Glucose Sample ($mmol.l^{-1}$)					
1	2	3	Mean BG ($mmol.l^{-1}$)	SD	CV (%)
3.1	3.2	3.0	3.1	0.1	3.2
3.8	3.8	3.4	3.7	0.2	6.3
4.6	4.6	4.6	4.6	0	0
5.4	5.4	5.5	5.4	0.1	1.1
6.8	7.6	7.7	7.4	0.5	6.7
9.4	9.4	10.1	9.6	0.4	4.2
11.4	11.5	10.9	11.3	0.3	2.9
12.3	11.2	12.6	12.0	0.7	6.1
15.7	15.9	15.9	15.8	0.1	0.7
17.4	17.7	15.8	17.0	1.0	6.0
21.4	22.2	21.4	21.7	0.5	2.1

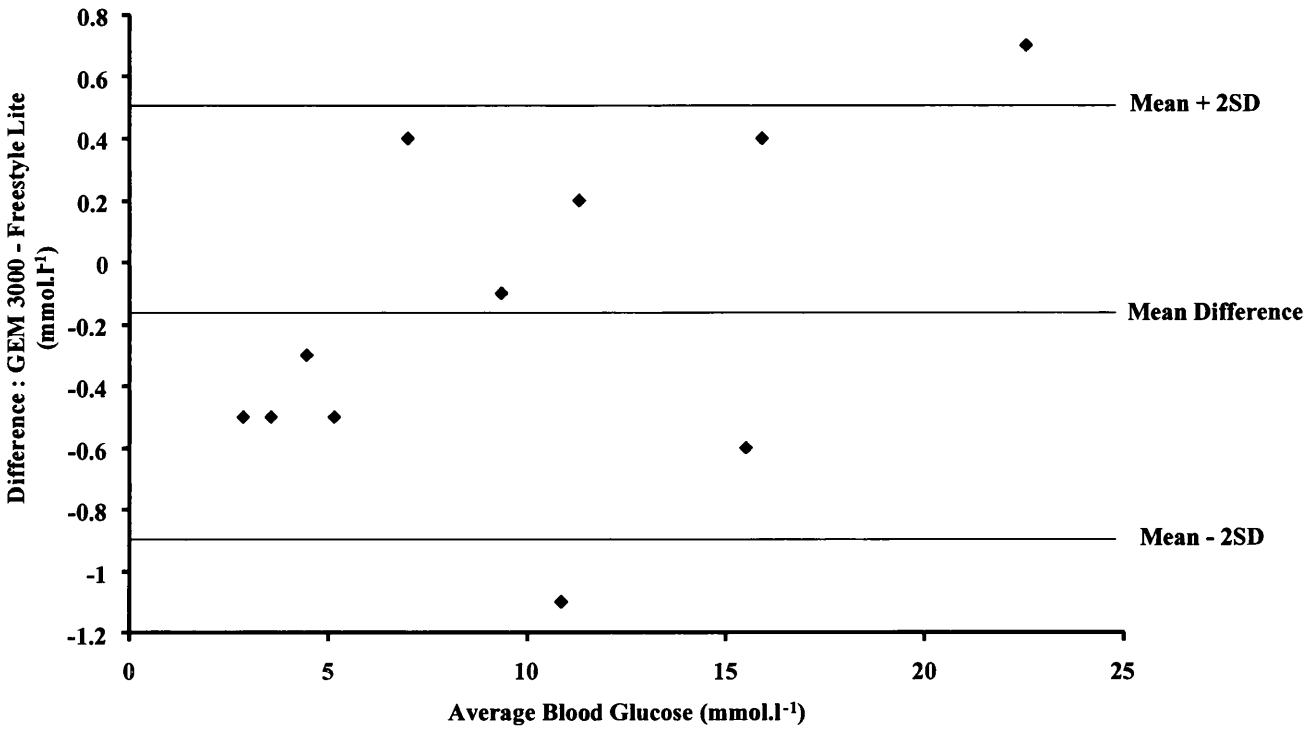


Figure 2.10: Bland and Altman (1986) plot comparing the agreement between the GEM 3000 and Freetyle lite. **Note**: $R^2 = 0.994$.

2.6.2 Post-laboratory activity

Post-laboratory activity was assessed through 60 s sampling of heart rate and steps taken (Sensewear Pro Armband™; Bodymedia, PA, USA). The Sensewear Pro Armband™ (Figure 2.11) is a two dimensional accelerometer, taking into account vertical and horizontal acceleration, worn on the right, upper arm. During a bout of exercise the device is highly reliable with coefficients of variation for steps taken of <10%. Moreover, the Sensewear Pro Armband™ recording of step count in free living has been shown to be in agreement with other valid activity monitors (Hanby et al., 2005).

Figure 2.11: The Sensewear Pro Armband™.

2.6.3 Self-recorded dietary intake and insulin administration

Before the trials began participants were provided with detailed instructions of how to complete dietary assessments in the most detail possible. For example participants were given a demonstration of how to obtain the dietary information from food stuffs (i.e. the nutritional content section on the food item). Moreover, participants were provided with example dietary assessment sheets (Appendix M). After completion of each experimental trial participants returned the dietary sheets to the research team, all sheets were immediately checked for detail and if any issues arose they could be explored further.

Participants were also instructed to record the number of rapid-acting insulin units administered with each meal. Moreover, participants were required to indicate if additional units had been added to correct for high blood glucose, or conversely units had been omitted to correct for low blood glucose.

2.7 Data analysis

2.7.1 Calculation of blood glucose area under the curve

The calculation of blood glucose area under the curve (BG_{AUC}) was calculated via the method of Wolever and Jenkins (1986) and was subsequently time averaged. Total BG_{AUC} was averaged to $mmol.l^{-1}.hour^{-1}$.

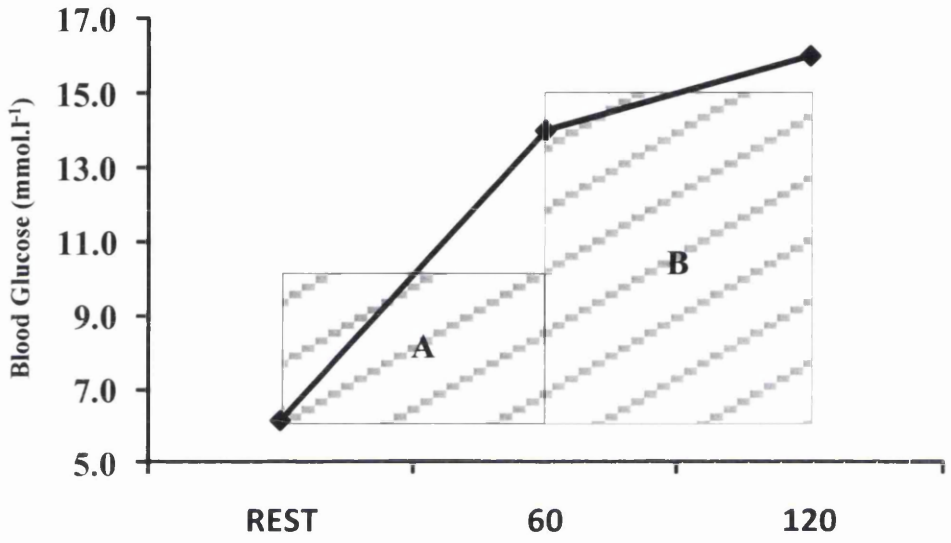


Figure 2.12: Example calculation of blood glucose area under the curve.

1) Calculations

A = $(((BG_{60} - BG_{REST})/2)+BG_{REST})$*Time (min)

- (14 – 6.2)/2 = 3.9
- 3.9 + 6.2 = 10.1
- 10.1 * 60 = 606 $mmol.l^{-1}.min^{-1}$

B = $(((BG_{120} - BG_{60})/2)+BG_{60})$*Time (min)

- (14.8 – 14)/2 = 0.4
- 0.4 + 14 = 14.4
- 14.4 * 60 = 864 $mmol.l^{-1}.min^{-1}$

Total BG_{AUC} = **A** + **B** = 1470 $mmol.l^{-1}.min^{-1}$

2) Time Average

Total BG_{AUC} ($mmol.l^{-1}.min^{-1}$) / (total hours * 60)

- 1470 / (2*60) = <u>12.3 $mmol.l^{-1}.hour^{-1}$</u>

2.7.2 Calculation of substrate oxidation rates and energy expenditure

The metasoft software (Cortex Biophysik, Liepzig) uses an automated calculation of inspired volume (V_I), using the Haldane Transformation method (below), to calculate inspired and expired VO_2 and VCO_2.

Haldane Transformation method:

$$V_I = \frac{F_EN_2\%}{F_IN_2\%} \times V_E$$

$F_IN_2\%$ (mass N_2 Inspired, constant) = 100% - 20.93 – 0.03 = 79.04

$F_EN_2\%$ (mass N_2 Expired) = $100 - F_EO_2 - F_ECO_2$

Calculating VO_2 and VCO_2:

$$VO_2 \text{ inspired} = V_I \times \frac{F_IO_2}{100}$$

$$VO_2 \text{ expired} = V_E \times \frac{F_EO_2}{100}$$

$$VCO_2 \text{ inspired} = V_I \times \frac{F_ICO_2}{100}$$

$$VCO_2 \text{ expired} = V_E \times \frac{F_ECO_2}{100}$$

Indirect Calorimetry

Non-protein respiratory exchange ratios were used to calculate the rates of carbohydrate and lipid oxidation, using the equations described by Frayn (1983).

$$\textbf{Carbohydrate oxidation (g.min}^{-1}\textbf{)} = 4.55\ VCO_2 - 3.21\ VO_2$$

$$\textbf{Fat oxidation (g.min}^{-1}\textbf{)} = 1.67\ VO_2 - 1.67\ VCO_2$$

Although ketone body formation can influence RER (Frayn, 1983), all individuals that took part in the experimental trials were being treated with a basal-bolus regimen, and only bolus insulin was manipulated, therefore, elevated ketone body formation was not considered an issue. Breath-by-breath respiratory data were time averaged to 1 minute.

Calculation of fuel oxidation and energy expenditure

Average carbohydrate and lipid oxidation rates were obtained through an average of either the 15 minute resting collection or the 45 minutes of running. Time course changes in carbohydrate and lipid oxidation were calculated in 10 minute blocks, not including the initial 5 minutes of the exercise bout.

Total energy expenditure was calculated through the sum of energy expended, within each minute, over the 45 minutes of running. For example:

- Total oxidation over 45 minutes: CHO = 110 g, lipids = 22.5 g
- (110*4) + (22.5*9) = <u>643 kcal</u>
- 643*0.0042 = <u>2.69 MJ</u>

2.7.3 Sample size calculation

Based on the data of Rabasa-Lhoret et al. (2001) for a statistical power of 80%, 33 subjects were required. However, due to the strict inclusion criteria and the time commitment required for completion of these trials, a sample size of 33 was outside of the pool of available T1DM individuals. Therefore, a sample of sufficient size to perform parametric statistics was attained. Statistical power across chapters 3 to 5 ranged from 45 to 59%.

2.7.4 Fasting blood glucose variability

For an inference into the variability that exists in fasted blood glucose concentrations within T1DM individuals, participants within chapter 3 completed a 3 week diary prior to participation in the trials.

Table 2.13: Twenty-one day morning fasted blood glucose concentrations of participants within chapter 3.

Participant ID	Mean Blood Glucose ($mmol.l^{-1}$)	SD	Range ($mmol.l^{-1}$)	COV (%)
1	7.9	4.6	2.2 - 19.8	59
2	4.8	1.9	2.7 - 8.6	39
3	7.6	3.0	2.8 - 13.4	40
4	6.9	2.3	4.4 - 10.8	32
5	7.1	2.9	2.5 - 11.7	40
6	7.0	3.5	3.4 - 13.3	51
7	4.6	1.4	2.9 - 8.2	29
8	7.7	4.6	2.3 - 16.3	59

2.7.5 Statistical analysis

Statistical analysis was performed using SPSS software (version 16; SPSS Inc., Chicago, IL), with significance set at $P \leq 0.05$. Data were tested for normal distribution (Shapiro-Wilk test) and subsequently analysed using repeated-measures ANOVA on two (chapter 4) or four (chapter 3 and 5) factors (treatment x time) with Bonferroni adjustment and dependent t-tests carried out where relevant. Where there were significant time and/or time*condition effects, P values and effect size (partial-eta^2) were reported. All results were reported as the mean ± SEM.

Data described as the change from rest (Δ) were calculated by subtracting resting values away from all subsequent sample time-points.

Chapter Three

The metabolic and glycaemic effects of reductions to pre-exercise rapid-acting insulin dose.

3.1 Introduction

Hypoglycaemia is a frequent occurrence in individuals with type 1 diabetes (T1DM; Cryer et al., 2003) and a major factor for the avoidance of exercise (Brazaeu et al., 2008). In an effort to better preserve blood glucose concentrations and combat the heightened risk of hypoglycaemia during and after exercise, individuals with T1DM are recommended to reduce their pre-exercise insulin dose (De Feo et al., 2006; Grimm, 2005; Mauvais-Jarvis et al., 2003; Rabasa-Lhoret et al., 2001).

Within the research examining pre-exercise insulin reductions, recommendations have varied from 10-40% (De Feo et al., 2006), 10-50% (Grimm, 2005), 50-90% (Mauvais-Jarvis et al., 2003) and 50-75% (Rabasa-Lhoret et al., 2001). Much of the variation in these findings can be attributed to differences in the insulin regimen used by the participants, e.g. regular/Neutral Protamine Hagedorn insulin (Mauvais-Jarvis et al., 2003) or Ultralente with prandial lispro (Rabasa-Lhoret et al., 2001), and differences in the intensity and duration of the exercise model. Another limitation, within the existing literature, is the examination of T1DM individuals performing cycling exercise only. Cycling is a primarily concentric form of exercise i.e. the muscle shortens as it contracts. However, in many daily activity patterns including body weight supporting exercises such as jogging or running there is a significant proportion of eccentric muscle actions, where the muscle lengthens in the performance of the movement. Eccentric muscle actions have been demonstrated to hinder insulin action and glucose uptake for as long as 48 hours following exercise (Asp et al., 1995; Asp et al., 1996). However to date, the impact of combined concentric and eccentric movement patterns, such as running, on post-exercise glucose responses has been neglected. Furthermore, an important point of the previous research has been the limited time window of examination of the potential for the development of hypoglycaemia after exercise with some studies monitoring

participants for just 1-2 hours after exercise (Mauvais-Jarvis et al., 2003; Rabasa-Lhoret et al., 2001). Low blood glucose, leading to hypoglycaemia may occur several hours after exercise (MacDonald, 1987; Tsalikian et al., 2005), so studies that increase the observation window following the cessation of exercise are required to characterise the impact of exercise on post-exercise glycaemia. Moreover, as pre-exercise insulin dose has proven implications for post-exercise glycaemia, and glycaemia is associated with hunger and satiety (Russell et al., 2001) an examination of how reductions to pre-exercise insulin dose affects the voluntary diet intake of T1DM individuals is also required. In addition, reduction or omission in insulin dose is a significant factor in the development of diabetic ketoacidosis (Wallace and Matthews, 2004); keeping this in mind, exercise stimulates increases in ketogenic hormones. Therefore, lower circulating insulin concentrations and exercise induced increases in counter-regulatory hormones could create a milieu for increased production of ketone bodies; however, there is limited data to refute this.

Therefore, this study examined the metabolic and glycaemic responses to reductions in pre-exercise rapid-acting insulin dose for 24 hours after running in T1DM individuals.

3.2 Methodology

Eight T1DM individuals (7 males, 1 female, 34 ± 2 years, BMI 27 ± 1 kg/m^2) with a duration of diabetes of 16 ± 1 years and HbA_{1c} of 8.3 ± 0.1% were recruited for this study. Participant anthropometric (Table 2.5), glycaemic control (Table 2.8), and insulin regimen (Table 2.2) characteristics are presented within chapter 2.

After preliminary testing (Table 2.6) participants attended the laboratory on four occasions after an overnight fast and having consumed similar evening meals prior to each trial. Upon arrival participants received catheterisation in their non-dominant arm and resting blood samples were processed for glucose, lactate, pH, insulin, glucagon, adrenaline, noradrenaline, cortisol and β-hydroxybutyrate (section 2.3.5). Participants were then instructed to ingest a 1.12 MJ meal (60 g carbohydrates, 2 g protein, 2 g fat; wheat and peaches) and administer either **Full** (7.3 ± 0.2 IU), **75%** (5.4 ± 0.1 IU), **50%** (3.7 ± 0.1 IU), or **25%** (1.8 ± 0.1 IU) of their rapid-acting insulin dose into the abdomen (subcutaneously). Subsequent blood samples were taken for 2 hours before performing 45 minutes of running at 70 ± 1% VO_{2peak} and for three hours post-exercise (Figure 3.1). Cardio-respiratory parameters were collected at rest and during exercise.

Participant activity and heart rate were recorded for 24 hours after exercise; moreover, for 21 hours after leaving the laboratory participants self-recorded blood glucose, dietary intake, insulin administration and hypoglycaemic incidences.

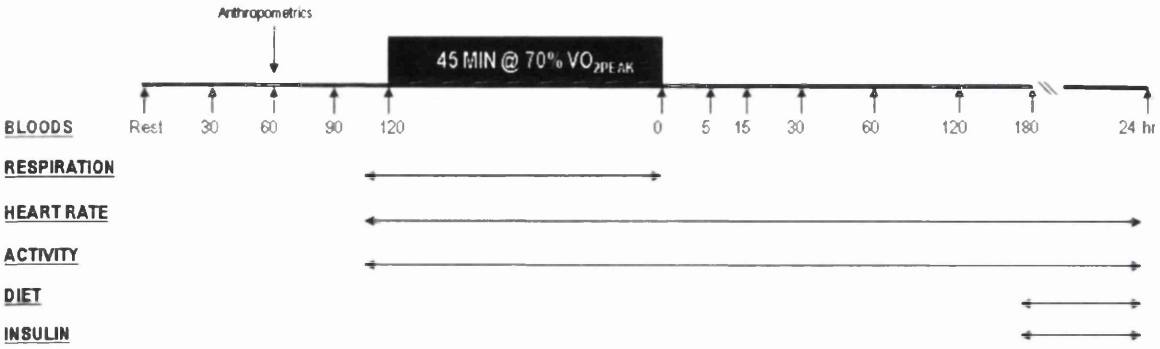


Figure 3.1: Schematic diagram of the experimental protocol of chapter 3.

3.2.1 Statistical Analysis

Statistical analysis was performed using SPSS software (version 16; SPSS Inc., Chicago, IL), with significance set at $P<0.05$. Data were tested for normal distribution (Shapiro-Wilk test) and subsequently analysed using repeated-measures ANOVA on four factors (treatment x time) with Bonferroni adjustments and dependent t-tests carried out where relevant. Data are presented as mean ± SEM.

3.3 Results

Due to insulin assay cross-reactivity with insulin detemir, only individuals treated with insulin glargine could be included in the analysis. Therefore, participant 6 was removed from analysis and n = 7.

3.3.1 Physiological responses to exercise

There were no effects of condition on the percentages of peak rate of O_2 consumption (**Full** 71 ± 0.6, **75%** 69 ± 0.8, **50%** 71 ± 0.5, **25%** 70 ± 0.5 %, P = 0.48) or $\%HR_{peak}$ (**Full** 83 ± 1, **75%** 82 ± 1, **50%** 83 ± 1, **25%** 83 ± 1 %, P = 0.43; Table 3.1) during exercise.

Exercise intensity fell within the current ACSM guidelines for diabetes with participants on average exercising at 67 ± 4% of VO_2 reserve and 58 ± 6% of HR reserve, during the 45 minute treadmill run.

Table 3.1: Cardio-respiratory responses to reductions in pre-exercise rapid-acting insulin dose at rest and during exercise..

	Rest				Exercise			
	Full	**75%**	**50%**	**25%**	**Full**	**75%**	**50%**	**25%**
HR (bpm)	67 ± 1	65 ± 1	63 ± 1	63 ± 1	158 ± 2*	155 ± 2*	159 ± 1*	160 ± 2*
V_E ($l.min^{-1}$)	9.2 ± 0.2	10.1 ± 0.2	10.1 ± 0.3	9.4 ± 0.2	71.5 ± 2.3*	70.1 ± 2.*1	70.6 ± 2.0*	72.5 ± 1.9*
VO_2 ($ml.kg^{-1}.min^{-1}$)	3.7 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	29.8 ± 0.5*	30.4 ± 0.6*	31.1 ± 0.6*	30.3 ± 0.5*
VCO_2 ($ml.kg.^{-1}.min^{-1}$)	3.3 ± 0.1	3.6 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	29.1 ± 0.5*	28.3 ± 0.6*	29.7 ± 0.7*	29.1 ± 0.5*
RER	0.90 ± 0.01	0.87 ± 0.01	0.89 ± 0.01	0.88 ± 0.01	0.97 ± 0.01*	0.93 ± 0.01*	0.95 ± 0.01*	0.95 ± 0.01*

Data presented as means ± SEM (n = 7). * indicates a significant increase when compared with rest ($P<0.05$).

3.3.2 Blood glucose responses

Absolute and delta blood glucose responses are illustrated in Figures 3.2 A-C. There were no differences in fasting blood glucose concentrations (**Full** 9.4 ± 0.5, **75%** 7.5 ± 0.4, **50%** 9.2 ± 0.7, **25%** 7.5 ± 0.6 mmol.l^{-1}, P = 0.58). There were interactions between insulin dose and both absolute (P = 0.014, *partial-eta*^{2} = 0.399) and delta blood glucose responses (P = 0.012, *partial-eta*^{2} = 0.401) (Figure 3.2).

On average, pre-exercise peak blood glucose concentrations occurred 60 minutes after meal ingestion and insulin administration under **Full**, **75%** and **50%**, however, occurred at 90 minutes under **25%**. Peak blood glucose concentrations under the reduction trials were greater than under **Full** (Table 3.2). At 120 min post-meal ingestion, blood glucose concentrations elicited under **Full** (+3.9 ± 0.4 mmol.l^{-1}), were lower than **75%** (P = 0.02) and **25%** (P = 0.02), but similar to **50%** (P = 0.11, Table 3.2). The reduced dose trials were statistically similar (P>0.05, Table 3). Exercise resulted in a decrease in blood glucose concentrations under all conditions (P<0.01) with the decline under **Full** (6.1 ± 0.4 mmol.l^{-1}) tending to be greater than **75%** (4.3 ± 0.5 mmol.l^{-1}, P = 0.08), and **25%** (3.2 ± 0.4 mmol.l^{-1}, P = 0.04) but similar to **50%** (5.5 ± 0.5 mmol.l^{-1}, P = 0.21). The insulin reduction trials were not different (Figure 3.2 B and C).

The change from 0 to 180 minutes was similar across conditions (**Full** Δ+0.3 ± 0.5; **75%** Δ-0.3 ± 0.5; **50%** Δ-2.2 ± 0.3; **25%** Δ-1.4 ± 0.7 mmol.l^{-1}, P>0.05) with blood glucose concentrations at 180 minutes similar to 0 minutes under all conditions (Figure 3.2 B and C). Post-exercise blood glucose area under the curve (BG_{AUC}) was lowest under **Full** in comparison with the reduction trials (**Full** 7.3 ± 0.4 vs. **75%** 9.2 ± 0.5; **50%** 8.5 ± 0.4; **25%** 11.2 ± 0.7 mmol.l^{-1}.hour^{-1}, P<0.05), with BG_{AUC} greater under **25%** in comparison with all other trials (P<0.05).

Hypoglycaemia and low blood glucose

During the trial period, under **Full**, there were 3 occasions where blood glucose reached hypoglycaemic concentrations (two at 0 minutes and one at 180 minutes post-exercise). There was a single case of low blood glucose under **75%** (180 minutes post-exercise) and **50%** (180 minutes post-exercise), and one case of blood glucose reaching hypoglycaemic concentrations under **25%** (180 minutes post-exercise).

3.3.3 Serum insulin responses

Serum insulin responses are reported in Figure 1A. There were no significant differences in fasting serum insulin (**Full** 115 ± 15, **75%** 125 ± 15, **50%** 127 ± 19, **25%** 126 ± 13 pmol.l^{-1}, P = 0.63). There was a significant time effect (P = 0.003 *partial-eta*^{2} = 0.611) and a significant time*condition interaction (P = 0.012, *partial-eta*^{2} = 0.321) within the serum insulin responses (Figure 3.2A). The peak insulin concentrations occurred 60 minutes after administration, regardless of dose (Table 3, Figure 3.2A). Insulin concentrations elicited at this time point under **Full** were similar to **75%** (P = 0.18) and **50%** (P = 0.14), but tended to be greater than **25%** (P = 0.06). The reduced dose trials were not statistically different (P>0.05, Table 3.2). Pre-exercise serum insulin concentrations were greatest under **Full**, however, these differences did not reach statistical significance (**Full** 112 ± 15; **75%** 86 ± 10, **50%** 56 ± 7, **25%** 55 ± 5 pmol.l^{-1},P>0.05).

The change in serum insulin concentrations with exercise was greater under **50%** and **25%** (**50%** Δ+31 ± 5; **25%** Δ+48 ± 9 pmol.l^{-1}) when compared to **Full** and **75%** (**Full** Δ+19 ± 8; Δ+15 ± 7 pmol.l^{-1}, P<0.05). Immediate post-exercise circulating insulin was similar to pre-exercise concentrations under **Full** and **75%**, but tended to increase under **50%** (P = 0.07)

and **25%** (P = 0.07) (Table 3.2 and Figure 3.2A). The decline in insulin in the 3 hour recovery period under **Full** (Δ-142 ± 13 pmol.l^{-1}) was greater under **25%** (Δ-92 ± 7 pmol.l^{-1}, P = 0.03) and tended to be greater than **75%** (Δ-115 ± 7 pmol.l^{-1}, P = 0.08) but similar to **50%** (Δ-138 ± 15 pmol.l^{-1}, P = 0.31); there were no statistical differences between the reduced dose trials. Insulin concentrations were not statistically different, between conditions, after 180 minutes of rest (P>0.05; Table 3.2 and Figure 3.2A).

Table 3.2: ΔSerum insulin and Δblood glucose responses to reductions in pre-exercise rapid-acting insulin dose.

	Condition			
Variable	**Full**	**75%**	**50%**	**25%**
Peak				
Insulin (pmol.l^{-1})	125 ± 11	114 ± 8	82 ± 12*	67 ± 9*†
BG (mmol.l^{-1})	6.0 ± 0.4	7.4 ± 0.3*	7.0 ± 0.5*	9.0 ± 0.6*
Pre-Exercise				
Insulin (pmol.l^{-1})	112 ± 15	86 ± 10	56 ± 7	55 ± 5
BG (mmol.l^{-1})	3.5 ± 0.4*	6.2 ± 0.3*	5.6 ± 0.4	7.7 ± 0.7*
0 min				
Insulin (pmol.l^{-1})	111 ± 15	87 ± 7	101 ± 19	80 ± 11*
BG (mmol.l^{-1})	-2.6 ± 0.7	1.9 ± 0.5*	0.1 ± 0.7	4.5 ± 0.9*
180 min				
Insulin (pmol.l^{-1})	15 ± 11	8 ± 12	7 ± 12	26 ± 11
BG (mmol.l^{-1})	-2.4 ± 0.8	1.1 ± 0.8	-2.1 ± 0.9	4.1 ± 0.6*

Data presented as means ± SEM (n = 7). * indicates difference when compared with **Full** (P<0.05). † indicates difference when compared with **75%** (P<0.05). BG indicates blood glucose.

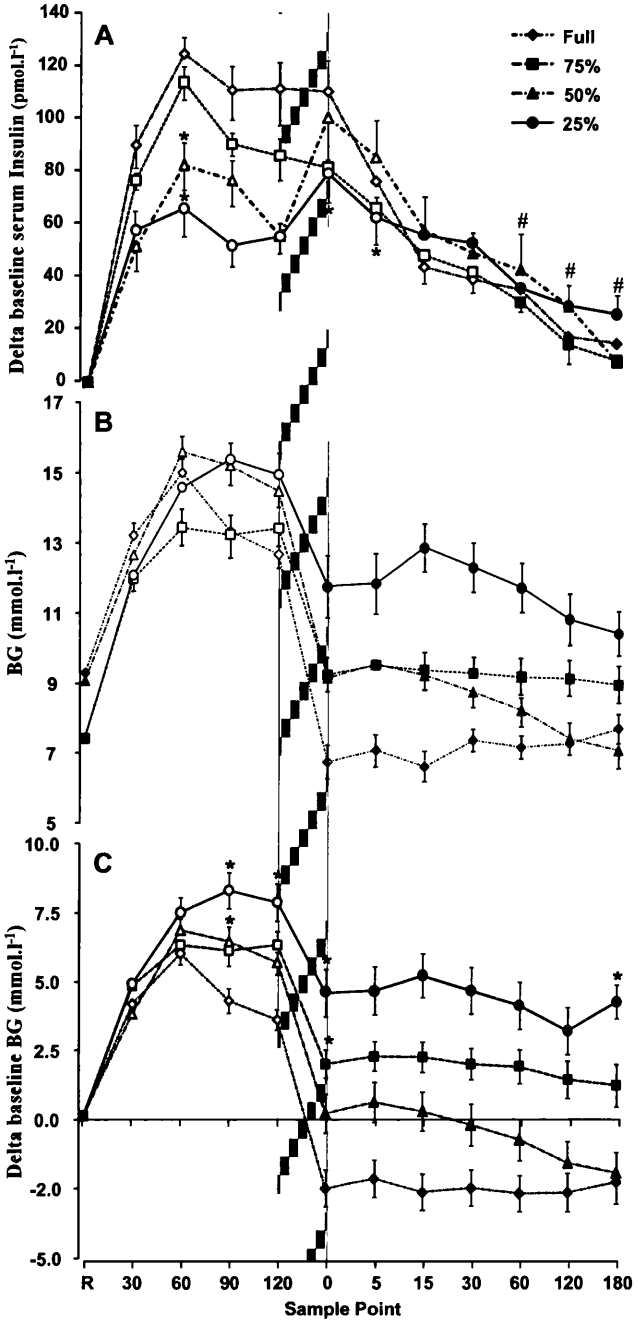


Figure 3.2: Time-course changes in serum insulin (A) and consequent blood glucose responses in absolute concentrations (B) and changes from baseline (C), after reductions in pre-exercise rapid-acting insulin dose. Data presented as mean ± SEM. Transparent sample point within a trial indicates significant difference when compared to rest ($P<0.05$).* indicates significant difference when compared with **Full** at the respective time point ($P<0.05$). # indicates significant difference when compared to 0 min post-exercise under all conditions ($P<0.05$). Thatched area indicates exercise. **Note**: Test meal and insulin were administered immediately following the resting sample.

3.3.4 Counter-regulatory hormonal responses

Fasting concentrations of all counter-regulatory hormones were similar across conditions ($P>0.05$; Table 3.3).

There was no time effect ($P = 0.12$) or effect of condition ($P = 0.13$) on the plasma glucagon response. Plasma glucagon did not differ from rest at 180 minute post-exercise, with similar changes with time between conditions (**Full** $\Delta+20 \pm 4$; **75%** $\Delta-46 \pm 11$; **50%** $\Delta-37 \pm 6$; **25%** $\Delta+10 \pm 5$ pmol.l^{-1}; $P>0.05$; Table 3.3).

There was a time effect ($P = 0.001$, Partial-eta$^2 = 0.769$), but no conditional influence ($P = 0.215$) on the plasma adrenaline response. Adrenaline significantly increased with exercise under all trials, with the change from rest to 0 minutes similar across conditions (**Full** $\Delta+1.2 \pm 0.1$; **75%** $\Delta+0.7 \pm 0.1$; **50%** $\Delta+1.0 \pm 0.1$; **25%** $\Delta+1.0 \pm 0.1$ nmol.l^{-1}; $P>0.05$). Adrenaline concentrations decreased from 0 to 180 min under all conditions, with similar changes across conditions (**Full** $\Delta-1.0 \pm 0.1$; **75%** $\Delta-0.7 \pm 0.04$; **50%** $\Delta-0.6 \pm 0.1$; **25%** $\Delta-0.8 \pm 0.1$ nmol.l^{-1}; $P>0.05$). At 180 minutes adrenaline concentrations were different to rest under **Full** only ($P<0.05$, Table 3.3).

There was a time effect ($P = 0.001$, Partial-eta$^2 = 0.816$), but no conditional influence ($P = 0.14$) on the plasma noradrenaline response. Noradrenaline significantly increased with exercise under all conditions, with the change from rest to 0 minutes similar across **Full**, **75%** and **25%** (**Full** $\Delta+19.8 \pm 1.5$; **75%** $\Delta+17.8 \pm 1.7$; **50%** $\Delta+10.8 \pm 0.9$; **25%** $\Delta+20.1 \pm 1.8$ nmol.l^{-1}; $P>0.05$); changes under **50%** were less than all other trials (**50%** $\Delta+10.8 \pm 0.9$ nmol.l^{-1}; $P<0.05$). Noradrenaline concentrations decreased to resting concentrations from 0 to 180 minutes post-exercise under all conditions (Table 3.3), with the change over this period

less under **50%** (Δ-8.4 ± 1.0 nmol.l^{-1}) in comparison with all other trials (**Full** Δ-17.7 ± 1.5; **75%** Δ-17.6 ± 1.8; **25%** Δ-17.7 ± 2.1 nmol.l^{-1}; P<0.05).

There was a time effect (P = 0.001, Partial-eta^2 = 0.759), but no conditional influence (P = 0.62) on the serum cortisol response. Cortisol did not change with exercise under any condition (Table 3.3). However, cortisol significantly increased from 0 to 15 minutes post-exercise under all conditions (Table 3.3). The change from 0 to peak was similar across conditions (**Full** Δ+54 ± 13; **75%** Δ+48 ± 15; **50%** Δ+86 ± 10; **25%** Δ+84 ± 14 nmol.l^{-1}; P>0.05). Cortisol concentrations declined to concentrations less than rest from 15 to 180 minutes (Table 3.4), with the change over this period similar between conditions (**Full** Δ-400 ± 24; **75%** Δ-342 ± 27; **50%** Δ-338 ± 30; **25%** Δ-397 ± 26 nmol.l^{-1}; P>0.05).

Table 3.3: Counter-regulatory hormonal responses to reductions in pre-exercise rapid-acting insulin dose.

		Sample Point			
Hormone		Rest	0 min	15 min	180 min
Glucagon (pmol.l^{-1})	**Full**	184 ± 29	-	-	205 ± 32
	75%	176 ± 29	-	-	130 ± 20
	50%	226 ± 36	-	-	189 ± 34
	25%	175 ± 30	-	-	166 ± 28
Adrenaline (nmol.l^{-1})	**Full**	0.56 ± 0.01	1.75 ± 0.10*	-	0.73 ± 0.02*
	75%	0.60 ± 0.02	1.26 ± 0.04*	-	0.54 ± 0.01
	50%	0.84 ± 0.01	1.83 ± 0.07*	-	1.27 ± 0.08
	25%	0.57 ± 0.03	1.57 ± 0.11*	-	0.80 ± 0.08
Noradrenaline (nmol.l^{-1})	**Full**	3.43 ± 0.18	23.22 ± 1.25*	-	5.54 ± 0.18
	75%	3.91 ± 0.38	21.75 ± 1.96*	-	4.17 ± 0.15
	50%	3.64 ± 0.37	14.44 ± 0.90*	-	6.03 ± 0.31
	25%	2.51 ± 0.18	22.59 ± 1.89*	-	5.41 ± 0.65
Cortisol (nmol.l^{-1})	**Full**	610 ± 20	585 ± 18	657 ± 20*†	265 ± 19*†
	75%	637 ± 24	574 ± 14	632 ± 19*†	298 ± 29 *†
	50%	596 ± 19	486 ± 19	591 ± 22*†	261 ± 19*†
	25%	650 ± 20	592 ± 14	685 ± 22*†	295 ± 21*†

Data presented as means ± SEM (n = 7). * indicates significant difference when compared with rest (P<0.05). † indicates difference when compared to 0 min post-exercise (P<0.05).

3.3.5 Serum β-hydroxybutyrate responses

There was a significant time effect ($P = 0.003$, partial-eta$^2 = 0.630$) within the serum β-hydroxybutyrate responses to alterations in rapid-acting insulin dose (Figure 3.3), however, there were no conditional differences ($P = 0.58$; Figure 3.3). There were small declines from rest to 120 minutes post-ingestion under all conditions (**Full** Δ-0.15 ± 0.03; **75%** Δ-0.07 ± 0.01; **50%** Δ-0.03 ± 0.01; **25%** Δ-0.03 ± 0.01 mmol.l^{-1}; $P>0.05$). Concentrations did not change with exercise under any condition (**Full** Δ+0.06 ± 0.01; **75%** Δ+0.04 ± 0.01; **50%** Δ+0.04 ± 0.01; **25%** Δ+0.03 ± 0.01 mmol.l^{-1}; $P>0.05$; Figure 3.3), however, there was a transient increase in concentrations over the three hour recovery period, with concentrations at 120 and 180 minutes significantly greater than both rest and 0 minutes post-exercise under all conditions ($P<0.05$; Figure 3.3). The change from 0 to 180 minutes post-exercise was similar across conditions (**Full** Δ+0.31 ± 0.04; **75%** Δ+0.24 ± 0.02; **50%** Δ+0.27 ± 0.03; **25%** Δ+0.39 ± 0.05 mmol.l^{-1}; $P>0.05$).

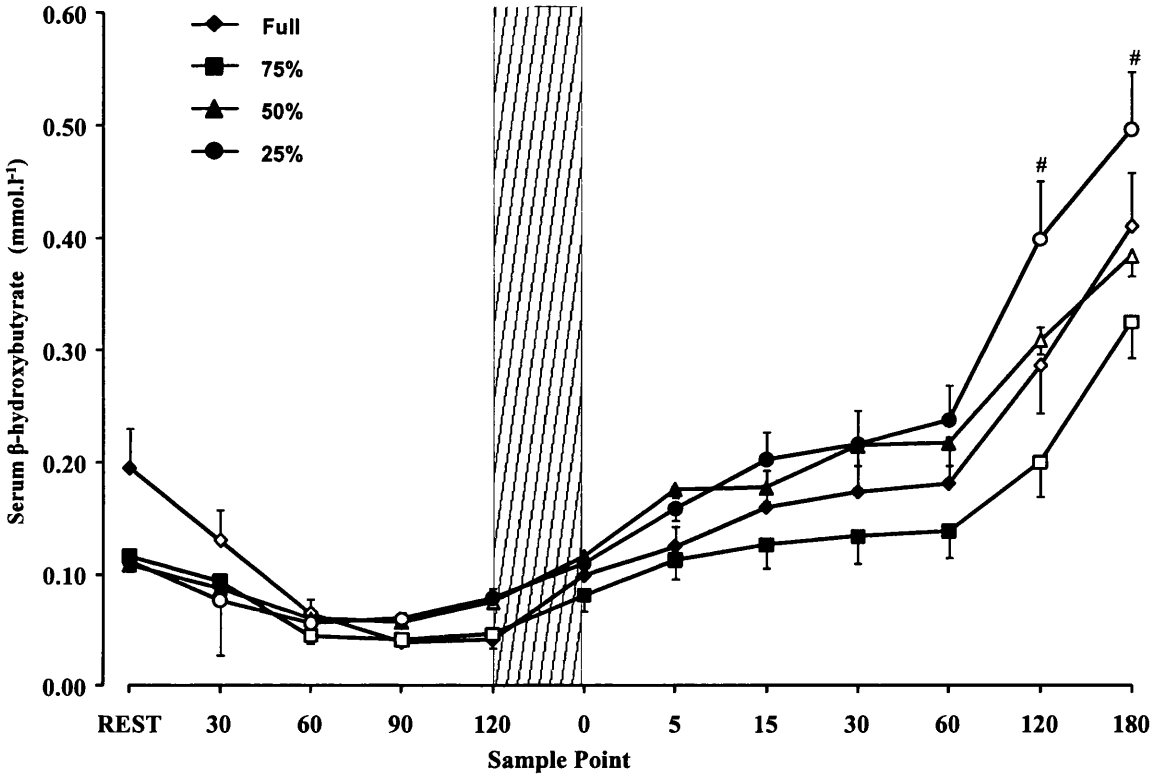


Figure 3.3: Time-course changes in serum β-hydroxybutyrate after reductions in pre-exercise rapid-acting insulin dose. Data presented as mean ± SEM. Transparent sample point within a trial indicates a significant difference when compared to rest ($P<0.05$), # indicates significant difference from 0 min post-exercise under all conditions ($P<0.05$). Thatched area indicates exercise. **Note**: Test meal and insulin were administered immediately following the resting sample.

3.3.6 Blood pH and lactate responses

Time course-changes in blood pH and lactate are presented in Figure 3.4 A and B. There was a significant time effect ($P = 0.002$, partial-eta$^2 = 0.674$) and a significant time*treatment interaction ($P = 0.002$, partial-eta$^2 = 0.251$) within the blood pH responses. There were no conditional differences in blood pH responses during the pre-exercise period (Figure 3.3 A). Blood pH increased significantly from the pre-exercise sample to 0 minutes post-exercise under all conditions ($P<0.05$). However, the change in pH with exercise was less under **25%** when compared to the other trials (**Full** $\Delta+0.07 \pm 0.01$; **75%** $\Delta+0.06 \pm 0.01$; **50%** $\Delta+0.06 \pm 0.01$; **25%** $\Delta+0.02 \pm 0.01$), but was only statistically different to **Full** ($P = 0.03$). Blood pH under **25%** at 0 and 5 minutes post-exercise was significantly lower than both **Full** and **75%** (Figure 3.4A). Moreover, after the 5 minute post-exercise sample, blood pH was not different between conditions for the remainder of the 180 minute recovery period (Figure 3.4A). Blood pH decreased from 0 to 180 minutes under **Full**, **75%** and **50%**, but not under **25%**. Moreover, the change in blood pH from 0 to 180 min was greatest under **Full** (**Full** $\Delta-0.07 \pm 0.01$; **75%** $\Delta-0.05 \pm 0.01$; **50%** $\Delta-0.05 \pm 0.01$; **25%** $\Delta-0.03 \pm 0.01$), however, only **Full** and **25%** were statistically different ($P = 0.04$).

There was a significant time effect within the blood lactate response to the protocol ($P = 0.024$, partial-eta$^2 = 0.532$). However, there were no effects of insulin dose on the blood lactate responses ($P = 0.27$). There were no conditional differences in blood lactate concentrations within the pre-exercise period, with concentrations just before exercise similar across conditions (**Full** 1.1 ± 0.03; **75%** 1.0 ± 0.03; **50%** 1.0 ± 0.4; **25%** 1.1 ± 0.03 mmol.l^{-1}, $P>0.05$). The change in blood lactate concentrations, with exercise, was greatest under **25%**; however, there were no statistical differences between the conditions (**Full** $\Delta+3.1 \pm 0.4$; **75%** $\Delta+3.3 \pm 0.5$; **50%** $\Delta+3.1 \pm 0.04$; **25%** $\Delta+4.3 \pm 0.4$ mmol.l^{-1}; $P>0.05$). Blood lactate

concentrations decreased with time after 0 minutes under all conditions and the change from 0 to 180 minutes was similar across trials (**Full** Δ-3.5 ± 0.4; **75%** Δ-3.5 ± 0.5; **50%** Δ-3.3 ± 0.0.4; **25%** Δ-4.5 ± 0.5 $mmol.l^{-1}$; P>0.05; Figure 3.4 B).

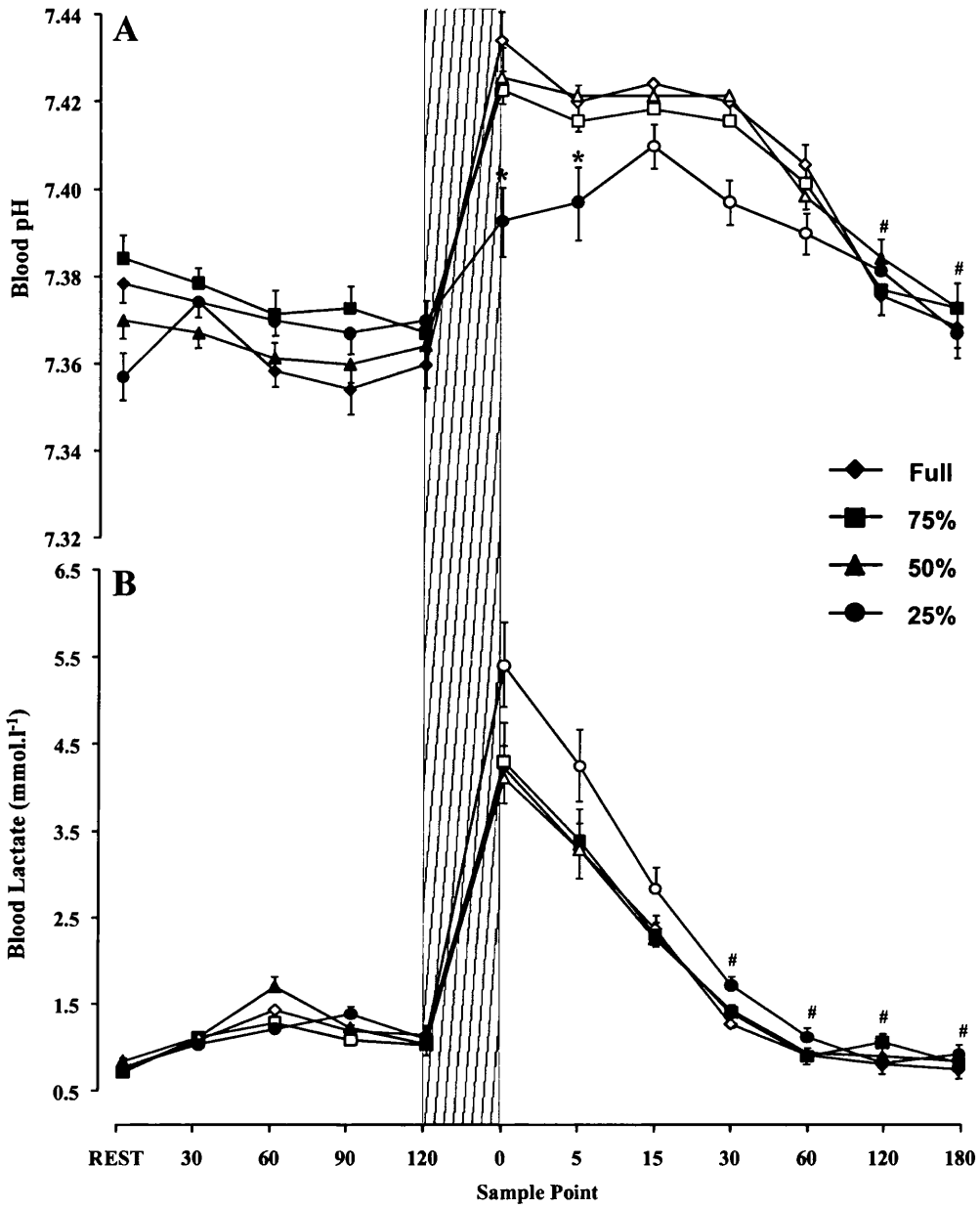


Figure 3.4: Time-course changes in blood pH (A) and lactate (B), in absolute values, after reductions in pre-exercise rapid-acting insulin dose. Data presented as mean ± SEM. Transparent sample point within a trial indicates a significant difference when compared to rest ($P<0.05$). * indicates significantly different to **Full** and **75%** ($P<0.05$). # indicates significantly different to 0 min post-exercise under all conditions ($P<0.05$). Thatched area indicates exercise. **Note**: Test meal and insulin were administered immediately following the resting sample.

3.3.7 Patterns of carbohydrate and lipid oxidation

There was no significant effect of insulin dose on resting or exercising fuel metabolism with similar rates of carbohydrate and lipid oxidation between conditions (P = 0.21; Table 3.4). Moreover, when expressing substrate oxidation rates as a change from rest to exercise, no conditional differences were evident (P = 0.13; Table 3.4).

Table 3.4: Substrate oxidation responses to reductions in pre-exercise rapid-acting insulin dose at rest and during exercise.

	Rest				Exercise			
	Full	**75%**	**50%**	**25%**	**Full**	**75%**	**50%**	**25%**
CHO ($g.min^{-1}$)	0.25 ± 0.01	0.23 ± 0.06	0.25 ± 0.1	0.23 ± 0.01	2.67 ± 0.08*	2.35 ± 0.05*	2.50 ± 0.09*	2.56 ± 0.07*
Lipids ($g.min^{-1}$)	0.05 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.15 ± 0.01*	0.30 ± 0.07*	0.27 ± 0.03*	0.21 ± 0.02*
ΔCHO ($g.min^{-1}$)	-	-	-	-	2.41 ± 0.07*	2.15 ± 0.08*	2.24 ± 0.09*	2.32 ± 0.07*
ΔLipids ($g.min^{-1}$)	-	-	-	-	0.10 ± 0.01*	0.24 ± 0.01*	0.20 ± 0.02*	0.06 ± 0.03*

Data presented as means ± SEM (n = 7). * indicates a significant increase when compared with rest ($P<0.05$).

Energy expended during exercise was similar across trials (**Full** 2.26 ± 0.04; **75%** 2.29 ± 0.05; **50%** 2.34 ± 0.04; **25%** 2.29 ± 0.04 MJ, P = 0.51). The contribution to energy demand from carbohydrates (**Full** 87.8 ± 1.1; **75%** 76.9 ± 1.5; **50%** 80.0 ± 2.2; **25%** 83.4 ± 1.4 %, P = 0.61) and lipids (**Full** 12.2 ± 1.1; **75%** 23.1 ± 1.5; **50%** 20.0 ± 2.2; **25%** 16.6 ± 1.4 %, P = 0.61) were similar across conditions.

3.3.8 Post-laboratory activity and self-recorded glycaemia and dietary intake

During the 21 hours after leaving the laboratory, low blood glucose and hypoglycaemia were encountered under all trials (Low blood glucose: **Full** 9 hours; **75%** 7 and 19 hours; **25%** 5 and 22 hours post-laboratory: Hypoglycaemia: **Full** 1, 2, 8 and 9 hours; **75%** 18 hours; **50%** 1, 6, and 12 hours; Table 3.5). Furthermore, participants consumed more energy under **Full** and **75%** compared with **25%**. Participants consumed lower amounts of carbohydrate under **25%** when compared to **Full** ($P = 0.02$), with similar trends under **75%** ($P = 0.09$) and **50%** ($P = 0.08$). There were no conditional differences in the percentage contribution to total energy from carbohydrate (**Full** 49 ± 1.4; **75%** 50 ± 1.8; **50%** 49 ± 1.4; **25%** 50 ± 0.9 %; $P>0.05$), protein (**Full** 17 ± 0.7; **75%** 19 ± 0.3; **50%** 16 ± 0.7; **25%** 19 ± 0.4 %; $P>0.05$) or fats (**Full** 35 ± 1.1; **75%** 31 ± 1.9; **50%** 34 ± 1.4; **25%** 28 ± 1.0 %; $P>0.05$). There were no differences in the number of rapid-acting insulin units administered or activity patterns between trials, as average heart rate and steps taken were similar ($P>0.05$; Table 3.5).

Table 3.5: Twenty-one hour post-laboratory blood glucose, dietary intake and activity patterns.

	Condition			
Variable	**Full**	**75%**	**50%**	**25%**
BG_{auc} ($mmol.l^{-1}.hour^{-1}$)	7.0 ± 0.3	6.8 ± 0.2	7.5 ± 0.3	8.0 ± 0.3†
CHO intake (g)	180 ± 7	169 ± 7	148 ± 7*	124 ± 7*
Protein intake (g)	64 ± 3	63 ± 4	48 ± 3	48 ± 3
Fat intake (g)	60 ± 4	49 ± 5	50 ± 4	31 ± 2*
Energy intake (MJ)	6.3 ± 0.3	5.6 ± 0.3	5.2 ± 0.2*	4.1 ± 0.2*†
Rapid-acting insulin (U)	20 ± 1	22 ± 1	24 ± 1	23 ± 1
Frequency BG < 4.0 $mmol.l^{-1}$	1	2	-	2
Frequency BG < 3.5 $mmol.l^{-1}$	4	1	3	2
Average heart rate (bpm)	74 ± 1	82 ± 1	74 ± 1	76 ± 1
Average steps taken	4017 ± 267	6382 ± 682	5223 ± 382	6473 ± 420

Data presented as means ± SEM (n = 7). * indicates significantly different from **100%** ($P<0.05$), † indicates significantly different from **75%** ($P<0.05$). BG_{AUC} indicates blood glucose area under the curve.

3.4 Discussion

The aim of this study was not to examine hypoglycaemia *per se*, but to examine the effects of the currently recommended pre-exercise insulin reductions on metabolic and glycaemic responses for 24 hours after running in T1DM individuals. The results demonstrate the most severe reduction to pre-exercise rapid-acing insulin dose (75%) best preserved blood glucose responses for 24 hours following running, without increasing the risk of developing ketoacidosis. Furthermore, less energy and carbohydrates were consumed under this trial over the same time period.

Rapid-acting insulin concentrations peaked 60 minutes after injection across all trials with the lowest peak values occurring under the most severe insulin reduction condition (Figure 1A). Our results are similar to other researchers' findings (Homko et al., 2003; Plank et al., 2002) and demonstrate the relationship between injected dose and resultant serum concentration as well as the consistency of modern rapid-acting analogues to reach peak effect in spite of differences in anthropometric (body fat percentage, body mass index), or physiological (subcutaneous blood flow) parameters (Kolendorf et al., 1983). Similar to other researchers (Mauvais-Jarvis et al., 2003; Rabsa-Lhoret et al., 2001), the effect of the reduction in insulin dose was to increase blood glucose concentrations such that, in our study, blood glucose was highest immediately prior to running under the **25%** condition. (15.2 ± 0.6 mmol.l^{-1}) (Figures 3.2 B and C, Table 3.2).

Running pace was predominantly aerobic being performed at 70% of maximal oxygen uptake, which corresponded to ~67% and ~58% of participants' VO_2 and HR reserves, respectively. This resulted in completion of an isocaloric 2.6 MJ bout of exercise across trials, comprised of carbohydrate and lipid oxidation rates of approximately 2.5 and 0.23 g.min^{-1}, respectively. The total carbohydrate breakdown of ~113 g would have necessitated a

significant contribution from liver and muscle glycogen stores whereas the relatively low contribution of lipids oxidised (~10 g) during running suggest minimal contribution from adipose tissue and/or intramuscular depots. Respiratory data revealed there were no differences in carbohydrate or lipid oxidation rates during running between any of the trials. It has been demonstrated that decreases in circulating insulin concentrations provide a milieu for increased rates of lipid oxidation (Chokkalingam et al., 2007), but this was not evident from our data. The high pre-exercise blood glucose concentrations (13-15 $mmol.l^{-1}$), irrespective of condition, may have caused an equal but elevated carbohydrate oxidation rate (Jenni et al., 2008) suppressing any shifts in the pattern of lipid oxidation.

There were no treatment effects on the gluco-regulatory hormones. Within the pathophysiology of T1DM is a progressive loss of α-cell function over time, potentially due a loss of β – α cell signalling (Banarer et al., 2002), such that after 5 years of diagnosis falling blood glucose will fail to stimulate the release of this key hormone (Mokan et al., 1994). Although glucagon was only measured at two time points across trials, it is unlikely that any conditional changes would occur. Furthermore, the glycaemic threshold for the initiation of a counter-regulatory hormonal response is typically shifted to lower concentrations, compared to non-diabetics, in T1DM individuals (Cryer et al., 2001). Within this study, out of 28 trials, there were only 4 occasions where blood glucose fell below 3.5 $mmol.l^{-1}$, therefore concentrations were simply not low enough to augment the counter-regulatory hormonal response, thus explaining the lack of conditional differences in these hormones.

There was a large decline in insulin concentrations in the immediate post-exercise period across all trials (Figure 3.2A) which may have been due to an increased insulin clearance rate (Tuominen et al., 1997). This rapid reduction in insulin concentration may have lessened its inhibitory effects on the circulating counter-regulatory hormones; glucagon, adrenaline,

noradrenaline and cortisol. Despite similar concentrations of insulin in the 3 hour post-exercise period, the preservation of blood glucose was greatest under **25%** over the same time period.

There were no conditional differences in blood lactate or serum β-hydroxybutyrate responses to the trials. As the exercise stimulus was the same across trials it was unlikely that a conditional difference in blood lactate appearance would be evident. With regards to β-hydroxybutyrate; although ketone formation is associated with reduced insulin concentrations and hyperglycaemia, the participants' basal insulin dose remained unaltered across conditions. One of the primary roles of the basal dose is to restrain ketogenic enzymes (Barnett, 2003), moreover, as there were no differences in important ketone stimulating hormones, such as glucagon and adrenaline (Laffel, 1999); it was unlikely that differences in β-hydroxybutyrate would be present. The conditional differences in pH demonstrated under **25%** may be related to an increased glycoltyic flux due to elevated blood glucose concentrations (Coyle et al., 1997; Jenni et al., 2008). Coyle et al. (1997) identified that an increased glucose availability, such as under hyperglycaemic conditions, results in an increased glycolytic flux. Moreover, multiple stages of the glycolytic pathway results in the generation of H^+ (Robergs et al., 2004); of which some may leave the cell in exchange with Na^+ (Peronnet and Aguilaniu, 2006). Potentially, the hyperglycaemic conditions under **25%** resulted in an increased glycolytic flux and release of H^+, resulting in a lesser rise in pH during the exercise bout. An increased glycoltyic flux may be a potential explanation for the slightly, but non-significant, elevated peak lactate concentrations under this condition immediately after exercise.

The greatest incidence of post-exercise low blood glucose following exercise occurred in the full rapid-acting insulin dose condition, a finding similar to that of Rabasa-lhoret et al.

(2001). Furthermore, the most severe insulin reduction condition, the **25%** trial, resulted in the greatest preservation of blood glucose. Despite this, one participant, who experienced hypoglycaemia on three of four trials within the laboratory, still experienced hypoglycaemia after a 75% reduction to pre-exercise insulin (180 minutes post-exercise), suggesting individuals differ in their sensitivity to exercise with an additional large rate of glucose uptake for a small increase in circulating insulin. This is an important factor to consider in future research as a source of variability in T1DM individuals. Participants consumed fewest carbohydrates under **25%**, yet still administered a similar number of rapid-acting insulin units. As blood glucose was elevated leaving the laboratory (11.0 ± 0.7 mmol.l^{-1}), the insulin units administered, as a corrective measure for high blood glucose, combined with an increased sensitivity to insulin following exercise (Wojtaszewski et al., 2001), may explain the low blood glucose encountered in the post-laboratory period by some individuals under this trial.

Similar to the 3 hours following exercise, the self-reported data in the post-laboratory period revealed the 75% reduction in pre-exercise insulin resulted in the greatest post-exercise blood glucose area under the curve. Furthermore, over the remaining 21 hour of the trial participants consumed ~2.2 MJ less energy, notably as a result of 54 g less carbohydrate and 30 g less fat intake, however, there were no differences in the percentage composition of energy intake as there were no differences between the total energy intake from carbohydrates (49-50%), fats (29-35%) and proteins (16-19%), across any of the trials. T1DM individuals have been suggested to over-consume food in order to avoid hypoglycaemia (Jacob et al., 2006), but the reduced food intake within the **25%** trial may be evidence of a reduced need to consume additional foodstuffs as a compensatory approach to maintain blood glucose and avoid hypoglycaemia following exercise. In addition, the greater

blood glucose concentrations may have contributed to a greater feeling of satiety and reduced participants' hunger over the course of the day (Russell et al., 2001).

A potential limitation to this study was that participants monitored their blood glucose more frequently than they would typically perform on a daily basis which is likely a contributing factor to why there was only ~1.2 $mmol.l^{-1}.hour^{-1}$ range between the highest and lowest blood glucose area under the curve over the 21 hour post-laboratory periods. Although it is established that frequent monitoring of blood glucose can improve glycaemic control (Evans et al., 1999) through normalising blood glucose concentrations, it is important to recognise that this data is based upon T1DM individuals in everyday situations. We questioned T1DM individuals who were physically active, and it was evident that after exercise there is a tendency to increase the frequency of monitoring; moreover, the number and frequency of blood glucose measurements was consistent across trials. Furthermore, although there were small differences in blood glucose area under the curve between conditions, the frequent monitoring of blood glucose allowed participants to adapt their insulin administration and dietary intake, which was detectable in their diaries and ultimately in this chapters findings. Participants were provided with sufficient information and examples of how to complete the dietary diaries accurately. Moreover, it should be noted that this method of monitoring the patients' diet is currently employed across diabetes clinics in the UK.

A limitation to this study is its lack of statistical power. Based on the data of Rabasa-Lhoret et al. (2001), for 80% power we would have needed a sample size of ~33 subjects. Due to our strict inclusion criteria (Table 2.1), the large time commitment required for the trials and we were limited to individuals using glargine as their basal insulin, due to assay cross reactivity with insulin detemir, we could only examine 7 participants. As a result this study had a statistical power of 57%. Future research in this area should be done so with a larger sample

size. However, despite this, the data is of clinical importance, in that there is a clear and consistent preservation of blood glucose when a severe pre-exercise rapid-acting insulin reduction is employed. Employing this strategy may allow T1DM individuals the ability to regularly exercise without experiencing a hypoglycaemic incident during and after exercise. Furthermore, with a greater preservation of blood glucose and a lesser risk of hypoglycaemia, T1DM individuals may not feel the need to over consume carbohydrates in the post-exercise period.

In conclusion, this study examined the effects of reductions in pre-exercise rapid-acting insulin dose on metabolic and glycaemic responses for 24 hours after running in T1DM individuals. These data demonstrate that reducing pre-exercise, rapid-acting insulin dose by 75% improved blood glucose responses for 24 hours following running, without increasing the risk of developing diabetic ketoacidosis.

Chapter Four

The metabolic and glycaemic effects of alterations in the glycaemic index of the carbohydrate ingested before exercise.

4.1 Introduction

Exercise causes large reductions in blood glucose concentrations in type 1 diabetes individuals (T1DM). Reductions to the pre-exercise insulin dose help preserve blood glucose during and after exercise (De Feo et al., 2006; Mauvais-Jarvis et al., 2003; Rabasa-Lhoret et al., 2001). Furthermore, current research advocates the consumption of carbohydrates before exercise to prevent falls in blood glucose during and after a bout of exercise (De Feo et al., 2006; Hibbert-Jones and Regan, 2005; Maahs et al., 2009). However, specific factors related to the physical and chemical composition of carbohydrates, such as the glycaemic index, have been under researched.

The effects of changes in the type of carbohydrate consumed by T1DM, before performing exercise, on blood glucose responses following exercise has been under researched. Carbohydrates with a low glycaemic index (LGI) digest at slower rates than high glycaemic index (HGI) carbohydrates and are unable to cross the mucosal cell membrane within the small intestine and enter the bloodstream unless hydrolysed into monosaccharides (Thomas et al., 2007). Research has established the importance of including LGI carbohydrates into the daily diets of T1DM, with observed benefits such as lower daily mean blood glucose (Nansel et al., 2008) and reduced incidence of hypoglycaemia and reductions in HbA_{1c} (Gilbertson et al., 2001; Thomas et al., 2007). Within the study of Nansel et al. (2008), consumption of LGI foodstuffs, such as peaches, kidney beans or brown rice, resulted in glucose concentrations (assessed using a continuous glucose monitor) being within a target range of 3.9 – 9.9 $mmol.l^{-1}$ significantly more of the time than under the HGI trial (67 vs. 47 %). Moreover, the participants elicited a lower mean blood glucose concentration (LGI 7.6 ± 2.0 vs. HGI 10.1 ± 2.6 $mmol.l^{-1}$) and required less bolus insulin per 10 g of carbohydrate. There is limited data

examining the post-exercise metabolic and glycaemic responses of T1DM following ingestion of differing glycaemic index carbohydrates.

In addition to the potential to improve glycaemia, research has demonstrated that LGI carbohydrates alter exercising fuel metabolism. Following consumption of a LGI meal, carbohydrate oxidation rates have been shown to increase less, with a concomitant lesser suppression of lipid utilisation, during a bout of isoenergetic exercise in non-T1DM individuals (Achten et al., 2007; Stevenson et al., 2006). Stevenson et al. (2006) demonstrated a 31 % lower carbohydrate oxidation rate and 56 % greater lipid oxidation rate during a one hour treadmill run at 65 % VO_{2max} 3 hours following consumption of a LGI or HGI meal in eight female participants. Similarly, ingestion of a 50 g bolus of isomaltulose reduced carbohydrate oxidation rate by ~0.2 $g.min^{-1}$ and increased lipid oxidation more than sucrose during 150 minutes of cycling at 60 % VO_{2max} (Achten et al., 2007). This raises the possibility that consumption of an LGI carbohydrate source may spare both endogenous and exogenous carbohydrate reserves and increase fat oxidation in T1DM during exercise, resulting in better preservation of blood glucose during the post-exercise period.

With the potential for LGI carbohydrates to offer improved glycaemia and fuel oxidation in T1DM; it would also be prudent to examine the role of the glycaemic index of the pre-exercise carbohydrate on the incidence of hypoglycaemia after exercise, when a heavily reduced insulin dose is implemented (findings of chapter 3). Potentially, in the avoidance of hypoglycaemia after exercise the glycaemic index of the pre-exercise carbohydrate may be secondary in importance to a heavy reduction in pre-exercise insulin dose. Therefore, the aim of this study was to examine the effects of ingesting a high and low glycaemic index carbohydrate, in combination with a heavily reduced insulin dose, on metabolic and glycaemic responses before, during, and for 24 hours after running in T1DM individuals.

4.2 Methodology

Eight participants with T1DM (7 males, 1 female, 35 ± 2 years, BMI 26 ± 0.3 kg/m^2) with duration of diabetes of 14 ± 1 years and HbA_{1c} of 8.0 ± 0.2 % volunteered to participate in this study. Participant anthropometric (Table 2.5), glycaemic control (Table 2.8), and insulin regimen (Table 2.3) characteristics are presented chapter 2.

After preliminary testing (Table 2.6) participants attended the laboratory on two occasions after an overnight fast and having consumed similar evening meals prior to each trial. Upon arrival participants received catheterisation in their non-dominant arm and blood samples were processed for glucose, lactate, pH, glucagon, adrenaline, noradrenaline, cortisol, β-hydroxybutyrate, triglycerides, non-esterified fatty acids and glycerol (section 2.3.5). Participants were then required to consume, in a randomised and counterbalanced fashion, 75 g of either a high GI (**HGI**; Dextrose; GI 96) or low GI (**LGI**; Isomaltulose; GI 32) carbohydrate, mixed with 750 ml of water (10 % solution, Perrone et al., 2005). Immediately before ingestion, participants were instructed to administer their rapid-acting insulin, which had been reduced by 75 % (2.1 ± 0.2 IU) into the abdomen (findings from chapter 3; Rabasa-Lhoret et al., 2001). Subsequent blood samples were taken for 2 hours before performing 45 minutes of running at 80 ± 1% VO_{2peak} and for three hours post-exercise (Figure 4.1). Cardio-respiratory parameters were collected at rest and during exercise.

Participant activity and heart rate were recorded for 24 hours after exercise; moreover, for 21 hours after leaving the laboratory participants self-recorded blood glucose, dietary intake, insulin administration and hypoglycaemic incidences.

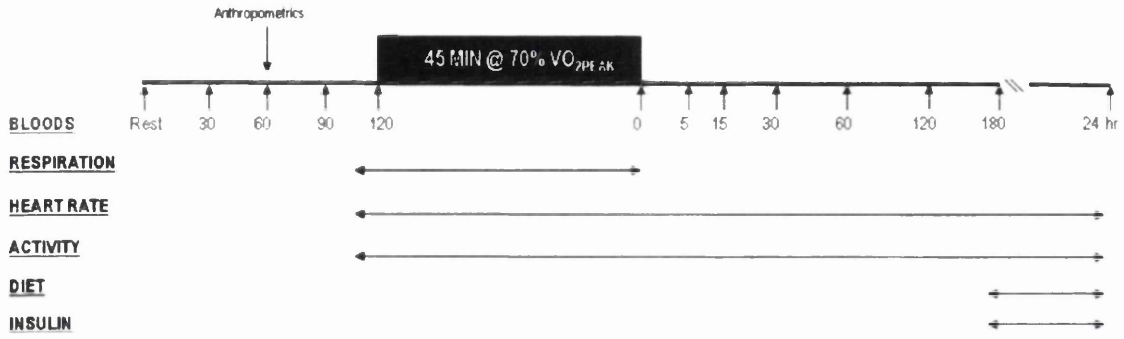


Figure 4.1: Schematic diagram of the experimental protocol of chapter 4.

4.2.1 Statistical Analysis

Statistical analysis was performed using SPSS software (version 16; SPSS Inc., Chicago, IL), with significance set at $P<0.05$. Data were tested for normal distribution (Shapiro-Wilk test) and subsequently analysed using repeated-measures ANOVA on two factors (treatment x time) with Bonferroni adjustments and dependent t-tests carried out where relevant. Data are presented as mean ± SEM.

4.3 Results

4.3.1 Physiological responses to exercise

The physiological responses to **LGI** and **HGI** are reported in Table 4.1. There were no differences in the resting rates of oxygen consumption or carbon dioxide production between **LGI** and **HGI** ($P>0.05$). Participants exercised at a similar exercise intensity under both conditions with similar $\%VO_{2peak}$ (**LGI** 80.8 ± 0.9% vs. **HGI** 78.2 ± 0.9 % VO_{2peak}, $P = 0.12$) and HR_{peak} (**LGI** 85 ± 1% vs. **HGI** 83 ± 1 % VO_{2peak}, $P = 0.12$) elicited during the 45 minutes of running. However, there was a tendency for a greater rate of oxygen use during exercise under **LGI** ($P = 0.05$; Table 1). Resting and exercising heart rates were similar between conditions (Table 4.1).

Table 4.1: Cardio-respiratory responses, at rest and during exercise, following pre-exercise ingestion of **LGI** or **HGI**.

	Rest		Exercise		
	HGI	**LGI**	**HGI**	**LGI**	$P_{(Ex)}$
HR (bpm)	65 ± 1	68 ± 1	159 ± 2*	163 ± 2*	0.12
V_E ($l.min^{-1}$)	10.8 ± 0.2	10.5 ± 0.5	73.8 ± 1.5*	75.5 ± 1.1*	0.28
VO_2 ($ml.kg^{-1}.min^{-1}$)	5.2 ± 0.1	5.3 ± 0.1	34.6 ± 0.5*	35.8 ± 0.6*	0.05
VCO_2 ($ml.kg.^{-1}.min^{-1}$)	4.7 ± 0.1	4.9 ± 0.1	33.6 ± 0.6*	34.0 ± 0.6*	0.32
RER	0.91 ± 0.01	0.93 ± 0.01	0.97 ± 0.01*	0.95 ± 0.01*	0.14

Data are presented as mean ± SEM (n = 8). $P_{(Ex)}$ demonstrates paired-samples t-test on exercising data only.* indicates significant differences from rest ($P<0.01$).

4.3.2 Blood glucose responses

Fasted blood glucose concentrations were not different between conditions (**LGI** 7.6 ± 0.2 vs. **HGI** 6.2 ± 0.3 mmol.l^{-1}, P = 0.11). The absolute and relative blood glucose responses to **LGI** or **HGI** ingestion are reported in Figure 4.2. There were no conditional differences when blood glucose was examined in absolute concentrations (Figure 4.2A); however, when expressed as change from baseline, significant conditional differences in blood glucose responses were evident (Figure 4.2B).

Peak blood glucose under **LGI** (Δ+4.5 ± 0.4 mmol.l^{-1}), occurred 120 minutes post-ingestion and was less than **HGI** (Δ+9.1 ± 0.6 mmol.l^{-1}, P<0.01) which peaked at 90 minutes. The drop in blood glucose with exercise was similar between conditions (**LGI** 4.4 ± 0.4 vs. **HGI** 5.8 ± 0.3 mmol.l^{-1}, P = 0.11). The drop in blood glucose under **LGI** was not as great as **HGI** in 6 of 8 participants. Immediately after exercise, blood glucose concentrations under **LGI** were significantly lower than **HGI** and were not different from resting concentrations (Figure 4.2B).

In the 3 hour post-exercise period, blood glucose concentrations under **LGI** were lower than those under **HGI** at all time points (Figure 4.2B). Blood glucose concentrations did not change from 0 to 180 minutes under either condition (**LGI** Δ+0.1 ± 0.3: **HGI** Δ+1.3 ± 0.3 mmol.l^{-1}, P = 0.46). The post-exercise blood glucose area under the curve (BG_{AUC}; **LGI** 7.9 ± 0.5: **HGI** 10.0 ± 0.5 mmol.l^{-1}.hour^{-1}, P = 0.03) and mean blood glucose (**LGI** +3.4 ± 0.3: **HGI** +0.5 ± 0.5 mmol.l^{-1}, P = 0.03) concentration, over the 180 minute recovery period, was 21 ± 3 % and 3.0 ± 0.4 mmol.l^{-1}, lower, respectively, under **LGI** in comparison to **HGI** (P<0.05).

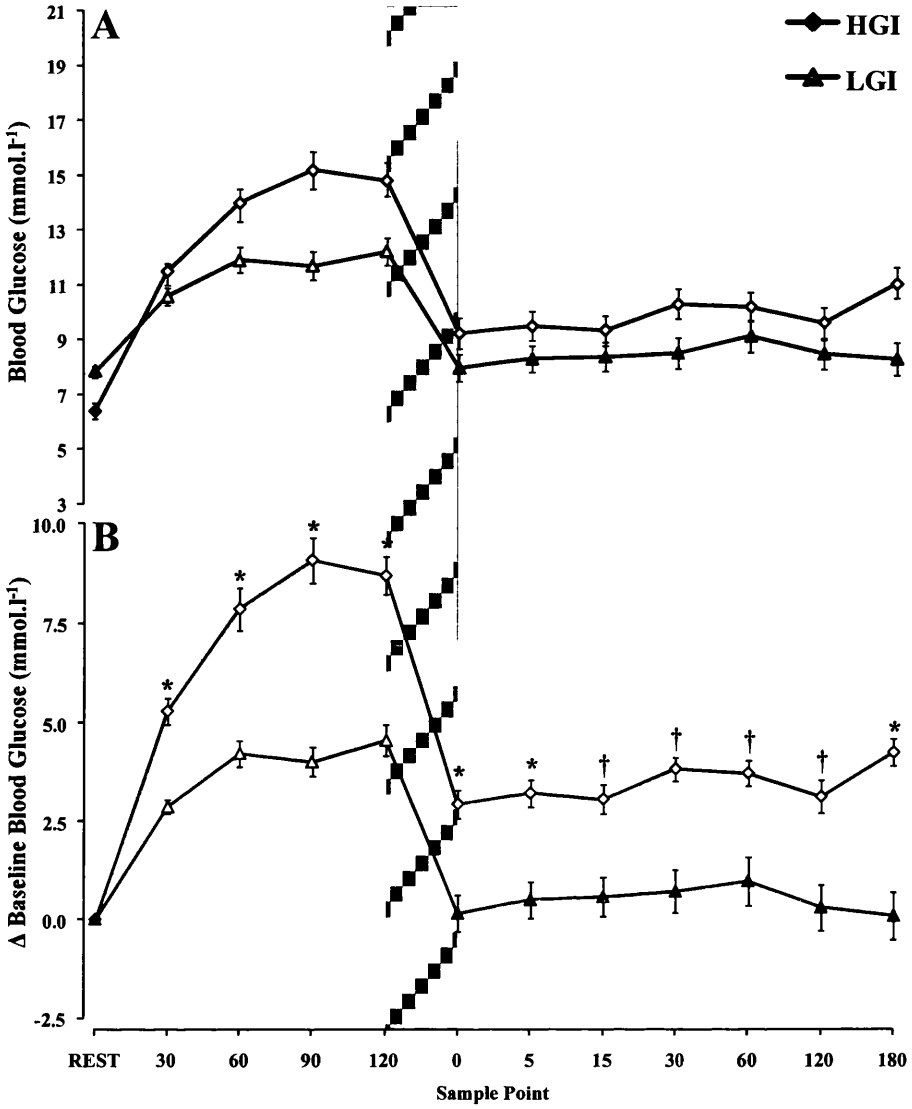


Figure 4.2: (**A**) Time-course changes in blood glucose following pre-exercise ingestion of **LGI** or **HGI**. There was a significant time effect (P = 0.001 partial-eta^2 = 0.601), there was a tendency for a treatment effect (P = 0.054, partial-eta^2 = 0.432), but no time*treatment interaction (P>0.05). (**B**) Time-course changes in blood glucose, relative to rest, following ingestion of **LGI** or **HGI**. There was a significant time (P = 0.001, Partial-eta^2 = 0.617) and treatment effect (P = 0.006, Partial-eta^2 = 0.686) and a significant time*treatment interaction (P = 0.04, Partial-eta^2 = 0.225). Data presented as mean ± SEM (n = 8). Transparent sample points indicate significant difference from rest (P<0.05). * indicates significant difference when compared to **LGI** (P<0.05). † indicates a trend when compared to **LGI** (P<0.1). Thatched area indicates exercise. **Note**: Insulin and carbohydrate solution was administered immediately following resting sample.

Hypoglycaemia

There were no incidences of hypoglycaemia during the 2 hour rest period or during treadmill running. In the post-exercise period, there were two occasions where blood glucose fell below 3.5 mmol.l^{-1} under both **HGI** (5 and 120 minutes post-exercise) and **LGI** trials (30 and 180 minutes post-exercise). One participant experienced a hypoglycaemic encounter on both occasions (**LGI** 120 minutes; **HGI** 180 minutes post-exercise).

4.3.3 Counter-regulatory hormonal responses

There were no conditional effects on any of the counter-regulatory hormonal responses to the trials (Table 4.2).

Plasma glucagon was not affected by time (P = 0.32) or condition (P = 0.46). Plasma glucagon remained similar to resting concentrations after exercise (Table 4.2), with similar changes from rest to 0 min post-exercise between conditions (**LGI** Δ+4 ± 6; **HGI** Δ-16 ± 4 pmol.l^{-1}; P = 0.44). Moreover, after 180 minutes post-exercise glucagon concentrations did not differ from rest or 0 minutes post exercise under either condition (Table 4.2); the change from 0 to 180 minutes was similar between conditions (**LGI** Δ+5 ± 1; **HGI** Δ-5 ± 1 pmol.l^{-1}; P = 0.72) .

There was a significant time effect (P = 0.02, Partial-eta^2 = 0.77) but no effect of condition (P = 0.77) on the plasma adrenaline response. Plasma adrenaline peaked at 0 minutes post-exercise (Table 4.2), with the no conditional differences in peak concentrations. Moreover, the change in concentrations from rest to 0 minutes post-exercise was similar between conditions (**LGI** Δ+1.47 ± 0.12; **HGI** Δ+1.72 ± 0.26 nmol.l^{-1}; P = 0.81). Plasma adrenaline concentrations were similar at 180 minutes post-exercise, and not different to rest under both

conditions (Table 4.2). Moreover, the change over the 180 minute post-exercise period was similar between conditions (**LGI** Δ-1.27 ± 0.12; **HGI** Δ-1.88 ± 0.28 nmol.l^{-1}; P = 0.58).

There was a significant time effect (P = 0.01, Partial-eta^2 = 0.76) but no effect of condition (P = 0.28) on the plasma noradrenaline response. Plasma noradrenaline peaked at 0 minutes post-exercise (Table 4.2), with the no conditional differences in peak concentrations. Moreover, the change in concentrations from rest to 0 minutes post-exercise was similar between conditions (**LGI** Δ+14.82 ± 0.93; **HGI** Δ+13.32 ± 1.19 nmol.l^{-1}; P = 0.49). Plasma noradrenaline concentrations were similar at 180 minutes post-exercise, and not different to rest under both conditions (Table 4.2). Moreover, the change over the 180 minute post-exercise period was similar between conditions (**LGI** Δ-14.46 ± 1.02; **HGI** Δ-11.23 ± 1.08 nmol.l^{-1}; P = 0.22).

There was a significant time effect (P = 0.02, Partial-eta^2 = 0.71) but no effect of condition (P = 0.44) on the serum cortisol response. Cortisol did not rise with exercise; however, concentrations began to increase immediately following exercise, peaking at 15 minutes post-exercise. Peak cortisol concentrations at 15 minutes (**LGI** 590 ± 16; **HGI** 595 ± 22 nmol.l^{-1}; P = 0.46) were not different from rest or 0 minutes post-exercise under either condition. At 180 minutes post-exercise serum cortisol concentrations were significantly lower than rest (**LGI** 276 ± 18; **HGI** 197 ± 6 nmol.l^{-1}; P = 0.20) under both conditions (P< 0.05; Table 4.2). Moreover, the change in concentrations from peak to 180 minutes were similar between conditions (**LGI** Δ-315 ± 29; **HGI** Δ-399 ± 25 nmol.l^{-1}; P = 0.20).

Table 4.2: Counter-regulatory hormonal responses following pre-exercise ingestion of **LGI** or **HGI**.

		Sample Point			
Hormone		Rest	0 min	15 min	180 min
Glucagon (pmol.l^{-1})	**HGI**	93 ± 9	75 ± 10	-	78 ± 10
	LGI	97 ± 9	87 ± 12	-	94 ± 10
Adrenaline (nmol.l^{-1})	**HGI**	0.28 ± 0.03	2.00 ± 0.28*	-	0.73 ± 0.02
	LGI	0.20 ± 0.03	1.67 ± 0.12*	-	0.40 ± 0.05
Noradrenaline (nmol.l^{-1})	**HGI**	2.64 ± 0.13	23.22 ± 1.25*	-	4.73 ± 0.35
	LGI	3.66 ± 0.29	18.48 ± 0.10*	-	4.02 ± 0.13
Cortisol (nmol.l^{-1})	**HGI**	532 ± 7	516 ± 22	595 ± 22*	197 ± 6*†
	LGI	553 ± 6	523 ± 21	590 ± 16*	276 ± 18*†

Data presented as mean ± SEM (n = 8).* indicates significant difference when compared with rest (P<0.05). † indicates difference when compared to 0 min post-exercise (P<0.05).

4.3.4 Serum β-hydroxybutyrate responses

The serum β-hydroxybutyrate responses to alterations in the GI of the pre-exercise carbohydrate are presented in Figure 4.3. Following carbohydrate ingestion and insulin administration there were small declines from rest to 120 minutes post-ingestion under both conditions (**LGI** Δ-0.09 ± 0.011 vs. **HGI** Δ-0.02 ± 0.005 $mmol.l^{-1}$, $P>0.05$). Concentrations did not change with exercise under either condition (**LGI** Δ+0.03 ± 0.004 vs. **HGI** +0.03 ± 0.004 $mmol.l^{-1}$, $P>0.05$), however, there was a transient rise in concentrations over the three hour recovery period, with concentrations at 180 minutes significantly greater than both rest and 0 minutes post-exercise under both conditions (Figure 4.3). The change from 0 to 180 minutes post-exercise was similar between conditions (**LGI** Δ+0.28 ± 0.033 vs. **HGI** Δ+0.38 ± 0.041 $mmol.l^{-1}$, $P = 0.52$).

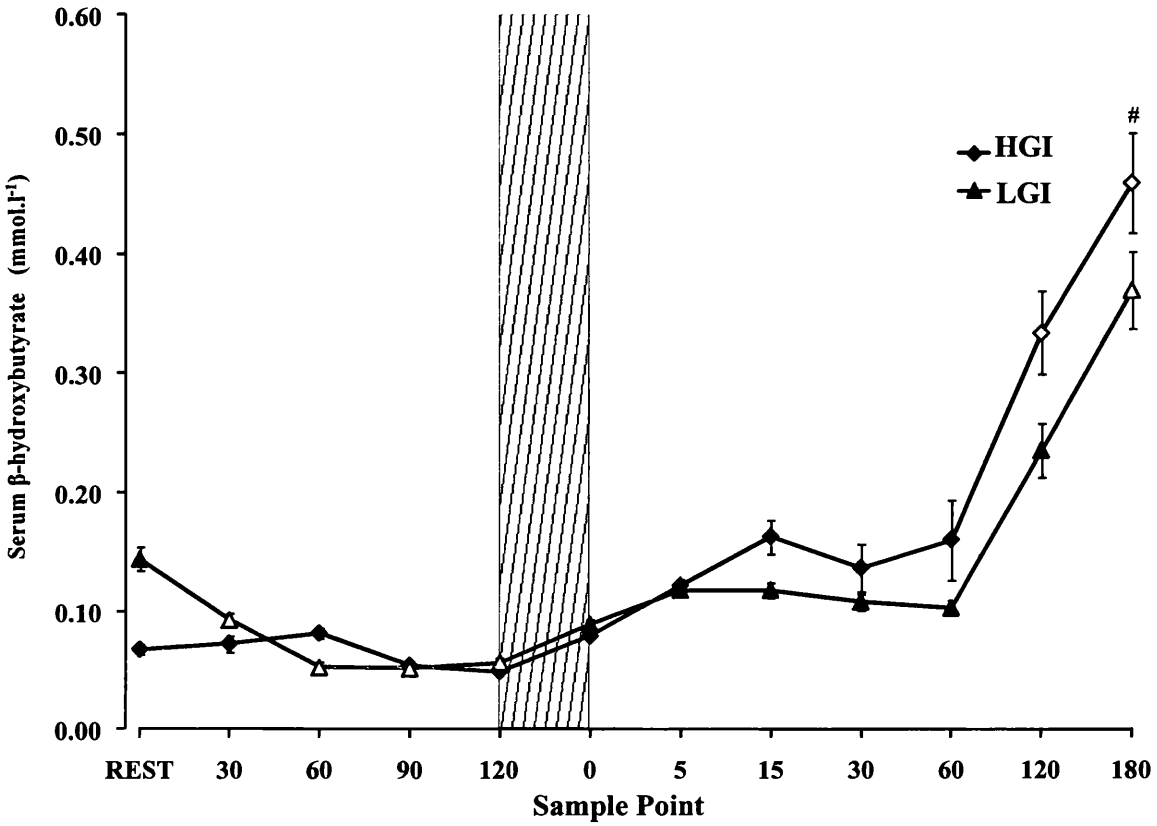


Figure 4.3: Time-course changes in serum β-hydroxybutyrate following pre-exercise ingestion of **LGI** or **HGI**. There was a significant time effect (P = 0.005, partial-eta^2 = 0.601) but no effect of condition (P = 0.414). Data presented as mean ± SEM. Transparent sample point within a trial indicates a significant difference when compared to rest (P<0.05). # indicates significantly different from 0 min post-exercise under both conditions (P<0.05). Thatched area indicates exercise. **Note**: Test meal and insulin were administered immediately following the resting sample.

4.3.5 Blood lactate and pH

The blood lactate responses are presented in Figure 4.4. After carbohydrate and insulin administration blood lactate increased from resting concentrations up to the pre-exercise sample under **LGI** (Figure 4.4). However, blood lactate under **HGI** remained similar to rest for the entire pre-exercise period (Figure 4.4). Two hours after consumption of the test meal the blood lactate concentration under **LGI** was 0.5 ± 0.1 mmol.l^{-1} greater than **HGI** (**LGI** 1.4 ± 0.1 vs. **HGI** 0.9 ± 0.03 mmol.l^{-1}, P = 0.02; Figure 4.4). These differences disappeared with exercise as similar peak lactate concentrations were observed immediately after exercise (**LGI** 4.5 ± 0.4 vs. **HGI** 4.4 ± 0.3 mmol.l^{-1}, P = 0.31). After exercise blood lactate concentrations decreased transiently, with similar declines in concentrations from 0 to 180 minutes post-exercise between conditions (**LGI** Δ-3.7 ± 0.4 vs. **HGI** Δ-3.6 ± 0.3 mmol.l^{-1}, P = 0.66). There were no differences in blood lactate concentrations between conditions over the post-exercise period (Figure 4.4).

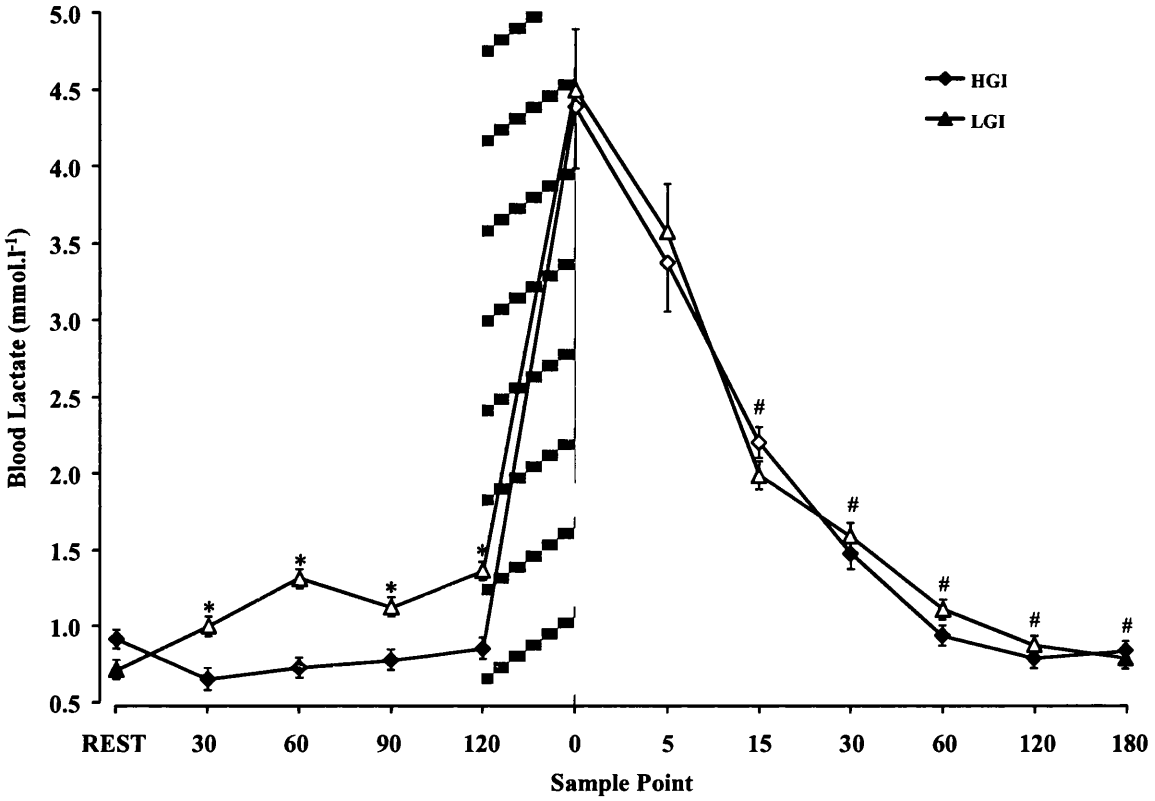


Figure 4.4: Time-course changes in blood lactate following pre-exercise ingestion of **LGI** or **HGI**. There was a significant time (P = 0.01, Partial-eta^2 = 0.623) and treatment effect (P = 0.04, Partial-eta^2 = 0.366) and a significant time*treatment interaction (P = 0.04, Partial-eta^2 = 0.221). Data are presented as mean ± SEM (n = 8). Transparent sample points indicate significant difference from rest (P<0.05). * indicates significant conditional difference (P<0.05). # indicates significantly different from 0 min post-exercise under both conditions (P<0.05). Thatched area indicates exercise. **Note**: Insulin and carbohydrate solution was administered immediately following resting sample.

The blood pH responses are presented in Figure 4.5. After carbohydrate and insulin administration blood pH concentrations did not change from rest over the pre-exercise period (Figure 4.5). Exercise resulted in an increase in blood pH, however, the change with exercise was similar between conditions (**LGI** Δ+0.05 ± 0.01 vs. **HGI** Δ+0.06 ± 0.01, P = 0.68). Blood pH remained greater than rest for 30 minutes post-exercise under both conditions (Figure 4.5), before reaching resting levels at 60 minutes. The change in pH from 0 to 180 min post-exercise was similar between conditions (**LGI** Δ-0.05 ± 0.01 vs. **HGI** Δ-0.03 ± 0.01, P = 0.39).

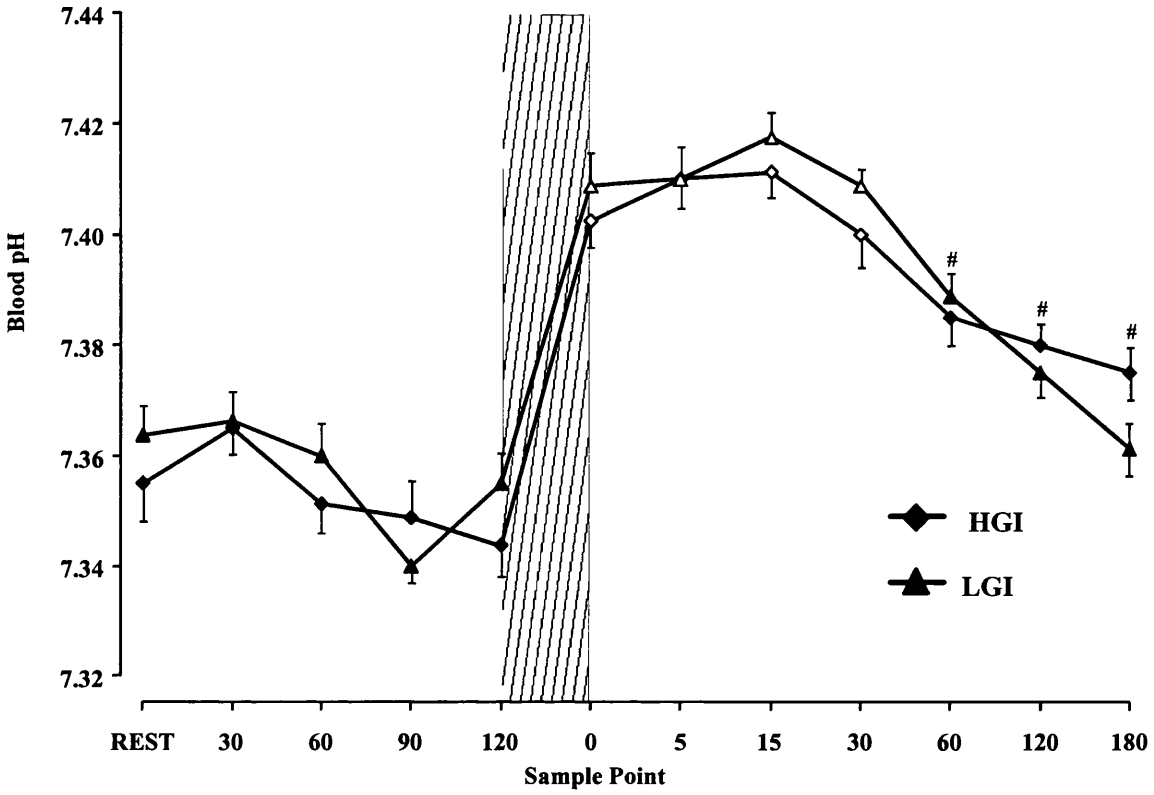


Figure 4.5: Time-course changes in blood pH following pre-exercise ingestion of **LGI** or **HGI**. There was a significant time (P = 0.01, Partial-eta^2 = 0.981) but no effects of condition (P = 0.805). Data are presented as mean ± SEM (n = 8). Transparent sample points indicate significant difference from rest (P< 0.05). * indicates significant conditional difference (P<0.05). # indicates significantly different to 0 min post-exercise under both conditions (P<0.05). Thatched area indicates exercise. **Note**: Insulin and carbohydrate solution was administered immediately following resting sample.

4.3.6 Serum triglyceride and NEFA responses

The serum triglyceride (TG) responses to alterations in the GI of the pre-exercise carbohydrate are reported in Figure 4.6A. Resting TG were not statistically different between the conditions (**LGI** 1.20 ± 0.10 vs. **HGI** 1.03 ± 0.06 mmol.l^{-1}). After carbohydrate consumption and insulin administration there were significant reductions in TG under **LGI** from rest up to 120 minutes (**LGI** Δ-0.30 ± 0.03 mmol.l^{-1}; P = 0.01), however, TG did not change under **HGI** (**LGI** Δ-0.03 ±0.03 mmol.l^{-1}; P = 0.78). Serum TG significantly increased with exercise under both conditions, with the change between conditions similar (**LGI** Δ0.24 ± 0.01 vs. **HGI** Δ0.19 ± 0.02 mmol.l^{-1}, P = 0.47). After exercise serum TG declined over the three hour post-exercise period, with the change from 0 to 180 minutes similar between conditions (**LGI** Δ-0.16 ± 0.03 vs. **HGI** Δ0.24 ± 0.03 mmol.l^{-1}, P = 0.49; Figure 4.6A). There were no conditional differences in serum TG concentrations.

The serum NEFA responses to alterations in the GI of the pre-exercise carbohydrate are reported in Figure 4.6B. Resting NEFA concentrations were similar (**LGI** 0.39 ± 0.03 vs. **HGI** 0.36 ± 0.02 mmol.l^{-1}, P = 0.71); after carbohydrate consumption and insulin administration there was no change in circulating NEFA concentrations up to 120 minutes (**LGI** Δ-0.10 ± 0.03 vs. **HGI** Δ-0.01 ± 0.02 mmol.l^{-1}, P>0.05). Moreover, serum NEFA concentrations did not increase when measured immediately after exercise (P = 0.60; Figure 4.6B), however, 5 minutes after the cessation of exercise there was a 2-fold increase in NEFA concentrations under both conditions (P<0.05; Figure 4.6B), with a similar increase in concentrations between conditions (**LGI** Δ+0.30 ± 0.02 vs. **HGI** Δ+0.37 ± 0.02 mmol.l^{-1}, P = 0.36). NEFA concentrations peaked at 3 hours post-exercise (**LGI** 0.86 ± 0.04 vs. **HGI** 0.92 ± 0.04 mmol.l^{-1}, P = 0.22).

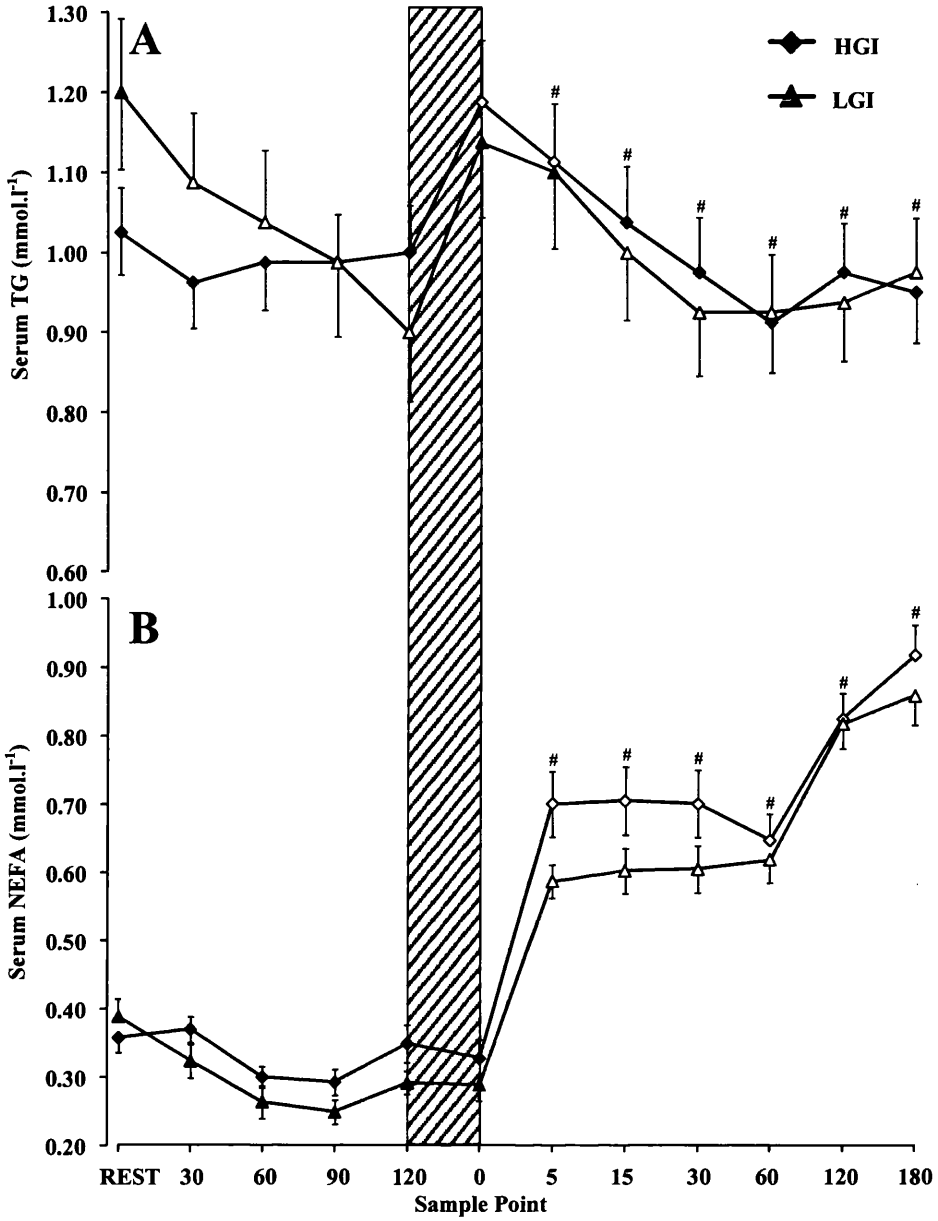


Figure 4.6: Time-course changes in serum triglyceride (TG; **A**) and non-esterified fatty acids (NEFA; **B**) following pre-exercise ingestion of **LGI** or **HGI**. There was a significant time effect on both TG ($P<0.01$, Partial-eta^2 = 0.45) and NEFA ($P<0.01$, Partial-eta^2 = 0.72), however, there were no effects of condition ($P>0.05$). Data are presented as mean ± SEM (n = 8). Transparent sample points indicate significant difference from rest ($P<0.05$). # indicates significantly different from 0 min post-exercise under both conditions ($P<0.05$). Thatched area indicates exercise. **Note**: Insulin and carbohydrate solution was administered immediately following resting sample.

4.3.7 Serum glycerol responses

Serum glycerol responses to alterations in the GI of the pre-exercise carbohydrate are presented in Figure 4.7. Resting serum glycerol concentrations were similar (**LGI** 12.2 ± 0.7 vs. **HGI** 12.2 ± 0.4 $mg.l^{-1}$, P = 0.68). After carbohydrate consumption and insulin administration glycerol concentrations declined up to 60 minutes under both conditions, however, concentrations remained similar for the remainder of the pre-exercise period under **HGI**, but continued to decline under **LGI** (Figure 4.7). Despite slight differences in pre-exercise time-course changes, pre-exercise concentrations were similar between conditions (P = 0.09; Figure 4.7). Glycerol concentrations increased with exercise under both conditions (**LGI** Δ+12.5 ± 0.6 vs. **HGI** Δ+10.7 ± 0.7 $mg.l^{-1}$, P = 0.46), before transiently declining over the post-exercise period under both conditions (Figure 4.7). The decline in glycerol concentrations from 0 to 180 minutes post-exercise was similar between conditions (**LGI** Δ-9.6 ± 0.6 vs. **HGI** Δ-8.7 ± 0.8 $mg.l^{-1}$, P = 0.68).

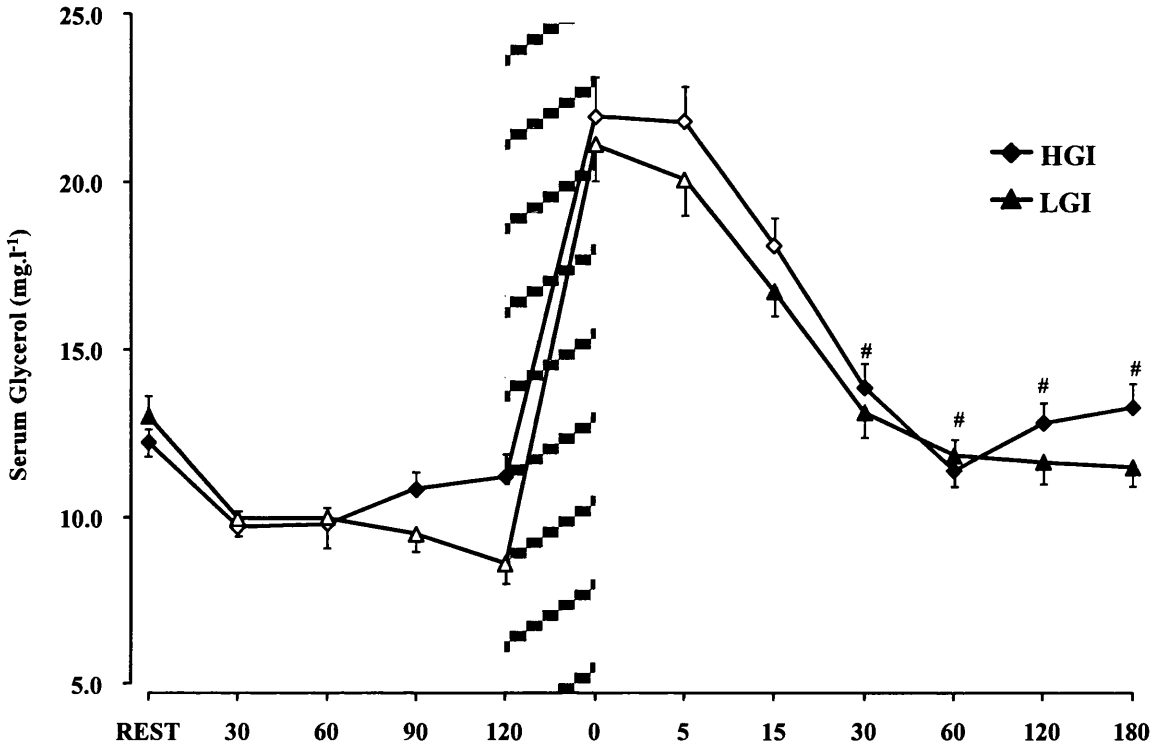


Figure 4.7: Time-course changes in serum glycerol following pre-exercise ingestion of **LGI** or **HGI**. There was a significant time effect ($P<0.01$; Partial-eta^2 = 0.79), however, there were no treatment or treatment*time interactions ($P>0.05$). Data are presented as mean ± SEM (n = 8). Transparent sample points indicate significant difference from rest ($P<0.05$). # indicates significantly different from 0 min post-exercise under both conditions ($P<0.05$). Thatched area indicates exercise. **Note**: Insulin and carbohydrate solution was administered immediately following resting sample.

4.3.8 Patterns of carbohydrate and lipid oxidation

The carbohydrate and lipid oxidation rates during rest are presented in Table 4.3 and oxidation rates during exercise are presented in Table 4.3 and Figure 4.8A. There were no conditional differences in resting rates of carbohydrate (P = 0.36) or lipid oxidation (P = 0.34; Table 4.3). When expressing oxidation rates as changes from rest, there was a tendency for a lower carbohydrate oxidation rate under **LGI** (P = 0.08) with a concomitant greater rate of lipid oxidation (P = 0.03; Table 4.3) when compared with **HGI**. As exercise progressed, there was a lower CHO and greater lipid oxidation rate, such that by the last 10 minutes carbohydrate and lipid oxidation rates were significantly different under **LGI** compared to **HGI**, respectively (Figure 4.8B).

Table 4.3: Substrate oxidation responses, at rest and during exercise, following pre-exercise ingestion of **LGI** or **HGI**.

	Rest		Exercise	
	LGI	**HGI**	**LGI**	**HGI**
CHO ($g.min^{-1}$)	0.38 ± 0.02	0.34 ± 0.01	2.90 ± 0.06*	3.08 ± 0.06*
Lipids ($g.min^{-1}$)	0.06 ± 0.01	0.08 ± 0.01	0.22 ± 0.03*	0.16 ± 0.03*
ΔCHO ($g.min^{-1}$)	-	-	2.52 ± 0.04*	2.74 ± 0.05*
ΔLipids ($g.min^{-1}$)	-	-	0.23 ± 0.03*†	0.09 ± 0.02*

Data presented as means ± SEM (n = 8). * indicates a significant increase when compared with rest (P<0.05). † indicates difference to **HGI** (P<0.05).

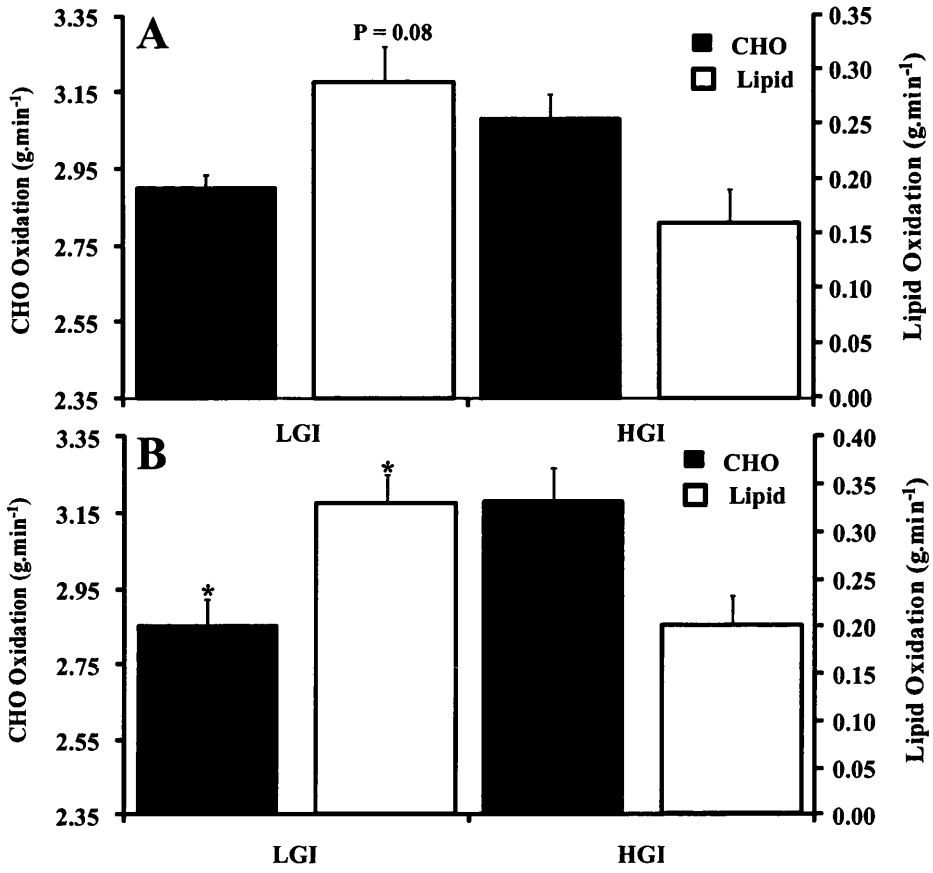


Figure 4.8: (A) Carbohydrate and lipid oxidation rates during 45 minutes of exercise after pre-exercise ingestion of **LGI** or **HGI**. (B) Carbohydrate and lipid oxidation rates over the final 10 minutes of exercise. Data are presented as mean ± SEM (n = 8). * indicates significant differences between **LGI** and **HGI** ($P<0.05$).

The energy expended in both trials was similar (**LGI** 2.67 ± 0.04 vs. **HGI** 2.63 ± 0.04 MJ, P = 0.41) with energy from carbohydrates similar between the conditions (**LGI** 2.18 ± 0.04 vs. **HGI** 2.32 ± 0.05 MJ, P = 0.13) and energy from lipids tending to be greater under **LGI** (**LGI** 0.49 ± 0.05 vs. **HGI** 0.31 ± 0.04 MJ, P = 0.06). There was a tendency for a lower percentage contribution to total energy expenditure from carbohydrate (**LGI** 83 ± 2 vs. **HGI** 89 ± 2 %, P = 0.07) and greater contribution from lipids (**LGI** 17 ± 2 vs. **HGI** 11 ± 2 %, P = 0.07) under **LGI**.

4.3.9 Post-laboratory activity and self-recorded glycaemia and dietary intake

There were no differences between conditions in any of the variables collected over the 21 hour post-laboratory period (Table 4.4). There were no conditional differences in the percentage contribution to total energy from carbohydrate (**LGI** 52 ± 1.5; **HGI** 49 ± 1.7 %; P>0.05), protein (**LGI** 19 ± 0.6; **HGI** 20 ± 0.6 %; P>0.05) or fats (**LGI** 37 ± 4.2; **HGI** 33 ± 1.8 %; P>0.05). There were 2 incidents of both low blood glucose (both 6 hours post laboratory) and hypoglycaemia (19 and 14 hours post-exercise) under **HGI** and 1 low blood glucose (19 hours post-laboratory) and 3 hypoglycaemic incidents under **LGI** (5, 16 and 19 hours post-laboratory).

Table 4.4*:* Twenty-one hour post-laboratory blood glucose, dietary intake and activity patterns.

	Condition		
Variable	**HGI**	**LGI**	P
BG_{auc} ($mmol.l^{-1}.hour^{-1}$)	7.7 ± 0.3	7.0 ± 0.3	0.31
CHO intake (g)	164 ± 10	193 ± 9	0.47
Protein intake (g)	62 ± 3	71 ± 3	0.48
Fat intake (g)	52 ± 5	46 ± 4	0.63
Energy intake (MJ)	5.7 ± 0.3	6.1 ± 0.3	0.70
Rapid-acting insulin (U)	19.8 ± 1.4	21.1 ± 1.4	0.23
Frequency BG < 4.0 $mmol.l^{-1}$	2	1	-
Frequency BG < 3.5 $mmol.l^{-1}$	2	3	-
Average heart rate (bpm)	76 ± 1	78 ± 1	0.73
Average steps taken	6197 ± 578	6295 ± 427	0.94

Data presented as mean ± SEM (n = 8).

4.4 Discussion

We compared the metabolic and glycaemic responses to ingestion of either a high GI or low GI carbohydrate before, during and after running in T1DM. The results demonstrate that compared to **HGI**, **LGI** increased blood glucose concentrations less before exercise and maintained blood glucose better for 24 hours after running. Furthermore, carbohydrate oxidation was reduced and lipid oxidation increased following **LGI** ingestion, during the latter stages of running.

There was a smaller increase in blood glucose under **LGI** compared to **HGI** in the pre-exercise period. Blood glucose concentrations increased above resting values by $\Delta+8.7 \pm 0.5$ $mmol.l^{-1}$ under **HGI**, whereas blood glucose increased to just half of this under **LGI** ($\Delta+4.5 \pm 0.4$ $mmol.l^{-1}$). The hydrolysation rate of **LGI** (isomaltulose) is very slow and only 20-25% of that of sucrose (Gunther and Heymann, 1998). Thus, the slow hydrolysation of **LGI**, into glucose and fructose, and subsequent absorption at the brush border membrane within the gastro-intestinal passage explains the later and lower peak in blood glucose in comparison with **HGI** (Lina et al., 2002). **HGI** can quickly cross via SGLT1 (sodium-glucose transporter 1), and can rapidly increase blood glucose concentrations (Figure 4.2). The reduced post-prandial glucose excursions under **LGI** prevented blood glucose reaching hyperglycaemic concentrations, as opposed to **HGI**. This is an important finding as maintaining glycaemia close to euglycaemic concentrations is the fundamental component in the management of T1DM (Cryer et al., 2003), especially when incorporating physical exercise into the lives of T1DM individuals.

There were greater lactate concentrations under **LGI** in the pre-exercise period (Figure 4.4). **LGI** (isomaltulose) is digested via the same sucrase/isomaltase complex, within the gastro-intestinal passage, as sucrose (Goda and Hosoya, 1983). During this process, within the

cytosol of the small intestine, some fructose derived from **LGI** is converted to lactate, however; most is metabolised within the liver (Ahlborg and Bjorkman, 1990). As fructose bypasses the phosphofructokinase regulatory point in glycolysis, there is an increased flux through the glycolytic pathway which results in an increased formation and subsequent release of lactate (Kaye et al., 1958; Sahebjami and Scalettar, 1971).

As exercise progressed there was a significantly lower carbohydrate oxidation rate evident under **LGI**, compared to **HGI**. This reduced carbohydrate oxidation rate under **LGI**, may, in part, be due to differences in blood glucose concentrations before and during exercise. Two hours following ingestion, blood glucose concentrations under **LGI** were near hyperglycaemic (12.2 ± 0.5 $mmol.l^{-1}$), whereas those under **HGI** were ~ 3 $mmol.l^{-1}$ greater at the beginning of exercise. This may have limited substrate oxidation to predominantly carbohydrates (Jenni et al., 2008). Jenni et al. (2008) demonstrated that when T1DM individuals perform prolonged exercise under blood glucose concentrations clamped at 11 $mmol.l^{-1}$ fuel metabolism was dominated by carbohydrate oxidation with concomitantly lower rates of lipid oxidation, compared with a euglycaemia condition; an effect due to the mass action of elevated blood glucose concentrations (Coyle et al., 1997). As blood glucose dropped Δ-4.4 ± 0.4 $mmol.l^{-1}$ with exercise under **LGI**, the blood glucose concentrations in the later stages of running returned to euglycaemic conditions sooner when compared to **HGI**, suggesting a lesser effect of high blood glucose concentrations in promoting carbohydrate combustion, and increasing the likelihood of observing differences in substrate oxidation in the latter stages of exercise.

As exercise progressed there was a greater lipid oxidation during exercise under **LGI**, in comparison with **HGI**, which became significantly greater during the final 10 minutes of exercise. Similar findings have been reported previously by Trenell et al. (2008) who found

low GI, pre-exercise, carbohydrate-based meals increased lipid oxidation by 10 % during 90 minutes of cycling at 70%VO_{2max}, in comparison to isoenergetic, high GI carbohydrate meals. Within our study, the increase in the combustion of lipids, with time, is possibly due to an increased mobilisation of intramuscular triglyceride stores, as NEFA concentrations did not differ between conditions. A potential mechanism behind the differences in lipid oxidation demonstrated in the latter stages of exercise may be related to differences in blood glucose concentrations between the conditions (Coyle et al., 1997). Coyle et al. (1997) investigated lipid oxidation, during 40 minutes of cycling at 50% VO_{2peak} in non-T1DM individuals, in a fasted state or having consumed 1.4 g/kg body mass of glucose. An examination of substrate oxidation revealed that an increased glucose availability and an increased glycolytic flux, and ultimately carbohydrate oxidation, directly suppresses lipid metabolism. Relating the findings of Coyle and colleagues to our study, the bolus of **HGI** ingested prior to exercise may have caused a similar suppression of lipid oxidation during exercise. Moreover, combining an increase in skeletal muscle hormone sensitive lipase activity (Watt et al., 2003) and lower glucose availability under **LGI** may have provided a milieu where the oxidation of intramuscular NEFAs was not as suppressed, compared to **HGI**, during exercise. Serum non-esterified fatty acid concentrations did not change with exercise; an effect likely the result of a reduction in adipose tissue blood flow, with exercise intensities >70% VO_{2max}, reducing the removal of NEFA (Jones et al., 1980; Romjin et al., 1995). A redistribution of blood flow to adipose tissue is also the likely mechanism behind the rapid rise in NEFA concentrations after the cessation of exercise (Romijn et al., 1995).

Post-exercise glycaemia was lower under **LGI**, compared with **HGI**, with BG_{AUC} and mean blood glucose being 21 ± 3 % and 3.0 ± 0.4 $mmol.l^{-1}$ lower, respectively, over the three hour recovery period. There were similar changes in blood glucose within each condition. The

preservation of blood glucose might potentially be due to exercise-induced increases in skeletal lipoprotein lipase activity (Kiens et al., 1989), increasing triglyceride breakdown and NEFA availability. Furthermore, the similar concentrations of counter-regulatory hormones may help explain the similar preservation of blood glucose with time, across conditions. From a T1DM individuals perspective this is important, as avoiding high blood glucose concentrations following exercise is not only beneficial for improved glycaemic control, but may also help avoid the occurrence of hypoglycaemia, as individuals are less likely to have to administer corrective insulin units, which in a post-exercise insulin-sensitised state could cause unexpected falls in blood glucose. Both types of carbohydrate were equally effective at preventing hypoglycaemia over the 3 hour recovery period, with two episodes under each condition. Despite the increased appearance of lactate under **LGI** during the pre-exercise period, the conditional differences in this metabolite were not evident after running was completed. Potentially, greater lactate concentrations under **LGI** resulted in lactate being channelled into oxidative pathways within the heart (Gertz et al., 1988) and the active musculature (Mazzeo et al., 1986), and/or hepatic gluconeogenesis (Ahlborg and Felig, 1982).

When leaving the laboratory blood glucose was near euglycaemic under **LGI** (~8 $mmol.l^{-1}$) and still elevated under **HGI** (~11 $mmol.l^{-1}$). However, despite this there were no statistical differences in 21 hour post-laboratory period BG_{AUC}, carbohydrate intake or insulin administration. Moreover, there were similar incidences of low blood glucose and hypoglycaemia under both conditions. Potentially, there was an elevated blood glucose uptake under **HGI** in comparison with **LGI**, due to lower post-exercise glycogen stores under **HGI**. Jenni et al. (2008) demonstrated that T1DM individuals elicit an increased intramuscular glycogen breakdown rate when exercising under hyperglycaemic conditions.

Potentially, as the exercise bout under **HGI** was under hyperglycaemic concentrations and was fuelled predominantly by carbohydrate oxidation (Coyle et al., 1991), blood glucose uptake to restore muscle glycogen stores may have been elevated under **HGI**; consequently resulting in a gradual reduction in blood glucose concentrations over the post-laboratory period. Additionally/conversely, participants were administering ~1 IU of insulin per 10 g of carbohydrates; however, ~20 IU were administered with ~164 g of carbohydrates under **HGI**. Potentially some participants applied corrective insulin units under the **HGI** condition to return glycaemia to normal.

In conclusion, this study examined the effects of consuming a low GI and high GI carbohydrate on metabolic and glycaemic responses before, during and for 24 hours after running in T1DM individuals. These data demonstrate that consuming a low GI carbohydrate improves blood glucose responses during and after exercise through reduced carbohydrate and increased lipid oxidation during exercise. Regularly employing this strategy could be beneficial for long-term glycaemic control within exercising T1DM individuals.

Chapter Five

The metabolic and glycaemic effects of alterations in the pre-exercise timing of carbohydrate and insulin administration.

5.1 Introduction

Individuals with type 1 diabetes (T1DM) are encouraged to engage in aerobic exercise due to the potential to improve long-term glycaemic control, i.e. reduce their HbA_{1c} percentage (Sideravičiūté et al., 2006), however, impaired gluco-regulatory responses results in an increased risk of hypoglycaemia during and after exercise. Hypoglycaemia, during or after exercise, is a major concern for individuals with T1DM (Brazeau et al., 2008). Current strategies to help combat the heightened risk of hypoglycaemia, associated with exercise, focus on reductions in insulin dose (De Feo et al., 2006; Rabasa-Lhoret et al., 2001;) as well as pre-exercise carbohydrate consumption (De Feo et al., 2006; Hibbert-Jones and Regan, 2005).

The type of carbohydrate consumed before performing exercise may be an important factor in blood glucose responses to exercise, within T1DM. Carbohydrates with a low glycaemic index (LGI) digest at slower rates than high glycaemic index (HGI) carbohydrates and are unable to cross the mucosal cell membrane within the small intestine and enter the bloodstream unless hydrolysed into monosaccharides (Southgate, 1995). Research has established the importance of including LGI carbohydrates into the daily diets of T1DM, with observed benefits such as lower daily mean blood glucose (Nansel et al., 2008) and reduced incidence of hypoglycemia and reductions in HbA_{1c} (Gilbertson et al., 2001; Thomas et al., 2007). As demonstrated within chapter 4, peak blood glucose responses were twice as great after consumption of a HGI carbohydrate in comparison with a LGI carbohydrate (Figure 4.2; moreover, blood glucose area under the curve was 21 % lower after a LGI carbohydrate for 3 hours post-exercise. Therefore, a low GI carbohydrate source may be beneficial for maintaining blood glucose within normal ranges after exercise.

Another important factor in preserving post-exercise blood glucose concentrations is reducing insulin dose prior to exercise. Within the research examining pre-exercise insulin reductions on blood glucose concentrations following exercise, results have demonstrated improved glucose concentrations following reductions of 10-40% (De Feo et al., 2006), 10-50% (Grimm, 2005), 50-90% (Mauvais-Jarvis et al., 2003) and 50-75% (Rabasa-Lhoret et al., 2001; findings of chapter 3). Much of the variation in these findings can be attributed to differences in the insulin regimen used by participants, e.g. regular/Neutral Protamine Hagedorn insulin (Mauvais-Jarvis et al., 2003) or Ultralente with prandial insulin lispro (Rabasa-Lhoret et al., 2001). The choice of insulin species is important when examining blood glucose responses to reductions in insulin. The rDNA insulins currently available (e.g. insulin aspart/lispro) offer very different, more favourable, action-time profiles and less variability than longer established insulins, such as regular human insulin (Brange and Vølund, 1999; Tuominen et al., 1995). These insulin analogues are structured such that they remain in the monomeric form (i.e. the form that diffuses across the capillary wall into circulation) at the subcutaneous injection site, and have a lesser tendency to convert into dimmers and hexamers (Brange and Vølund, 1999). As a result these insulins elicit intense and rapid increases in insulin concentrations, taking peak effect just 45 – 60 minutes after administration (Plank et al., 2002). It is for these reasons that it is currently recommended to avoid administration of rapid-acting insulin within 90-120 minutes of exercise due to the risk of over-insulinisation of the active musculature during exercise (De Feo et al., 2006; Perry and Gallen, 2009). However, with current research demonstrating improved blood glucose concentrations with reductions in rapid-acting insulin dose by 75% (Rabasa-Lhoret et al., 2001; findings of chapter 3), there is potential that low doses of insulin administered within 90-120 minutes of exercise may not increase the risk of developing hypoglycaemia during or after exercise.

At present, to our knowledge, there is no literature that has examined the metabolic effects of alterations in the timing of a combined LGI carbohydrate and reduced rapid-acting insulin strategy before exercise. In light of this, the aim of this study was to examine metabolic and glycaemic responses to alterations in the timing of a combined LGI carbohydrate and insulin reduction strategy before, during and for 24 hours following running in individuals with T1DM.

5.2 Methods

Seven participants with T1DM (7 males, 31 ± 2 years, BMI 26 ± 0.3 kg/m^2) with a duration of diabetes of 19 ± 2 years and HbA_{1c} of 8.3 ± 0.1 % volunteered to participate in this study. Individual participant anthropometric (Table 2.5), glycaemic control (Table 2.8), and insulin regimen characteristics (Table 2.4) are presented in chapter 2.

After preliminary testing (Table 2.6) participants attended the laboratory on four occasions after an overnight fast and having consumed similar evening meals prior to each trial. Upon arrival participants received catheterisation in their non-dominant arm and blood samples were processed for glucose, lactate, pH, adrenaline, noradrenaline, cortisol, β-hydroxybutyrate, triglycerides and non-esterified fatty acids (section 2.3.5). Participants were then required to consume 75 g of a low GI carbohydrate (Isomaltulose; GI 32; findings from chapter 4), mixed with 750 ml of water (10 % solution, Perrone et al., 2005). Immediately before ingestion, participants were instructed to administer their rapid-acting insulin, which had been reduced by 75 % (2.1 ± 0.1 IU) into the abdomen (Rabasa-Lhoret et al., 2001; findings from chapter 3). After carbohydrate and insulin administration participants remained at rest for 120 (**120min**), 90 (**90min**), 60 (**60min**) or 30 minutes (**30min**) before completing 45 minutes of running at 71 ± 1% VO_{2peak}. Blood samples were taken every 30 minutes in the pre-exercise period and for three hours post-exercise (Figure 5.1). Cardio-respiratory parameters were collected at rest and during exercise.

Participant activity and heart rate were recorded for 24 hours after exercise; moreover, for 21 hours after leaving the laboratory participants self-recorded blood glucose, dietary intake, insulin administration and hypoglycaemic incidences.

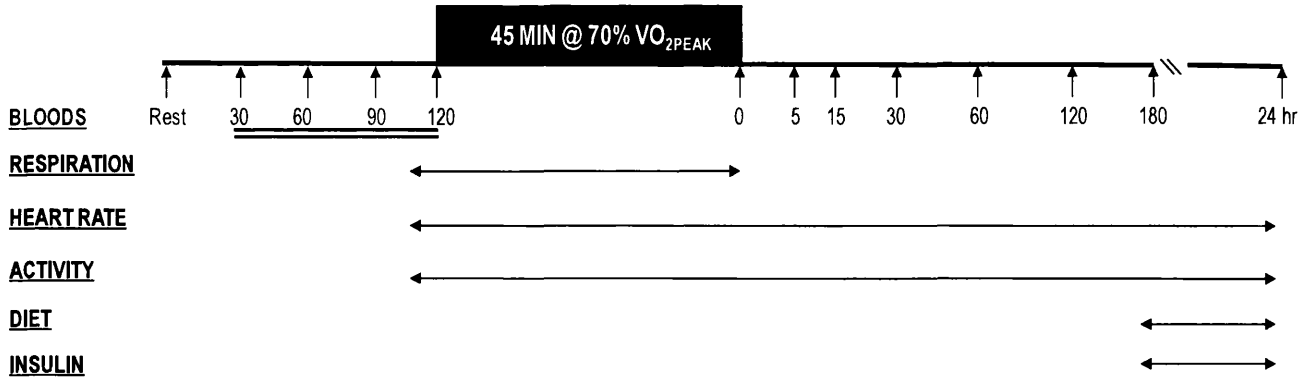


Figure 5.1: Schematic diagram of the experimental protocol of chapter 5. **Note**: Double line indicates changes in pre-exercise sample point.

5.2.1 Data Analysis

Statistical analysis was performed using SPSS software (version 16; SPSS Inc., Chicago, IL), with significance set at $P<0.05$. Data were tested for normal distribution (Shapiro-Wilk test) and subsequently analysed using repeated-measures ANOVA on two factors (treatment x time) with Bonferroni adjustment and dependent t-tests carried out where relevant. Blood glucose area under the curve (BG_{AUC}) was calculated using the method of Wolever and Jenkins (1988) and subsequently time averaged. Data are presented as mean ± SEM.

5.3 Results

5.3.1 Physiological responses to exercise

The physiological responses to the trials are presented in Table 5.1. Resting rates of oxygen consumption and CO_2 production were significantly greater under **30min**, when compared to all other trials (Table 5.1). There were no differences in the resting rates of oxygen consumption or carbon dioxide production across **120min**, **90min** and **60min** ($P>0.05$; Table 5.1).

Participants exercised at a similar exercise intensity with similar $\%VO_{2peak}$ (**120min** 72 ± 3; **90min** 70 ± 5; **60min** 71 ± 7; **30min** 71 ± 8 $\%VO_{2peak}$; $P = 0.62$) and HR_{peak} (**120min** 81 ± 4; **90min** 82 ± 4; **60min** 82 ± 4; **30min** 83 ± 4 $\%HR_{peak}$; $P = 0.59$) elicited across trials.

Table 5.1: Cardio-respiratory responses, at rest and during exercise, to alterations in the timing of pre-exercise carbohydrate and insulin administration.

	Rest				Exercise			
	120min	**90min**	**60min**	**30min**	**120min**	**90min**	**60min**	**30min**
HR (bpm)	65 ± 1	68 ± 1	64 ± 1	69 ± 1	157 ± 2*	158 ± 2*	158 ± 1*	159 ± 2*
V_E ($l.min^{-1}$)	10.4 ± 0.3	11.0 ± 0.2	10.7 ± 0.3	11.7 ± 0.2	81.2 ± 1.2*	82.6 ± 1.3	81.0 ± 1.0*	80.6 ± 1.0*
VO_2 ($ml.kg^{-1}.min^{-1}$)	4.3 ± 0.1	4.8 ± 0.1	4.4 ± 0.1	5.5 ± 0.2†	36.8 ± 0.3*	35.6 ± 0.4 *	35.9 ± 0.6*	36.0 ± 0.5*
VCO_2 ($ml.kg.^{-1}.min^{-1}$)	4.2 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	4.8 ± 0.2†	37.4 ± 0.4*	35.4 ± 0.4*	35.9 ± 0.6*	35.6 ± 0.5*‡
RER	0.99 ± 0.01	0.95 ± 0.01	0.98 ± 0.01	0.89 ± 0.01†	1.02 ± 0.01*	0.99 ± 0.01*	1.00 ± 0.01*	0.99 ± 0.01*‡

Data presented as means ± SEM (n = 7). † indicates significantly different to all other trials ($P<0.05$). ‡ indicates significantly different to **120min** ($P<0.05$). * indicates a significant increase when compared with rest ($P<0.05$).

5.3.2 Blood glucose responses

The absolute and relative blood glucose responses are presented in Table 5.2 and Figure 5.2. Pre-exercise responses are reported in Table 5.2. Fasted blood glucose concentrations were similar between conditions (P = 0.23; Table 5.2). After consumption of the LGI carbohydrate there were increases in blood glucose under all conditions, with immediate pre-exercise values lowest under **30min** (**30min** Δ+2.8 ± 0.2; **60min** Δ+3.9 ± 0.2; **90min** Δ+4.3 ± 0.2; **120min** Δ+4.1 ± 0.6 mmol.l^{-1}; Figure 5.2; Table 5.2); concentrations under **30min** were only statistically different to **120min** and **90min** (P<0.05; Figure 5.2B).

On average, the drop in blood glucose with exercise was least under **30min** (**30min** 3.7 ± 0.4 vs. **60min** 5.0 ± 0.7; **90min** 5.4 ± 0.5; **120min** 6.4 ± 0.3 mmol.l^{-1}), however, only **30min** and **120min** were statistically different (P = 0.02). When looking at the participants blood glucose responses to exercise individually, five of seven elicited the lowest drop in BG under **30min**.

In the 3 hour post-exercise period blood glucose concentrations were greater under **30min** and **60min**, when compared to **120min** (Figure 5.2B). However, these differences were only significant for 60 minutes post-exercise (Figure 5.2B). Blood glucose concentrations significantly increased from 0 to 60 minutes under both **30min** and **60min** (Figure 5.2), with the change under **30min** greater (**30min** Δ+3.1 ± 0.2 vs. **60min** Δ+1.7 ± 0.3 mmol.l^{-1}, P = 0.02). Blood glucose concentrations under both **90min** and **60min** remained similar to 0 minutes under both conditions (Figure 5.2). Mean post-exercise blood glucose was lowest under **120min**, in comparison all other trials (**120min** 5.9 ± 0.5 vs. **90min** 7.6 ± 0.3; **60min** 10.1 ± 0.6; **30min** 9.0 ± 0.5 mmol.l^{-1}; P<0.05). Blood glucose area under the curve (BG_{AUC}) for the 3 hour post-exercise period under **120min** and **90min** were lower than **60min** and **30min** (**120min** 6.0 ± 0.5; **90min** 7.0 ± 0.3 vs. **60min** 9.4 ± 0.6; **30min** 7.8 ± 0.5 mmol.l^{-1}.hour^{-1}; P<0.05); BG_{AUC} under **60min** and **30min** were not different (P = 0.32).

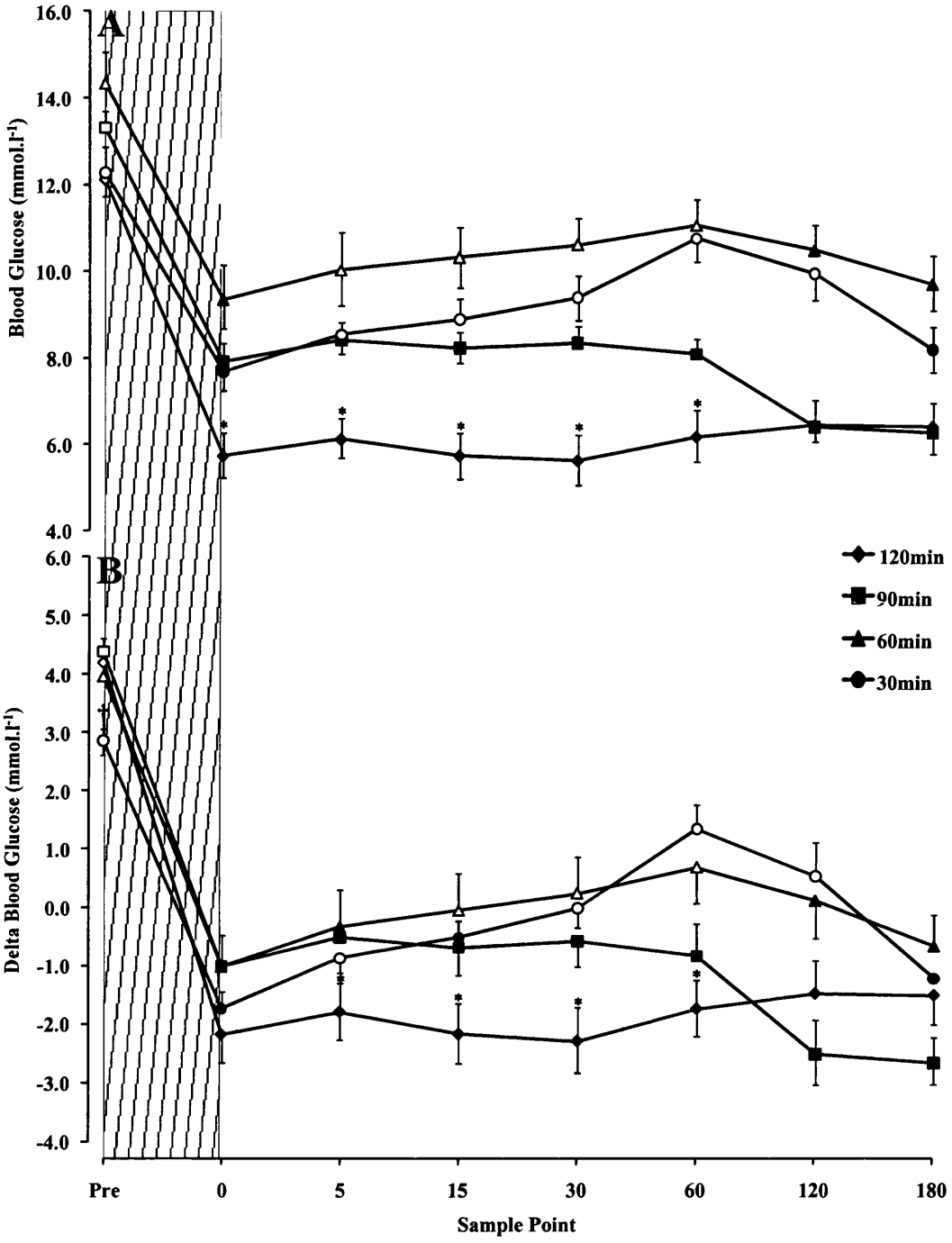


Figure 5.2: Time-course changes in blood glucose after alterations in the pre-exercise timing of carbohydrate and insulin administration, presented as absolute concentrations (A) and relative to rest (B). Under both absolute and relative concentrations there was a significant time effect ($P = 0.001$, partial-eta$^2 = 0.794$) and a significant time*condition interaction ($P = 0.002$, partial-eta$^2 = 0.307$). Data presented as mean ± SEM. * indicates significantly different from **60min** and **30min** at the respective time point ($P<0.05$). † indicates different to **120min** and **90min** at the respective time point ($P<0.05$). Transparent sample points indicate significant change from 0 min post exercise ($P<0.05$). Thatched area indicates exercise.

Hypoglycaemia

There were no hypoglycaemic occurrences under **30min**, however, there was 1 under **60min** (60 minutes post-exercise), 2 under **90min** (1 at 30 and 1 at 120 minutes post-exercise) and 5 under **120min** (1 at 5, 1 at 30 and 3 at 60 minutes post-exercise).

5.3.3 Serum insulin responses

The serum insulin responses are presented in Table 5.2 and Figure 5.3. There were no differences in fasting serum insulin concentrations across conditions (Table 5.2). There were significant increases in serum insulin concentrations after administration of the rapid-acting dose with the change from rest to pre-exercise similar across conditions (**120min** Δ+25 ± 3; **90min** Δ+18 ± 3; **60min** Δ+27 ± 6; **30min** Δ+20 ± 5 pmol.l^{-1}; P>0.05; Table 5.2). Moreover, there were no significant changes in serum insulin concentrations between 30 minutes post-administration and the pre-exercise sample under **120min**, **90min** and **60min** (**120min** Δ+5 ± 2; **90min** Δ+6 ± 3; **60min** Δ+13 ± 7 pmol.l^{-1}; P>0.05).

Serum insulin concentrations significantly increased with exercise under all conditions, with the change under **120min** and **90min** significantly greater than the changes under **60min** and **30min** (**120min** Δ+83 ± 7; **90min** Δ+81 ± 12 vs. **60min** Δ+49 ± 9; **30min** Δ+48 ± 3 pmol.l^{-1}, P<0.05); changes within **120min** and **90min** were similar (P = 0.52), moreover, the changes within **60min** and **30min** were also similar (P = 0.62).

In the three hour post-exercise period serum insulin concentrations followed similar time-course changes under each condition (Figure 5.3). However, the change in concentrations from 0 to 180 minutes was greater under **120min** and **90min**, in comparison with **30min** (**120min** Δ-101 ± 9; **90min** Δ-107 ± 9 vs. **30min** Δ-69 ± 6 pmol.l^{-1}, P<0.05); changes under **60min** were not statistically different to all other trials (**60min** Δ-78 ± 16, P>0.05).

Table 5.2: Pre-exercise blood glucose and serum insulin responses, in absolute concentrations and changes (Δ) from rest, to alterations in the pre-exercise timing of carbohydrate and insulin administration.

			Sample Point			
Trial	Variable	Rest	30	60	90	120
120min	BG ($mmol.l^{-1}$)	7.9 ± 0.4	10.7 ± 0.5*	11.9 ± 0.7*	12.4 ± 0.7*	12.0 ± 0.8*
	ΔBG	-	2.8 ± 0.2*	4.1 ± 0.4*	4.5 ± 0.5*	4.1 ± 0.6*
	Serum insulin ($pmol.l^{-1}$)	114 ± 6	134 ± 7*	128 ± 8*	129 ± 5*	139 ± 7*
	ΔSerum insulin	-	20 ± 3*	14 ± 3*	15 ± 2*	25 ± 3*
90min	BG ($mmol.l^{-1}$)	8.9 ± 0.4	11.4 ± 0.4*	12.9 ± 0.4*	13.2 ± 0.4*	
	ΔBG	-	2.5 ± 0.2*	4.1 ± 0.2*	4.3 ± 0.2*	
	Serum insulin ($pmol.l^{-1}$)	123 ± 7	135 ± 5*	133 ± 4*	141 ± 6*	
	ΔSerum insulin	-	12 ± 4*	10 ± 5*	18 ± 3*	
60min	BG ($mmol.l^{-1}$)	10.3 ± 0.7	12.7 ± 0.8*	14.2 ± 0.9*		
	ΔBG	-	2.3 ± 0.1*	3.9 ± 0.2*		
	Serum insulin ($pmol.l^{-1}$)	111 ± 8	126 ± 14*	139 ± 9*		
	ΔSerum insulin	-	14 ± 10*	27 ± 6*		
30min	BG ($mmol.l^{-1}$)	9.4 ± 0.6	12.2 ± 0.6*			
	ΔBG	-	2.8 ± 0.2*			
	Serum insulin ($pmol.l^{-1}$)	107 ± 8	127 ± 11*			
	ΔSerum insulin	-	20 ± 5*			

Data presented as mean ± SEM (N = 7). * indicates difference when compared to rest ($P<0.05$).

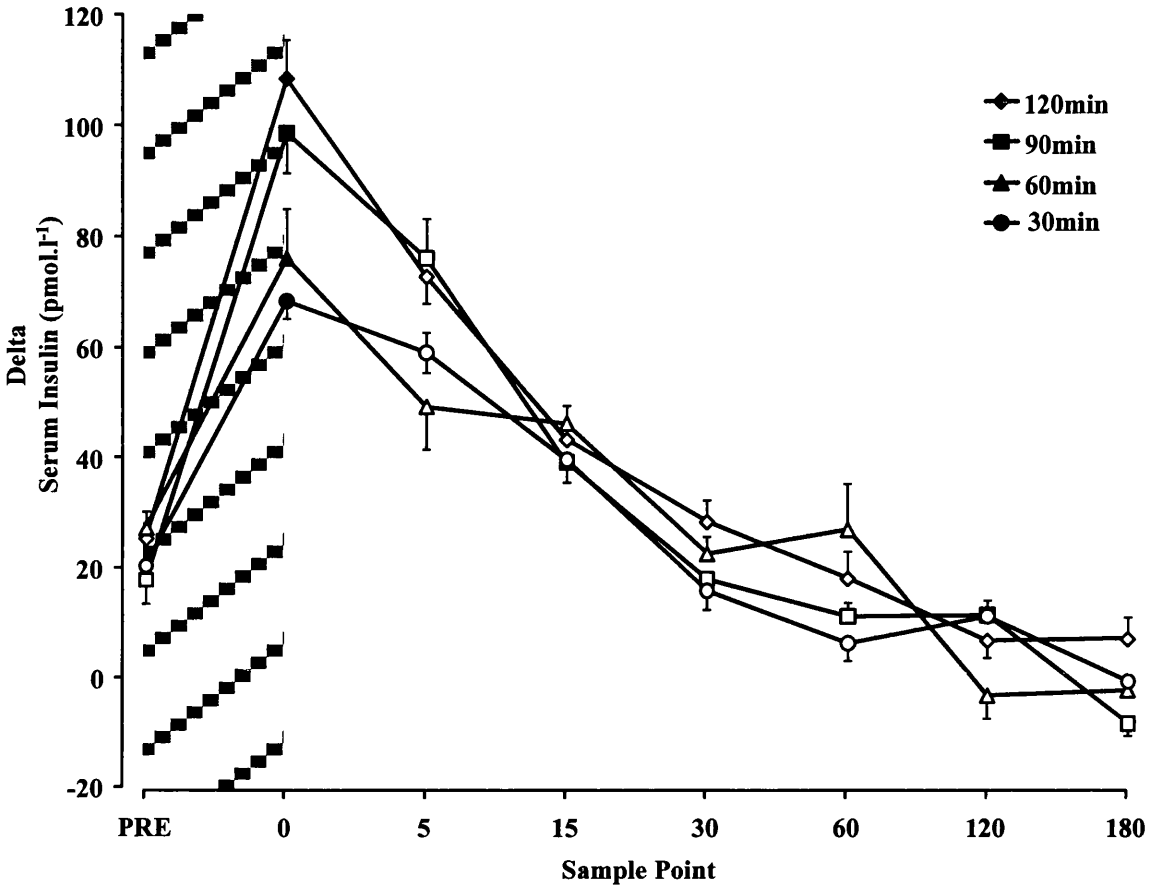


Figure 5.3: Time-course changes in serum insulin, relative to rest, after alterations in the pre-exercise timing of carbohydrate and insulin administration. There was a significant time effect (P = 0.009, partial-eta^2 = 0.627) however, there were no significant time*condition interactions (P = 0.354). Data presented as mean ± SEM. Transparent sample points indicate significant change from 0 min post exercise (P<0.05). Thatched area indicates exercise.

5.3.4 Counter-regulatory hormonal responses

There were no conditional effects on any of the counter-regulatory hormonal responses to the trials (Table 5.3). Moreover, there were no differences in fasting concentrations of all measured counter-regulatory hormones (Table 5.3).

There were changes with time ($P = 0.001$, partial-eta$^2 = 0.778$), however no effects of condition ($P = 0.62$) on the plasma adrenaline response. Plasma adrenaline peaked at 0 minutes post-exercise (Table 5.3), with no differences in peak concentrations across conditions ($P = 0.47$). Moreover, the change in concentrations from rest to 0 minutes post-exercise was greatest under **120min**, however, there were no significant differences across conditions (**120min** $\Delta+1.15 \pm 0.16$; **90min** $\Delta+1.07 \pm 0.23$; **60min** $\Delta+0.90 \pm 0.16$; **30min** $\Delta+0.64 \pm 0.11$ nmol.l^{-1}, $P = 0.13$). Plasma adrenaline concentrations were greatest under **120min** at 60 and 180 minutes post-exercise under all conditions, however were not significantly different to the other conditions; moreover at 180 minutes, concentrations were not different to rest under **90min**, **60min**, and **30min**, but remained elevated under **120min** (Table 5.3). The change over the 3 hour post-exercise period was similar across conditions (**120min** $\Delta-1.47 \pm 0.17$; **90min** $\Delta-1.76 \pm 0.22$; **60min** $\Delta-2.48 \pm 0.13$; **30min** $\Delta-1.24 \pm 0.15$ nmol.l^{-1}, $P>0.05$).

There was a significant time effect ($P = 0.01$, Partial-eta$^2 = 0.781$) but no effect of condition ($P = 0.28$) on the plasma noradrenaline response. Plasma noradrenaline peaked at 0 minutes post-exercise (Table 5.3), with peak concentrations similar across conditions ($P = 0.22$). Moreover, the change in concentrations from rest to 0 minutes post-exercise was similar between conditions (**120min** $\Delta+2.79 \pm 0.07$; **90min** $\Delta+2.28 \pm 0.19$; **60min** $\Delta+2.09 \pm 0.18$; **30min** $\Delta+2.35 \pm 0.46$ nmol.l^{-1}, $P>0.05$). Plasma noradrenaline concentrations were similar at 60 and 180 minutes post-exercise under all conditions; moreover at 180 minutes,

concentrations were not different to rest under **90min**, **60min**, and **30min**, but remained elevated under **120min** (Table 5.3). The change over the 3 hour post-exercise period was similar between conditions (**120min** Δ-1.39 ± 0.10; **90min** Δ-1.57 ± 0.18; **60min** Δ-1.16 ± 0.18; **30min** Δ-1.53 ± 0.47 $nmol.l^{-1}$, $P>0.05$).

There was a significant time effect ($P = 0.002$, Partial-$eta^2 = 0.784$) but no effect of condition ($P = 0.44$) on the serum cortisol response. Cortisol did not rise with exercise; however, concentrations began to increase significantly immediately following exercise, peaking at 15 minutes post-exercise. Peak cortisol concentrations at 15 minutes (Table 5.3) were not different from rest but were greater than 0 minutes post-exercise, under all conditions. Concentrations decreased with time under all conditions with similar concentrations at 60 minutes post-exercise (Table 5.3). At 180 minutes post-exercise serum cortisol concentrations were significantly lower than rest (Table 5.3) under all conditions ($P<0.05$; Table 5.3). Moreover, the change in concentrations from peak to 180 minutes were similar between conditions (**120min** Δ-281 ± 21; **90min** Δ-277 ± 21; **60min** Δ-332 ± 23; **30min** Δ330 ± 27 $nmol.l^{-1}$, $P>0.05$).

Table 5.3: Counter-regulatory hormonal responses to alterations in the pre-exercise timing of carbohydrate and insulin administration.

		Sample Point				
	Trial	Rest	0	15	60	180
Adrenaline ($nmol.l^{-1}$)	**120min**	1.33 ± 0.11	2.47 ± 0.15*	-	1.17 ± 0.10	1.00 ± 0.09*
	90min	1.22 ± 0.14	2.29 ± 0.19*	-	0.93 ± 0.07	0.61 ± 0.05
	60min	1.96 ± 0.14	2.86 ± 0.14*	-	0.84 ± 0.03	0.38 ± 0.03
	30min	1.17 ± 0.10	1.81 ± 0.11*	-	0.65 ± 0.05	0.38 ± 0.07
Noradrenaline ($nmol.l^{-1}$)	**120min**	0.90 ± 0.09	3.79 ± 0.09*	-	1.64 ± 0.14	2.30 ± 0.15*
	90min	1.15 ± 0.10	3.48 ± 0.21*	-	1.81 ± 0.14	1.86 ± 0.12
	60min	0.51 ± 0.06	2.61 ± 0.17*	-	0.98 ± 0.06	1.45 ± 0.17
	30min	0.89 ± 0.09	3.77 ± 0.48*	-	1.21 ± 0.14	1.71 ± 0.12
Cortisol ($nmol.l^{-1}$)	**120min**	560 ± 10	496 ± 18	579 ± 16†	378 ± 12*	215 ± 128†
	90min	540 ± 20	533 ± 29	598 ± 35†	419 ± 24*	275 ± 12*†
	60min	497 ± 21	532 ± 23	590 ± 21†	395 ± 14*	200 ± 7*†
	30min	580 ± 17	592 ± 19	617 ± 17†	414 ± 8*	263 ± 16*†

Data presented as mean ± SEM (N = 7). * indicates significantly different from rest ($P<0.05$). † indicates significantly different from 0 min post-exercise ($P<0.05$).

5.3.5 Serum β-hydroxybutyrate responses

The serum β-hydroxybutyrate responses are presented in Table 5.4 and Figure 5.4. There were small declines from rest to pre-exercise under all conditions (**120min** Δ-0.05 ± 0.01; **90min** Δ-0.08 ± 0.01; **60min** Δ-0.06 ± 0.01; **30min** Δ-0.02 ± 0.01 mmol.l^{-1}), however, only under **120min**, **90min** and **60min** was the decline significant ($P<0.05$; Table 5.4). β-hydroxybutyrate concentrations did not change with exercise under any condition ($P>0.05$; Figure 5.4), however, there was a transient increase in concentrations over the three hour recovery period, with concentrations at 120 minutes only greater than rest and 0 post-exercise under **120min** (Figure 5.4). At 180 minutes, concentrations were significantly greater than both rest and 0 minutes post-exercise under all conditions ($P<0.05$; Figure 3). The change from 0 to 180 minutes post-exercise was similar across conditions (**120min** Δ+0.07 ± 0.013; **90min** Δ+0.13 ± 0.015; **60min** Δ+0.09 ± 0.019; **30min** Δ+0.07 ± 0.015 mmol.l^{-1}; $P>0.05$).

Table 5.4: Pre-exercise serum β-hydroxybutyrate (β-OHB) responses to alterations in the pre-exercise timing of carbohydrate and insulin administration.

		Sample Point				
Trial	Variable	Rest	30	60	90	120
120min	β-OHB (mmol.l^{-1})	0.09 ± 0.006	0.10 ± 0.009	0.06 ± 0.003*	0.06 ± 0.03*	0.04 ± 0.002*
90min	β-OHB (mmol.l^{-1})	0.13 ± 0.013	0.07 ± 0.006*	0.05 ± 0.004*	0.04 ± 0.003*	
60min	β-OHB (mmol.l^{-1})	0.13 ± 0.009	0.11 ± 0.007*	0.08 ± 0.005*		
30min	β-OHB (mmol.l^{-1})	0.15 ± 0.012	0.13 ± 0.014			

Data presented as mean ± SEM (N = 7). * indicates significantly different from rest ($P<0.05$).

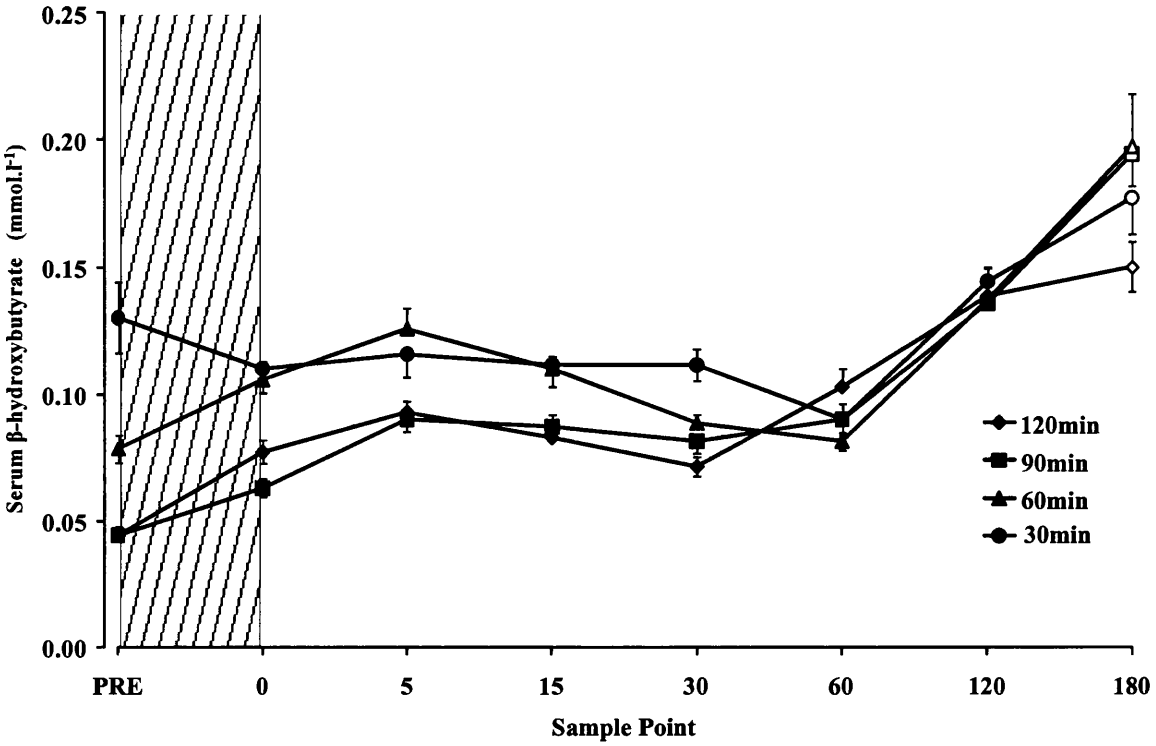


Figure 5.4: Time-course changes in serum β-hydroxybutyrate after alterations in the pre-exercise timing of carbohydrate and insulin administration. There was a significant time effect (P = 0.002, Partial-eta^2 = 0.646) but no effect of condition (P = 0.569). Data are presented as mean ± SEM (n = 7). Transparent sample points indicate significant difference from 0 min post-exercise (P<0.05). Thatched area indicates exercise.

5.3.6 Blood lactate and pH responses

The blood lactate responses are presented in Table 5.5 and Figure 5.5. There were no differences in fasting blood lactate and pH across conditions (Table 5.4). After carbohydrate and insulin administration blood lactate increased from resting concentrations at 90 minutes post-ingestion under both **90min** and **120min** (Table 5.5). However, remained unchanged under **60min** and **30min** (Table 5.5). Pre-exercise concentrations were lowest under **30min**, however, were not statistically different to the other trials ($P>0.05$; Figure 5.5). Blood lactate increased with exercise under all conditions, peaking at 0 minutes post-exercise (**120min** 3.0 ± 0.3; **90min** 3.9 ± 0.7; **60min** 3.2 ± 0.3; **30min** 2.9 ± 0.3 $mmol.l^{-1}$; $P>0.05$), the change with exercise was similar between conditions (**120min** Δ+1.6 ± 0.3; **90min** Δ+2.7 ± 0.7; **60min** Δ+1.8 ± 0.4; **30min** Δ+2.1 ± 0.3 $mmol.l^{-1}$; $P>0.05$). After exercise blood lactate concentrations decreased transiently, with similar declines in concentrations from 0 to 180 minutes post-exercise between conditions (**120min** Δ-2.2 ± 0.3; **90min** Δ-3.1 ± 0.7; **60min** Δ-2.5 ± 0.4; **30min** Δ-2.2 ± 0.3 $mmol.l^{-1}$; $P = 0.57$; Figure 5.5).

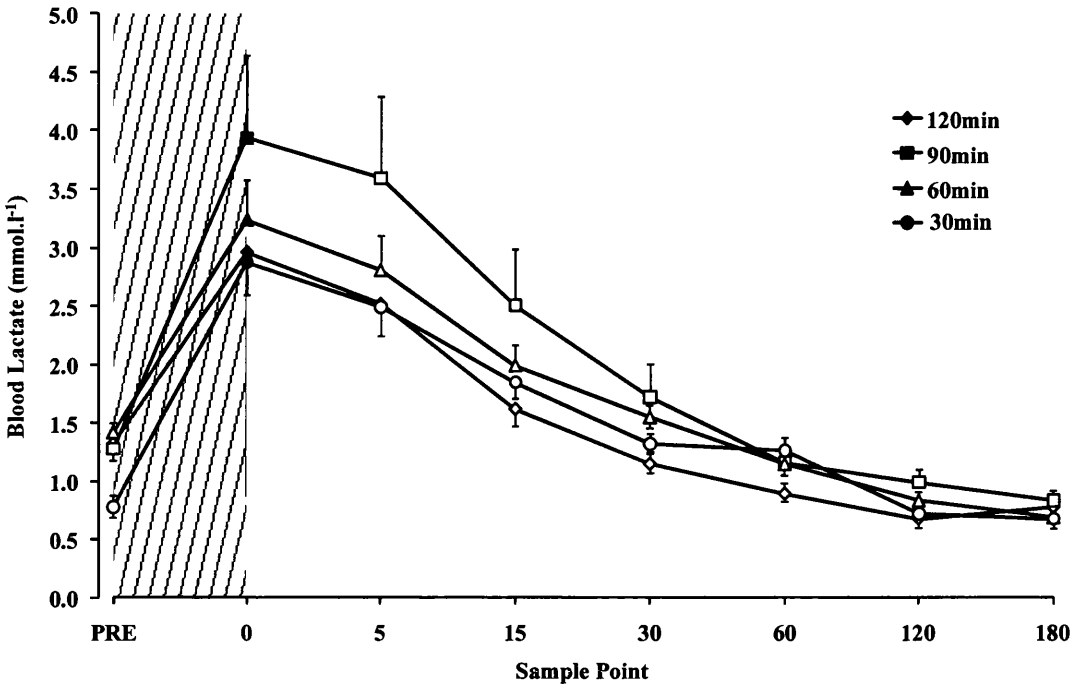


Figure 5.5: Time-course changes in blood lactate after alterations in the pre-exercise timing of carbohydrate and insulin administration. There was a significant time effect (P = 0.01, Partial-eta^2 = 0.420) but no effect of condition (P = 0.79). Data are presented as mean ± SEM (n = 7). Transparent sample points indicate significant difference from 0 min post-exercise (P< 0.05). Thatched area indicates exercise.

The blood pH responses are presented in Table 5.5 and Figure 5.6. After carbohydrate and insulin administration blood pH concentrations did not change from rest over the pre-exercise period under all conditions (Table 5.5). Exercise resulted in an increase in blood pH, however, the change with exercise was similar across conditions (**120min** Δ+0.07 ± 0.01; **90min** Δ+0.01 ± 0.02; **60min** Δ+0.04 ± 0.01; **30min** Δ+0.04 ± 0.01; P>0.05). Over the post-exercise period, there were no differences in blood pH responses between conditions; at 180 minutes post-exercise blood pH was similar across conditions (Figure 5.6). The change from 0 to 180 minutes post-exercise was similar across conditions (**120min** Δ-0.06 ± 0.01; **90min** Δ+0.01 ± 0.02; **60min** Δ-0.04 ± 0.01; **30min** Δ-0.03 ± 0.01; P = 0.51).

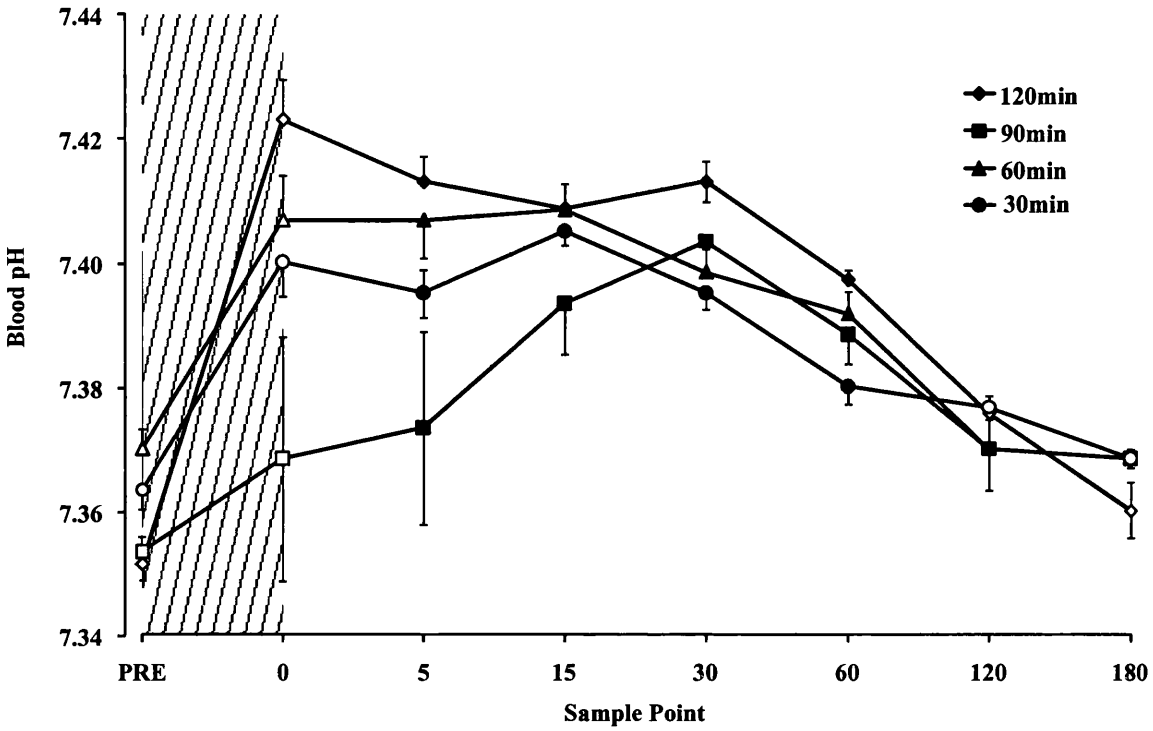


Figure 5.6: Time-course changes in blood pH after alterations in the pre-exercise timing of carbohydrate and insulin administration. There was a significant time effect (P = 0.002, Partial-eta^2 = 0.397) but no effect of condition (P = 0.518). Data are presented as mean ± SEM (n = 7). Transparent sample points indicate significant difference from 0 min post-exercise (P<0.05). Thatched area indicates exercise.

Table 5.5: Pre-exercise blood lactate and pH responses to alterations in the pre-exercise timing of carbohydrate and insulin administration.

		Sample Point				
Trial	Variable	Rest	30	60	90	120
120min	Lactate (mmol.l^{-1})	0.7 ± 0.02	1.0 ± 0.05	1.3 ± 0.07	1.4 ± 0.04*	1.3 ± 0.03*
	pH	7.36 ± 0.004	7.38 ± 0.004	7.37 ± 0.004	7.36 ± 0.004	7.35 ± 0.002
90min	Lactate (mmol.l^{-1})	0.7 ± 0.05	1.1 ± 0.08	1.4 ± 0.12	1.3 ± 0.07*	
	pH	7.38 ± 0.004	7.38 ± 0.003	7.38 ± 0.003	7.35 ± 0.002	
60min	Lactate (mmol.l^{-1})	0.7 ± 0.05	1.0 ± 0.04	1.4 ± 0.08		
	pH	7.38 ± 0.004	7.37 ± 0.003	7.37 ± 0.003		
30min	Lactate (mmol.l^{-1})	0.6 ± 0.02	0.8 ± 0.02			
	pH	7.36 ± 0.002	7.36 ± 0.003			

Data presented as mean ± SEM (N = 7). * indicates significantly different from rest (P<0.05).

5.3.7 Serum triglyceride and NEFA responses

The serum triglyceride (TG) responses are presented in Table 5.6 and Figure 5.7. After consumption of the LGI carbohydrate and insulin administration there were no changes in TG concentrations from rest to pre-exercise under all conditions (Table 5.6). However, serum TG concentrations increased with exercise under all conditions (Figure 5.7, $P<0.05$), with the change in serum TG similar across conditions (**120min** $\Delta+0.2 \pm 0.01$; **90min** $\Delta+0.2 \pm 0.01$; **60min** $\Delta+0.2 \pm 0.01$; **30min** $\Delta+0.2 \pm 0.01$ mmol.l^{-1}; $P = 0.91$). After exercise, serum TG concentrations decreased over the 180 minute post-exercise period under all conditions (Figure 5.7); the change from 0 to 180 minutes was similar across conditions (**120min** $\Delta-0.0 \pm 0.03$; **90min** $\Delta-0.1 \pm 0.02$; **60min** $\Delta-0.2 \pm 0.03$; **30min** $\Delta-0.2 \pm 0.02$ mmol.l^{-1}; $P = 0.66$).

The serum NEFA responses are presented in Table 5.6 and Figure 5.8. After carbohydrate consumption and insulin administration there was no change in NEFA concentrations from rest to pre-exercise under **30min** ($\Delta-0.06 \pm 0.01$ mmol.l^{-1}, $P = 0.32$), however, concentrations declined under **120min**, **90min** and **60min** (**120min** $\Delta-0.18 \pm 0.03$; **90min** $\Delta-0.17 \pm 0.03$; **60min** $\Delta-0.19 \pm 0.02$ mmol.l^{-1}; $P<0.05$; Table 5.6). The change from rest to pre-exercise was less under **30min** when compared to the other trials ($P<0.05$). There were no changes in NEFA concentrations with exercise; however, there were large increases in concentrations from 0 to 5 minutes post-exercise (Figure 5.8). After 5 minutes post-exercise, NEFA concentrations increased transiently over the remainder of the post-exercise period (Figure 5.8).

Table 5.6: Pre-exercise serum triglyceride and NEFA responses to alterations in the pre-exercise timing of carbohydrate and insulin administration.

		Sample Point				
Trial	Variable	Rest	30	60	90	120
120min	Triglyceride (mmol.l^{-1})	0.8 ± 0.05	0.9 ± 0.06	0.8 ± 0.04	0.7 ± 0.05	0.7 ± 0.04
	NEFA (mmol.l^{-1})	0.39 ± 0.5	0.36 ± 0.04	0.23 ± 0.02*	0.20 ± 0.02*	0.22 ± 0.03*
90min	Triglyceride (mmol.l^{-1})	1.0 ± 0.07	1.0 ± 0.06	0.9 ± 0.07	0.9 ± 0.06	
	NEFA (mmol.l^{-1})	0.47 ± 0.02	0.37 ± 0.02*	0.24 ± 0.01*	0.30 ± 0.02*	
60min	Triglyceride (mmol.l^{-1})	0.9 ± 0.06	0.8 ± 0.05	0.8 ± 0.05		
	NEFA (mmol.l^{-1})	0.46 ± 0.02	0.35 ± 0.02	0.20 ± 0.02*		
30min	Triglyceride (mmol.l^{-1})	1.0 ± 0.05	0.8 ± 0.05			
	NEFA (mmol.l^{-1})	0.46 ± 0.04	0.40 ± 0.04			

Data presented as mean ± SEM (N = 7). * indicates significantly different from rest (P<0.05).

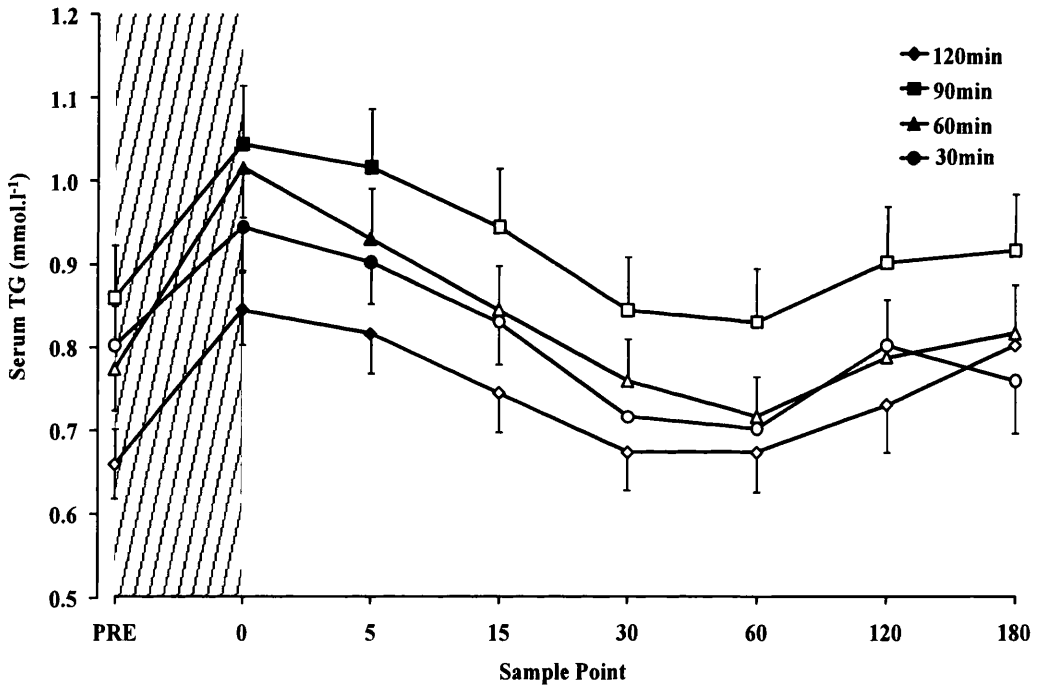


Figure 5.7: Time-course changes in serum triglycerides (TG) after alterations in the pre-exercise timing of carbohydrate and insulin administration. There was a significant time effect (P = 0.001, partial-eta^2 = 0.900) however, there were no significant time*condition interactions (P = 0.635). Data presented as mean ± SEM. Transparent sample points indicate significant change from 0 min post exercise (P<0.05). Thatched area indicates exercise.

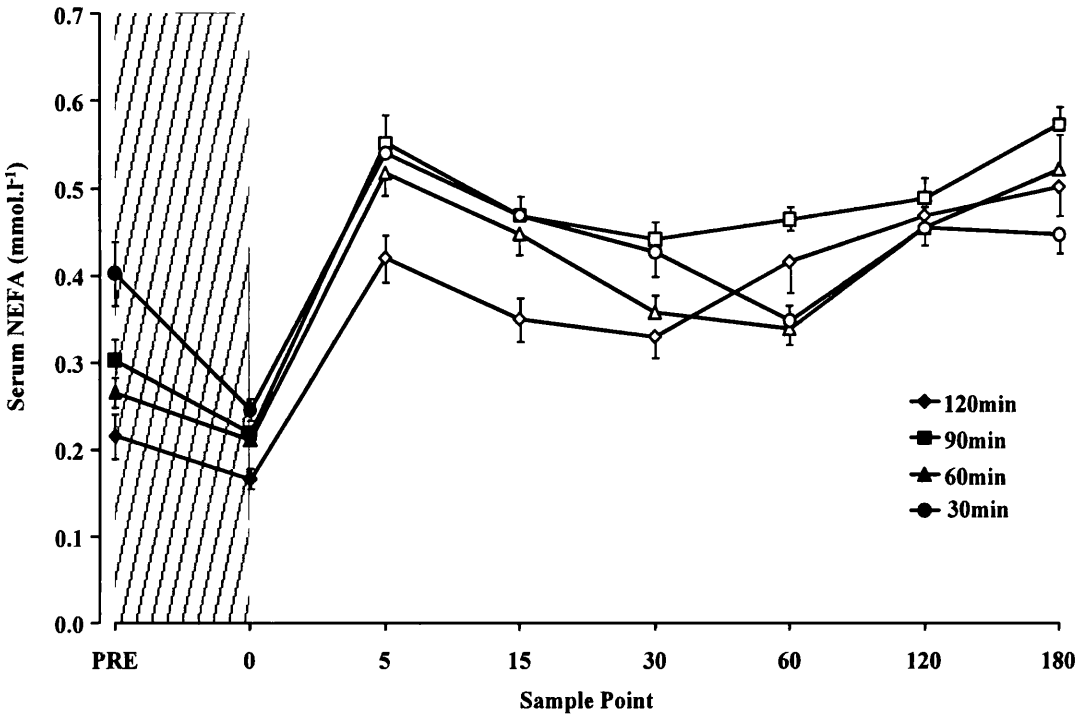


Figure 5.8: Time-course changes in serum NEFA's after alterations in the pre-exercise timing of carbohydrate and insulin administration. There was a significant time effect (P = 0.001, partial-eta^2 = 0.808) however, there were no significant time*condition interactions (P = 0.334). Data presented as mean ± SEM. Transparent sample points indicate significant change from 0 min post exercise (P<0.05). Thatched area indicates exercise.

5.3.8 Patterns of carbohydrate and lipid oxidation

Resting lipid and carbohydrate oxidation rates were greater and lower, respectively, under **30min**, when compared to all other trials (Table 5.7). During exercise lipid oxidation was greater and carbohydrate oxidation was lower under **30min**, when compared with **120min** (Table 5.7). When expressed as the change from rest, lipid oxidation was greater under **60min** and **90min**, in comparison with **120min** (Table 5.7). Moreover, carbohydrate oxidation was greater under **120min**, in comparison with all other trials (Table 5.7).

The energy expended across trials was similar (**120min** 3.56 ± 0.09; **90min** 3.21 ± 0.03; **60min** 3.24 ± 0.05; **30min** 3.14 ± 0.04 MJ, P = 0.32), however, there was a greater contribution to energy expenditure from lipids under **30min**, when compared with **120min** (**30min** 5.9 ± 0.7 vs. **120min** 2.0 ± 0.4 %, P = 0.02). This was also the case for carbohydrate contribution to energy demand (**30min** 94.1 ± 0.7 vs. **120min** 98 ± 0.4 %, P = 0.02). There were no between trial statistical differences in percentage contribution to energy demand from lipids and carbohydrates under **90min** and **60min** (lipids: **90min** 7.9 ± 1.1 **60min** 4.9 ± 1.3 %; carbohydrates: **90min** 92.1 ± 1.1 **60min** 95.1 ± 1.3 %; P>0.05).

Table 5.7: Substrate oxidation responses, at rest and during exercise, to alterations in the pre-exercise timing of carbohydrate and insulin administration.

	Rest				Exercise			
	120min	**90min**	**60min**	**30min**	**120min**	**90min**	**60min**	**30min**
CHO ($g.min^{-1}$)	0.47 ± 0.02	0.43 ± 0.01	0.45 ± 0.02	0.35 ± 0.02†	4.38 ± 0.05*	3.87 ± 0.06*	4.02 ± 0.08*	3.88 ± 0.08*‡
Lipids ($g.min^{-1}$)	0.02 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.10 ± 0.01†	0.07 ± 0.01*	0.17 ± 0.02*	0.12 ± 0.02*	0.13 ± 0.01*‡
ΔCHO ($g.min^{-1}$)	-	-	-	-	3.9 ± 0.04	3.4 ± 0.1‡	3.6 ± 0.1‡	3.5 ± 0.1‡
ΔLipids ($g.min^{-1}$)	-	-	-	-	0.04 ± 0.06	0.12 ± 0.02‡	0.09 ± 0.02‡	0.03 ± 0.01

Data presented as means ± SEM (n = 7). † indicates significantly different to all other trials ($P<0.05$). ‡ indicates significantly different to **120min** ($P<0.05$). * indicates a significant increase when compared with rest ($P<0.05$).

5.3.9 Post-laboratory activity and self-recorded glycaemia and dietary intake

Over the 21-hour post-laboratory period BG_{AUC} under **120min** and **90min** was lower than **60min** and **30min** ($P<0.05$; Table 5.8). With regards diet, participants consumed less carbohydrates under **60min** and **30min**, in comparison with **120min**, moreover, participants consumed less fat under **30min**, in comparison with **120min** (Table 5.8). As a result, total energy intake was lower under **60min** and **30min** in comparison with **120min** (Table 5.8). There were no conditional differences in the percentage contribution to total energy from carbohydrates (**120min** 52 ± 1.3; **90min** 51 ± 1.5; **60min** 49 ± 1.5; **30min** 53 ± 1.1 %; P = 0.62) and protein (**120min** 22 ± 0.7; **90min** 21 ± 0.8; **60min** 21 ± 0.9; **30min** 23 ± 0.8 %; P = 0.59), however, the contribution from fats under **30min** was lower than **90min** and **60min** (**30min** 23 ± 0.9 vs. **90min** 27 ± 1.2; **60min** 29 ± 1.1 %; $P<0.05$), but not **120min** (**120min** 25 ± 0.9 %, P = 0.22).

There were 3 incidents of hypoglycaemia (8, 15 and 17 hours post-laboratory) and 1 low blood glucose (6 hours post-laboratory) under **120min**. There were 2 incidents' of hypoglycaemia under **90min** (5 and 11 hours post-laboratory) and 1 incident of both hypoglycaemia (4 hours post-laboratory) and low blood glucose (20 hours post-laboratory) under **60min**. Under **30min** there were 2 incidents of hypoglycaemia (both at 4 hours post-laboratory). One participant experienced hypoglycaemia, in the post-exercise period, twice under **90min** and **30min**. There were no differences in rapid-acting insulin administration or participant activity, with similar average heart rate and steps taken over the 21 hour post-laboratory period (Table 5.8).

Table 5.8: Twenty-one hour post-laboratory blood glucose, dietary intake and activity patterns.

	Condition			
Variable	**120min**	**90min**	**60min**	**30min**
BG_{auc} ($mmol.l^{-1}.hour^{-1}$)	8.0 ± 0.1	6.9 ± 0.2	8.9 ± 0.3#	9.0 ± 0.2#
CHO intake (g)	160 ± 4	144 ± 4	131 ± 5*	137 ± 4*
Protein intake (g)	66 ± 3	59 ± 3	55 ± 3	60 ± 3
Fat intake (g)	34 ± 2	33 ± 2	35 ± 2	27 ± 2†
Energy intake (MJ)	5.1 ± 0.8	4.6 ± 0.9	4.4 ± 0.1*	4.3 ± 0.1*
Rapid-acting insulin (U)	16 ± 1	15 ± 1	16 ± 1	16 ± 1
Frequency BG < 4.0 $mmol.l^{-1}$	1	0	1	0
Frequency BG < 3.5 $mmol.l^{-1}$	3	2	1	2
Average heart rate (bpm)	79 ± 1	78 ± 1	78 ± 1	80 ± 1
Average steps taken	4826 ± 293	3153 ± 394	7469 ± 560	5525 ± 402

Data presented as means ± SEM (n = 7). * indicates significantly different from **120min** ($P<0.05$). # indicates different from **120min** and **90min**. † indicates significantly different from all other trials ($P<0.05$). BG_{AUC} indicates blood glucose area under the curve.

5.4 Discussion

This study examined the metabolic and glycaemic effects of alterations in the timing of carbohydrate feeding and insulin administration prior to running in individuals with T1DM. Results demonstrate that administration of both a LGI carbohydrate and a 75% reduction in rapid-acting insulin dose 30 minutes before exercise results in reduced carbohydrate and increased lipid oxidation during exercise and improved blood glucose responses after exercise.

Pre-Exercise

Resting carbohydrate oxidation was lower and lipid oxidation higher under **30min**, in comparison with **120min**. The slow digestion of the LGI carbohydrate, and a delayed gastric emptying (Achten et al., 2007), evident with lower pre-exercise blood glucose concentrations under **30min**, results in a slow absorption rate of glucose. With this in mind, after just 15-30 minutes, the slow digestion, and ultimately slow delivery of carbohydrate, resulted in a need for a greater contribution from lipids to meet energy demand. Conversely, after 105-120 minutes greater carbohydrate availability would have directly suppressed lipid oxidation (Coyle et al., 1997). Prior to exercise, blood glucose was lowest under **30min** in comparison with **120min**. As demonstrated in Chapter 4, blood glucose peaked 120 minutes after ingestion of an LGI carbohydrate (isomaltulose). Based on these data, just before starting running the LGI carbohydrate would not have been fully digested under **30min**. There were no significant differences in pre-exercise insulin concentrations, despite the manipulation of time prior to exercise. An investigation in to time-course changes in rapid-acting insulin has demonstrated these insulin analogues peak at 45 to 60 minutes after subcutaneous

administration, and decrease to 50% of peak concentrations at 120 minutes post administration (Plank et al., 2002). Within our study, peak insulin concentrations varied between 30 – 120 minutes after administration, across trials. Moreover, within **120min**, **90min** and **60min**, concentrations did not significantly change within the pre-exercise period after the 30 minute sample (Table 5.2). Within the research of Plank et al. (2002), participants received a bolus of 7.1 ± 1.3 IU of insulin aspart or lispro, whereas participants within this study administered just 2.1 ± 0.1 IU. To our knowledge there is no literature that has examined the pharmacokinetics of insulin aspart and lispro when administered at such low doses. Potentially, smaller doses may result in altered uptake kinetics and/or increased inter and intra individual variability in its appearance in circulation.

During Exercise

During exercise, oxidation rates of carbohydrate and lipids were lower and greater, respectively, under **30min** in comparison with **120min**. The differences in lipid oxidation were likely due to increased combustion of intramuscular derived lipids as serum NEFA responses were not different across trials (Figure 5.8). Two hours after ingestion would have been sufficient time for complete digestion of the LGI carbohydrate, resulting in a greater availability of carbohydrate to the active tissue, which may have resulted in a promotion of carbohydrate oxidation (Coyle et al., 1997); potentially lipid metabolism was directly suppressed by greater glucose availability (Coyle et al., 1997). Therefore, under **30min** the delayed absorption, similar to a fasted state, may have promoted lipid combustion. The drop in blood glucose with exercise was less under **30min** than **120min**. A lesser increase in serum insulin and a reduced carbohydrate oxidation rate and a greater contribution to energy demand from lipids would have aided in preserving blood glucose concentrations under

30min. Running resulted in an increase in serum insulin concentrations under all conditions. This is in accordance with previous research which has demonstrated exercise to increase the appearance of exogenous insulin (Dandona et al., 1980). Within our study concentrations increased 1.5-fold with exercise across conditions, with the increase with exercise under **30min** less than **120min**. According to Gallen (2003), after administration, exogenous insulin can lie trapped as a subcutaneous depot, which may take time to absorb and dissipate. Potentially, the bolus of insulin under **30min** would have had less time to dissipate and be absorbed into circulation at rest; this may potentially explain the tiered insulin concentrations after exercise, i.e. concentrations were greatest (but not significantly) under **120min** and lowest under **30min**. Serum non-esterified fatty acid concentrations did not change with exercise; an effect likely the result of a reduction in adipose tissue blood flow, with exercise intensities at and above 70% VO_{2max}, reducing the removal of NEFA (Jones et al., 1980; Romijn et al., 1995).

Post-Exercise

After exercise, blood glucose concentrations under **30min** were greater than **120min** and also significantly increased for 60 minutes post-exercise. The rise in glucose availability may be important in preventing post-exercise hypoglycaemia, as an increased glucose availability would aid in replenishing both muscle and liver glycogen stores. It has been suggested that preferential repletion of muscle glycogen over liver glycogen stores, resulting in hepatic glucose production being unable to meet glucose demand by the exercised tissue, is a contributing factor in the development of post-exercise hypoglycaemia (Steppel and Horton, 2003). Although the blood glucose responses under **30min** and **60min** are similar, the prevention of hypoglycaemia under **30min** is important in establishing the optimal pre-

exercise strategy. Over the post-exercise period, there were no differences in serum insulin responses with a similar and transient decline over the 3 hour post-exercise period. This is likely related to an increased insulin clearance that occurs in both T1DM and non-T1DM individuals after exercise (Tuominen et al., 1997); post-exercise insulin clearance is important for removing the inhibitory effects of insulin on the actions of counter-regulatory hormones (e.g. adrenaline, noradrenaline and cortisol). The sharp rise in NEFA concentrations from 0 to 5 minutes, under all conditions, is likely due to a redistribution of blood flow back to the adipose tissue at the cessation of exercise (Romijn et al., 1995).

Upon leaving the laboratory blood glucose concentrations were lowest under **120min** in comparison with **60min** and **30min** (**120min** 6.3 ± 0.6; **60min** 9.6 ± 0.6; **30min** 8.1 ± 0.5 $mmol.l^{-1}$). Over the 21 hour post-laboratory period BG_{AUC} was greater under **60min** and **30min** in comparison with **120min**, moreover, despite the greater blood glucose concentrations participants consumed less carbohydrates and overall less energy under both **60min** and **30min**, when compared to **120min** (Table 5.8). Participants were familiar with carbohydrate counting, administering ~ 1 IU of insulin per 10 g of carbohydrates; this reflected in the ~160 g of carbohydrate consumed under **120min**, with ~16 IU of insulin. However, under **60min** and **30min**, 131 and 137 g of carbohydrates, respectively, were consumed with ~ 16 IU of insulin. The two participants who experienced hypoglycaemia under **30min** left the laboratory with elevated blood glucose concentrations of 10.4 and 12.5 $mmol.l^{-1}$, and both experienced hypoglycaemia within 4 hours of leaving the laboratory, suggesting a rapid fall in blood glucose occurred, potentially due to administration of corrective insulin units. The contrasting post-laboratory blood glucose responses under **120min** and **30min** are both indicative of a lack of knowledge of increased post-exercise insulin sensitivity (Table 5.8). As blood glucose concentrations were lower after **120min**,

participants may have benefited from lowering their insulin to carbohydrate ratio in the hours after exercise, as recommended by De Feo et al. (2006). Conversely, under **30min**, applying corrective insulin units in a post-exercise insulin sensitised state may have caused unexpected falls in blood glucose.

This study has modified the existing strategies available to T1DM individuals that help combat hypoglycaemia during and after exercise. For the greatest protection against hypoglycaemia, our data suggest that a 75% reduced dose of rapid-acting insulin (Chapter 3; Rabasa-Lhoret et al., 2001), combined with a low GI carbohydrate (Chapter 4), should be administered 30 minutes before exercise. Moreover, despite administering a rapid-acting insulin dose so close to an intense, full body exercise bout, there were no incidences of hypoglycaemia under **30min**. These findings are of clinical importance as current recommendations do not advocate the administration of rapid-acting insulin less than 2 hours before exercise (De Feo et al., 2006). Moreover, current research demonstrates heavy pre-exercise insulin reductions are effective at avoiding exercise induced hypoglycaemia, however, may increase blood glucose concentrations such that individuals are exposed to pre and post-exercise hyperglycaemia (Rabsa-Lhoret et al., 2001); this could be detrimental to long-term glycaemic control if employed on a regular basis. Based on this study's findings, consumption of a LGI carbohydrate source and administration of a 75% reduced insulin dose 30 minutes before exercise reduces exposure to high blood glucose before exercise, promotes lipid combustion and reduces carbohydrate usage during exercise, preserving blood glucose concentrations such that both hyper- and hypoglycaemia are avoided after exercise.

In conclusion, this study examined blood glucose and serum insulin responses to alterations in the timing of carbohydrate feeding and insulin administration prior to running in individuals with T1DM. These data demonstrate that the T1DM individual may expect

improved glycaemic control after exercise and higher lipid oxidation rates during exercise if the pre-exercise rest period is restricted to 30 minutes following administration of a low glycaemic index carbohydrate and reduced insulin dose.

Chapter Six

General Discussion

6.1 Summary of aims and findings

The overarching aim of this thesis was to examine factors that affect post-exercise glycaemia and contribute to minimising the risk of hypoglycaemia after exercise. An inability to regulate circulating insulin concentrations is considered the primary gluco-regulatory defect within T1DM. Therefore, the aim of chapter 3 was to examine the effects of pre-exercise rapid-acting insulin reductions on blood glucose responses before and after running in T1DM individuals, to test the hypothesis that reducing pre-exercise insulin dose may help preserve post-exercise glycaemia. The results demonstrate that a 75% reduction to pre-exercise rapid-acing insulin dose best preserved blood glucose before and after exercise, without increasing the risk of ketoacidosis, and reduced the risk of hypoglycaemia in free living conditions for 24 hours following running.

An important factor determining blood glucose concentrations and subsequent patterns of fuel oxidation is the rate of appearance of carbohydrate into the circulation. Potentially, low GI carbohydrates may raise blood glucose less and increase the percentage contribution of lipids as a fuel because of a slower digestion. Therefore, the aim of chapter 4 was to examine the metabolic and blood glucose responses to ingestion of a high or low GI carbohydrate, combined with a 75% reduced insulin dose, before, during and for 24 hours after running. The results demonstrate that compared to a high GI carbohydrate, the low GI carbohydrate increased blood glucose concentrations less before exercise and maintained blood glucose better for 24 hours after running, via lower carbohydrate and higher lipid oxidation rates during the latter stages of running.

After manipulating both the insulin dose and the pre-exercise carbohydrate GI, to improve post-exercise blood glucose concentrations, the timing of the ingestion of carbohydrate (alongside a reduced insulin dose) before exercise is an important factor which may further

refine these strategies. Therefore, chapter 5 examined the metabolic and blood glucose responses to alterations in the timing of carbohydrate feeding and insulin administration prior to running. Our results demonstrated that administration of both a reduced rapid-acting insulin dose and low GI carbohydrate 30 minutes before exercise improved glycaemia for 24 hours after running, by reductions in carbohydrate oxidation, leading to increased carbohydrate availability post-exercise.

6.2 Impact of insulin reductions, carbohydrate administration and timing on glycaemia before and after running

Within T1DM, the primary defect in blood glucose regulation during exercise is the inability to regulate circulating insulin concentrations (Steppel and Horton, 2003). In chapter 3 pre-exercise insulin dose was reduced such that blood glucose concentrations were elevated above rest to a greater extent under the largest insulin reduction trial. Reducing pre-exercise insulin dose by 75% resulted in reductions in peak (and pre-exercise) insulin concentrations (**25%** 55 vs. **Full** 112 pmol.l^{-1}), and elevated blood glucose by ~4 mmol.l^{-1} before exercise (**25%** Δ+7.7 vs. **Full** Δ+3.5 mmol.l^{-1}). Manipulating the available insulin before exercise resulted in a reduced clearance of glucose into insulin sensitive tissues after ingestion of the meal and elevated blood glucose prior to exercise (Figure 6.1).

This difference in pre-exercise insulin concentrations is of importance, as with the same exercise bout the drop in blood glucose with exercise was less under **25%** due to the synergistic effect of insulin and contracting muscle (Nesher et al., 1985; Plough et al., 1984) being less under this condition. This would have reduced peripheral uptake of blood glucose and subsequent use by the exercising musculature (Chokkalingam et al., 2007), increasing hepatic glucose output through reduced inhibition at the adipocyte and hepatocyte allowing greater mobilisation of gluconeogenic substrate and less inhibition of hepatic enzymes involved in glycogenolysis (Cherrington et al., 2007). Keeping this in mind, higher pre-exercise blood glucose concentrations and a lesser drop in blood glucose during exercise under **25%** meant a preservation of blood glucose above resting concentrations was elicited, similar to the findings of Rabasa-Lhoret et al. (2001) (Figure 6.1; 6.2; 6.3).

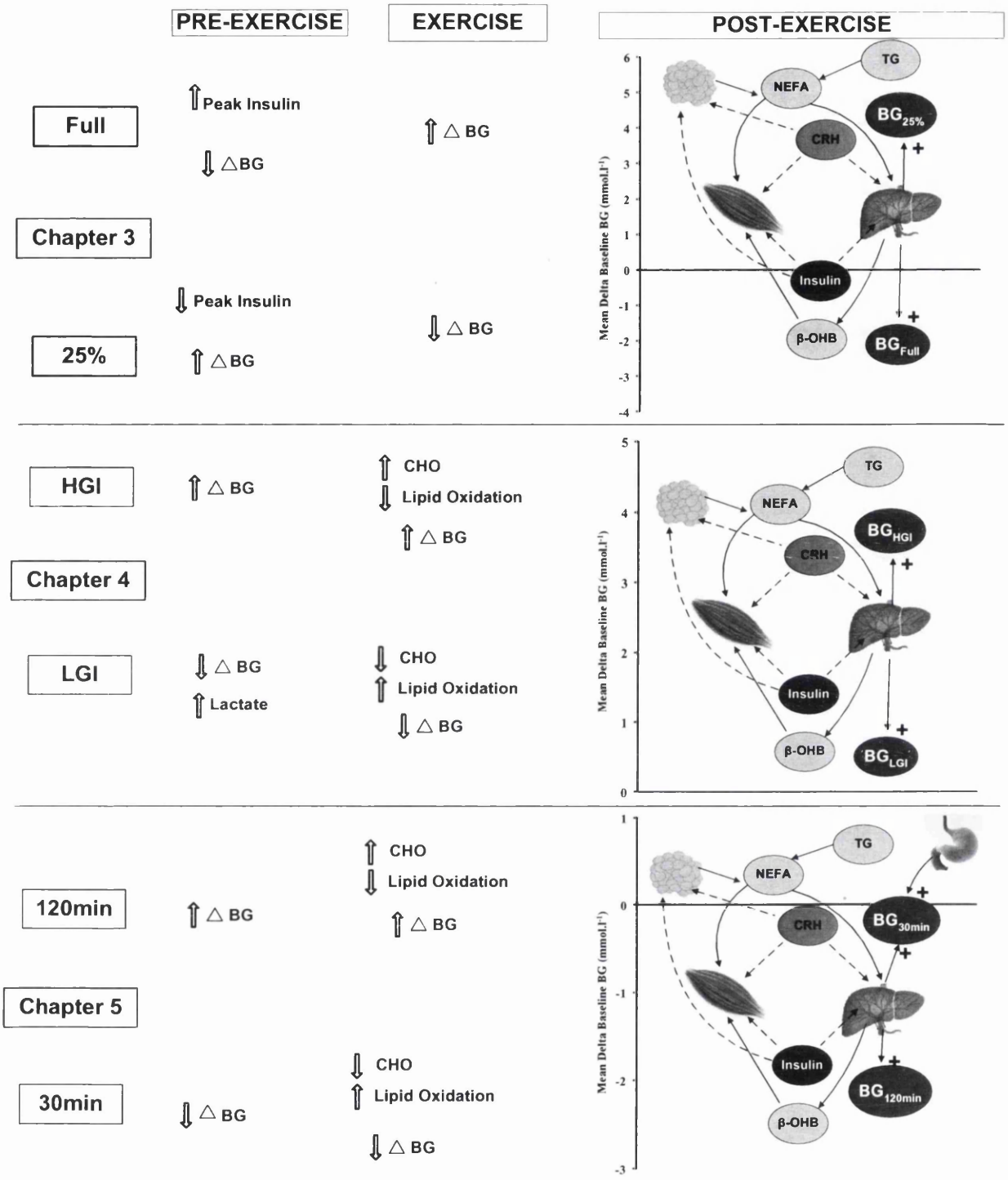


Figure 6.1: Schematic diagram of blood glucose regulation pre and post-exercise. **Note**: CRH = counter-regulatory hormones (catecholamines and cortisol); β-OHB = β-hydroxybutyrate; Dashed arrow indicates decreasing effect on tissue with time; Blue arrow indicates increased appearance with time.

Chapter 4 aimed to manipulate the glycaemic index of the administered carbohydrate given in conjunction with a reduced bolus insulin dose. The consumption of **HGI** resulted in a rapid rise in blood glucose, with peak concentrations double that of **LGI** occurring at 90 min post-ingestion, compared to 120 min under **LGI**. In contrast to chapter 3, where pre-exercise insulin concentrations were important in subsequent blood glucose responses during and after exercise, within chapter 4 gross differences in pre-exercise blood glucose concentrations had implications for subsequent glycaemic responses. Six of eight participants experienced a smaller drop in blood glucose under **LGI**. A potential mechanism behind this may be related to the hyperglycaemic blood glucose concentrations elicited under **HGI** at the onset of exercise. Research has demonstrated that when T1DM individuals exercise under hyperglycaemic conditions they elicit an increased disposal of blood glucose (Coyle et al., 1991; Jenni et al., 2008). The research of Jenni et al. (2008) demonstrated that when hyperglycaemic (11 $mmol.l^{-1}$), as under **HGI** (~15 $mmol.l^{-1}$), T1DM individuals elicit greater rates of glucose disposal and lower rates of endogenous glucose production. The mechanisms behind the greater drop in blood glucose under **HGI** may be due to blood glucose becoming the primary fuel source for the exercising muscles (Coyle et al., 1991). Other contributing factors to the lower drop in blood glucose during exercise under **LGI** may include a reduced oxidation of blood glucose by the active musculature due to a greater oxidation of lipids, and potentially fructose (Adopo et al., 1994). A further contributing mechanism could be greater rates of hepatic glucose output under **LGI**, due to the metabolism of fructose into glucose (Wahren et al., 1975; Ahlborg and Björkman, 1990) and the use of lactate as a gluconeogenic substrate (Ahlborg and Felig, 1982). Therefore, under **LGI** lower pre-exercise blood glucose concentrations and a lesser drop in blood glucose during exercise resulted in blood glucose concentrations being closer to euglycaemia, when compared to **HGI**, post-exercise (Figure 6.2; Figure 6.3).

Both types of carbohydrate were equally effective at preventing hypoglycaemia over the 3 hour recovery period, with two incidences under each condition. Based on this, for the prevention of hypoglycaemia it seems the glycaemic index of the carbohydrate consumed prior to exercise is secondary in importance to the 75% reduction in pre-exercise insulin dose. However, comparing the post-exercise glycaemia of chapter 3 and chapter 4 (Figure 6.2), it is apparent that the 75% reduction in insulin dose is important for the preservation of post-exercise blood glucose, regardless of carbohydrate type, however, less change in blood glucose under **LGI** with more euglycaemic concentrations post-exercise may be a more efficacious strategy for the T1DM individual, without an increased risk if hypoglycaemia.

As demonstrated within chapter 4, after consumption of the LGI carbohydrate blood glucose peaked at 120 minutes post-ingestion. Therefore, manipulation of pre-exercise timing would, as expected, result in a graded effect on pre-exercise blood glucose concentrations, i.e. lowest under **30min** and greatest under **120min**. Similar to the progression from chapter 3 to 4, where the exposure to high pre-exercise blood glucose concentrations was lessened; employing the **30min** strategy reduced blood glucose excursions further (pre-exercise blood glucose: chapter 3 **25%** Δ+9.0; chapter 4 **LGI** Δ+4.5; chapter 5 **30min** Δ+2.8 $mmol.l^{-1}$). Moreover, it is reasonable to suggest a similar tiered effect on serum insulin responses would be present; however, this was not the case. Pre-exercise insulin concentrations were similar across conditions, suggesting timing of administration of a heavily reduced insulin dose is secondary in importance to timing of carbohydrate consumption on pre-exercise blood glucose responses.

The incomplete digestion of the low GI carbohydrate at the onset of exercise under **30min** has important implications for blood glucose regulation during exercise. Two hours after

ingestion would have been sufficient time for complete digestion of the LGI carbohydrate under **120min**, resulting in a greater availability of carbohydrate to the active tissue, which may have resulted in a promotion of carbohydrate oxidation and a direct suppression of lipid metabolism (Coyle et al., 1997; Spriet, 2002). Therefore, under **30min** the delayed absorption, similar to a fasted state, may have allowed continued lipid combustion. The drop in blood glucose with exercise was less under **30min** than **120min**, with 5 of 7 participants experiencing a lesser drop under **30min**. A lesser increase in serum insulin concentrations, resulting in a lower rate of insulin mediated glucose uptake (Figure 6.1), lower rates of carbohydrate oxidation and a greater contribution to energy demand from lipids would have aided in preserving blood glucose concentrations under **30min**.

The drop in blood glucose with exercise is seemingly not just dependent upon a reduction in circulating insulin. When employing a 2 hour rest period between insulin and carbohydrate feeding and exercise (i.e. chapters 4 and 5), **Full** resulted in a blood glucose decline of ~6.1 $mmol.l^{-1}$, however, a 75% (**25%**) insulin reduction resulted in a drop in blood glucose of ~3.2 $mmol.l^{-1}$, with slightly greater declines thereafter under **LGI** and **HGI** (**LGI** ~4.4; **HGI** ~5.8 $mmol.l^{-1}$). However, the manipulation of pre-exercise timing also resulted in a range in drops in blood glucose (**30min** ~3.7; **60min** ~5.0; **90min** ~5.4; **120min** ~6.4 $mmol.l^{-1}$). Potentially, factors such as pre-exercise blood glucose concentrations, substrate oxidation and appearance of both insulin and carbohydrate in circulation may play a role in the drop in blood glucose with exercise. Moreover, based on the differences in the drop in blood glucose and the incidence of hypoglycaemia between **LGI** and **120min**, the duration of T1DM may be an important factor.

120min, being exactly the same protocol as the **LGI** trial of chapter 4, resulted in 5 of 7 participants experiencing hypoglycaemia within the laboratory, whereas just 2 of 8 experienced hypoglycaemia within the laboratory under **LGI**. Participants had similar anthropometric characteristics (chapter 4; body mass 84 ± 2; chapter 5; body mass 84 ± 1 kg) and were in similar glycaemic control (chapter 4; HbA_{1c} 8.0 ± 0.2; chapter 5; HbA_{1c} 8.3 ± 0.2 %), however, the duration of T1DM was different between studies (chapter 4; 14 ± 2; chapter 5; 19 ± 2 years), with duration of T1DM ranging from 1 - 34 years within chapter 4 and 6 - 35 years within chapter 5. Specifically, within chapter 4 half of participants had a duration of T1DM of less than 7 years, whereas there was just 1 individual under chapter 5. Therefore, there is potential that the longer duration of T1DM within chapter 5 meant participants within this study had a greater impairment of gluco-regulatory responses. Within the pathophysiology of T1DM is a progressive loss of α-cell function over time, and the glucagon response, potentially due a loss of β – α cell signalling (Banarer et al., 2002), such that after 5 years of diagnosis falling blood glucose will fail to stimulate the release of this key hormone (Mokan et al., 1994). Based on this research and the glucagon responses within chapters 3 and 4, differences in the release of this key hormone are unlikely to explain the different glycaemic responses and incidences of hypoglycaemia between the trials.

The differences in the glycaemic responses between **120min** and **LGI** may be related to adrenaline secretion. In addition to α-cell failure, T1DM experience progressive attenuation of autonomic, sympathetic neural and adrenomedullary responses (Cryer, 2002), with the adrenaline response becoming attenuated (Amiel et al., 1988; Bolli et al., 1983; Dagogo-Jack et al., 1993) (the attenuation of these responses is the likely explanation behind the lack of conditional differences in gluco-regulatory hormonal responses across conditions, despite gross differences in blood glucose responses). Moreover, there is potential that longer

duration T1DM individuals have lower adrenomedullary stores of adrenaline (De Galan et al., 2004). Although different participants, when examining the adrenaline response to exercise there was ~30% lesser rise in adrenaline, to the same exercise model, under chapter 5 when compared to chapter 4 (chapter 4 Δ+1.1 ± 0.16 vs. chapter 5 Δ+1.5 ± 0.02 nmol.l^{-1}). When considering the role of adrenaline in reducing blood glucose uptake (Howlett et al., 1999), increasing breakdown of intramuscular triglycerides and glycogen (Watt et al., 2001), and stimulating hepatic glucose output (Howlett et al., 1999), there may have been more reliance on blood glucose as a fuel during the **120min** condition, evident with a greater drop in blood glucose concentrations during exercise under **120min** in comparison with **LGI** (**120min** ~6.4 vs. **LGI** ~4.4 mmol.l^{-1}).

After exercise, blood glucose concentrations under **30min** were greater than **120min** and also significantly increased for 60 minutes post-exercise. As there were similar concentrations of gluco-regulatory hormones (e.g. insulin, catecholamines and cortisol) and blood lactate across conditions post-exercise, it is reasonable to suggest that the time-course changes in blood glucose concentrations under **30min** and **60min** are due to the appearance of ingested glucose, as opposed to changes in endogenous glucose production (Figure 6.1). At the cessation of exercise, a redistribution of blood flow back to the digestive system may have resulted in a flux of glucose into circulation, which raised blood glucose concentrations for 60 minutes. When comparing the conditions where a 25% insulin dose was employed (i.e. **25%**, chapter 4 and chapter 5) despite mean delta post-exercise blood glucose concentrations of just ~-0.4 mmol.l^{-1}, **30min** elicited the lowest incidences of hypoglycaemia, completely preventing hypoglycaemia. Moreover, **60min** elicited mean post-exercise blood glucose of ~-0.2 mmol.l^{-1}, yet only elicited 1 hypoglycaemic incident (Figure 6.2). Potentially, the rise in blood glucose after exercise is important in preventing post-exercise hypoglycaemia, as

increased glucose availability would aid in replenishing both muscle and liver glycogen stores, which may reduce the susceptibility to post-exercise hypoglycaemia (Steppel and Horton, 2003).

The maintenance of blood glucose (i.e. blood glucose concentrations did not decline below concentrations elicited at 0 minutes over the 3 hour post-exercise period) elicited in the post-exercise period under all conditions and chapters is potentially primarily regulated by decreasing insulin concentrations with time (Figure 6.1). Glucagon, the primary counter-regulatory hormone did not change with time or condition across chapters 3 and 4; moreover, the catecholamines and cortisol, also not different across conditions and chapters, decreased with time (Figure 6.1). Therefore, the decrease in insulin concentrations, likely due to an increased insulin clearance (Tuominen et al., 1997), resulted in the suppressive effect of insulin at the adipocyte and hepatocyte being lessened with time (Zinman et al., 1977; Figure 6.1) and concomitantly reducing insulin-stimulated blood glucose uptake (Figure 6.1). As glucose uptake would have been elevated to replenish glycogen stores within the exercised musculature (Wojtaszewski et al., 2002; Steppel and Horton, 2003), declining insulin concentrations would compensate for increased post-exercise insulin sensitivity (Wojtaszewski et al., 2002) and reduce the synergistic promotion of glucose uptake. Although NEFA concentrations were not measured within chapter 3, concentrations are assumed to follow similar time course changes as within studies 4 and 5. Moreover, β-hydroxybutyrate, which is the result of NEFA metabolism within the hepatocyte (Laffel, 1999) increased with time (Figure 6.1). The increase in NEFA concentrations, likely due to increased triglyceride breakdown and potentially lipolysis (Figure 6.1), and β-hydroxybutyrate concentrations may be important in providing an alternative energy substrate

for the exercised tissue (Laffel, 1999; Tuominen et al., 1997), and reducing the reliance on blood glucose as a fuel.

When examining the progression in the pre-exercise strategies from chapter 3 to 5 and their influence on post-exercise glycaemia (Figure 6.2; 6.3), it is evident that a heavy insulin reduction preserves post-exercise glycaemia the most, however, combining a 75% reduced insulin dose with a low GI carbohydrate preserves blood glucose less, but results in less change in blood glucose before and after exercise, and results in more euglycaemic concentrations after exercise, which may be beneficial for glycaemic control. However, this strategy was not fully effective at combating hypoglycaemia. Implementing the recommendations of chapter's 3 and 4 30 minutes before exercise results in less change in blood glucose before and after exercise, and completely prevented hypoglycaemia (Figure 6.2). Although **60min** also reduces the risk of hypoglycaemia and displays similar post-exercise glycaemic responses, it is the improved fuel oxidation, a lesser drop in blood glucose during exercise and the prevention of hypoglycaemia post-exercise which makes **30min** the recommended strategy. Furthermore, under **30min** the average fasting blood glucose of participants was ~8.5 $mmol.l^{-1}$, therefore, a mean decline in post-exercise blood glucose of ~0.4 $mmol.l^{-1}$ is negligible considering participants had completed 45 minutes of running and a further three hours of recovery, without feeding, yet did not experience hypoglycaemia.

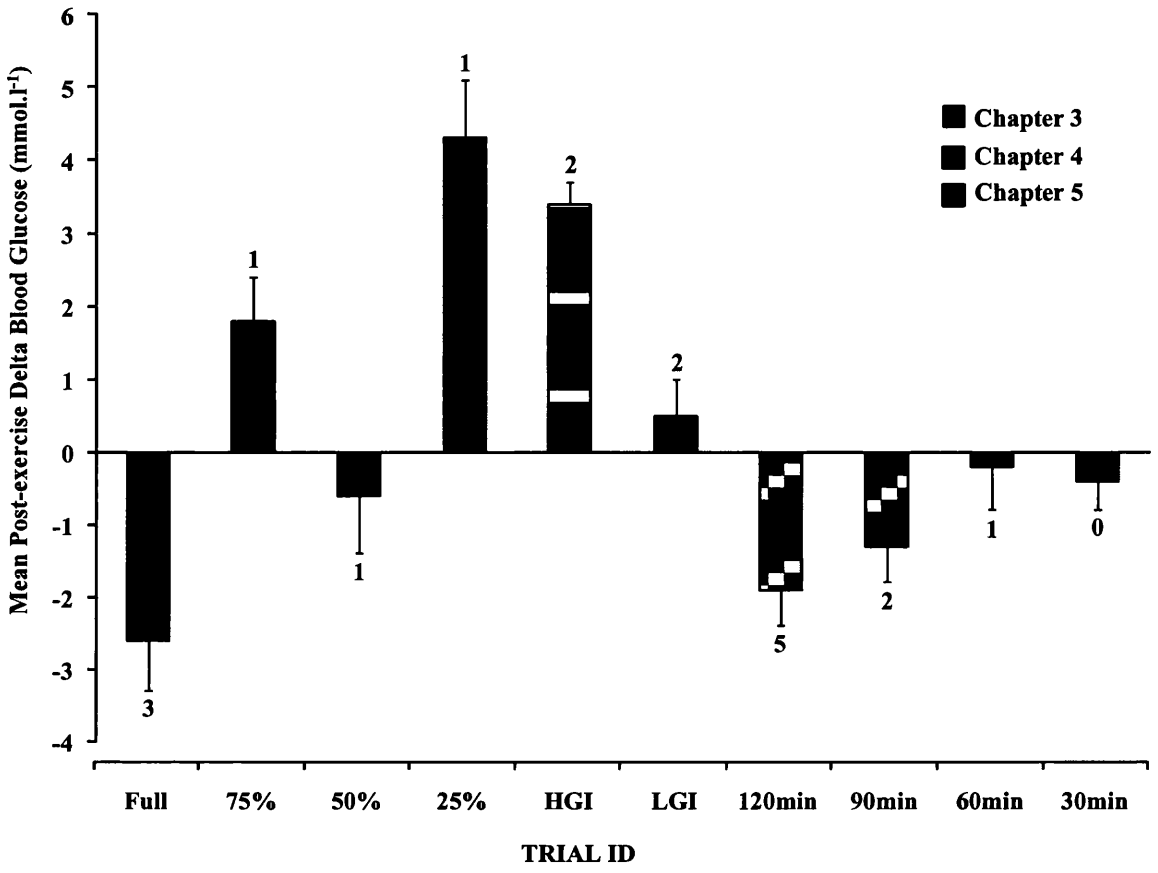


Figure 6.2: Mean post-exercise Δ blood glucose concentrations across chapters 3 to 5. Data presented as mean ± SEM. **Note**: Numbers indicate incidence of post-exercise hypoglycaemia.

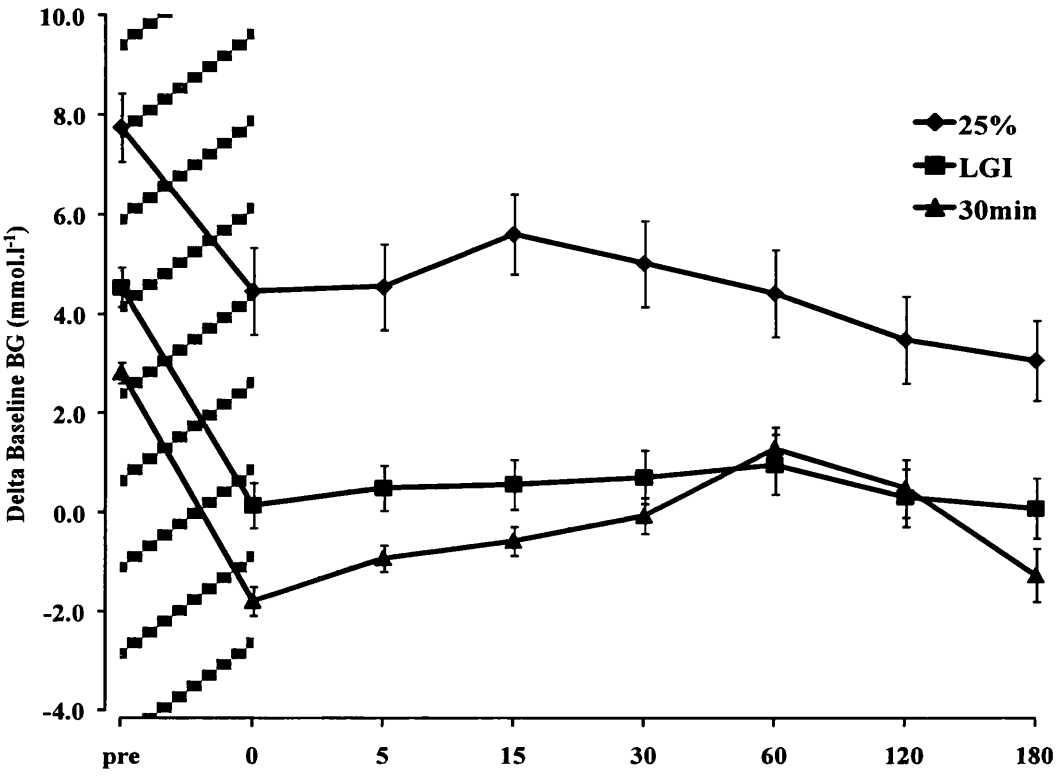


Figure 6.3: Blood glucose responses, relative to resting concentrations, presented from the pre-exercise sample to 180 minutes post-exercise under the three recommended strategies from chapters 3 to 5. Data presented as mean ± SEM.

6.2.1 Post-laboratory glycaemia

Determining the long-term effectiveness of our interventions following exercise based on the incidence of hypoglycaemia is difficult as participants resumed their normal daily routines. Participants were required to indicate if additional insulin units were added/or units were omitted to attempt to correct their blood glucose concentrations (e.g. correcting blood glucose from low to high, high to low or to maintain concentrations). Self-recorded data indicate that some participants administered additional units to correct for high blood glucose, e.g. under **25%** and **HGI** (evidenced by an insulin to carbohydrate ratio of more than 1 IU to 10 g of carbohydrate or administration of insulin units without feeding), moreover, when concentrations were within more euglycaemic concentrations, such as under **LGI**, **120min**, **90min** and **30min**, participants administered their usual insulin dose with the meal after exercise. Therefore, a lack of knowledge of increased post-exercise insulin sensitivity may have led to unexpected falls in blood glucose. This factor likely explains the incidences of low blood glucose and hypoglycaemia that were experienced by some participants under trials with well preserved blood glucose concentrations, e.g. **LGI**, **60min** and **30min**. Therefore, T1DM individuals need a greater awareness of an increase in insulin sensitivity after exercise, i.e. an increased potency of their rapid-acting insulin, and the importance of reducing their insulin to carbohydrate ratio after exercise (De Feo et al., 2006).

The effectiveness of our strategies was assessed based on a combination of glycaemic and metabolic responses during the laboratory period and post-laboratory period glycaemia and dietary intake. As a result, **30min** was deemed the most effective pre-exercise strategy for T1DM individuals to engage in. Implementing this strategy offers the T1DM individual improved resting and exercising fuel oxidation, lesser drops in blood glucose with exercise and improved blood glucose concentrations for 24 hours post-exercise.

6.3 Changes in the pattern of substrate oxidation

Making direct across chapter comparisons on the rates of substrate oxidation may be difficult due to different participants. However, it is important to recognise the within-chapter improvements in substrate oxidation and the preservation of blood glucose post-exercise.

Rest

Within chapter 3, despite alterations to insulin dose, and ultimately glycaemia, respiratory data revealed no differences in resting carbohydrate and lipid oxidation rates. This is in contrast to other literature that has demonstrated that gross alterations in insulin dose can significantly alter resting substrate metabolism within T1DM (Chokkalingham et al., 2007). Chokkalingham and colleagues demonstrated that low insulin concentrations, in comparison with high, can reduce resting carbohydrate oxidation by ~40% and increase lipid oxidation by ~80%. Moreover, within chapter 4 alterations to the GI of the ingested carbohydrate did not alter resting fuel metabolism. The lack of differences in fuel oxidation rates within chapters 3 and 4 can be explained by a similar mechanism. According to Jenni et al. (2008) hyperglycaemic blood glucose concentrations suppress lipid oxidation (Jenni et al., 2008) through a mass action effect of glucose (Coyle et al., 1997). Therefore, within chapter 3 a reduction in insulin dose and a concomitant rise in blood glucose, and hyperglycaemic (>11 $mmol.l^{-1}$; Jenni et al., 2008) blood glucose concentrations under both conditions within chapter 4, may have suppressed any changes in oxidation rates.

The mechanism behind the suppression of lipid oxidation may be related to a suppression of β-oxidation through a reduced transport of long chain fatty acids (LCFA) into the mitochondria (Coyle et al., 1997; Sidossis et al., 1996; Rasmussen et al., 2002). Figure 6.4 below describes the transport of LCFA into the matrix of the mitochondria for β-oxidation.

The carnitine palmitoyltransferase (CPT) complex, consisting of CPT 1, acylcarnitine transferase and CPT II, has a major regulatory role in the transport of LCFA into the mitochondria for subsequent β-oxidation in skeletal muscle (Figure 6.4; McGarry and Brown, 1997). CPT1, located on the outer surface of the outer mitochondrial membrane, catalyses the transfer of a variety of LCFA acyl groups from CoA to carnitine (Figure 6.4). The generated acylcarnitine can then permeate the inner membrane, via acylcarnitine/carnitine translocase (described as translocase in Figure 6.5). The acyl-CoA is then reformed in the matrix of the mitochondria by CPT II. This enzyme is located on the inner mitochondrial membrane and catalyses the transfer of the acyl group from carnitine to CoA and the reformed acyl-CoA enters the β-oxidation pathway (McGarry and Brown, 1997). CPT1 is considered the rate limiting step in the oxidation of LCFA and is reversibly inhibited by malonyl-CoA (Spriet, 2002). It has been demonstrated that hyperglycaemia and hyperinsulinaemia reduce LCFA oxidation through an inhibition of CPT1 (Sidossis et al., 1996). Moreover, hyperglycaemia has been demonstrated to increase the concentration of malonyl-CoA, and suppress LCFA transport into the mitochondria (Rasmussen et al., 2002). Therefore, an increased glycolytic flux, resulting in an increase in malonyl-CoA (Figure 6.5), may result in an inhibition of CPT1 and a concomitant suppression of lipid oxidation.

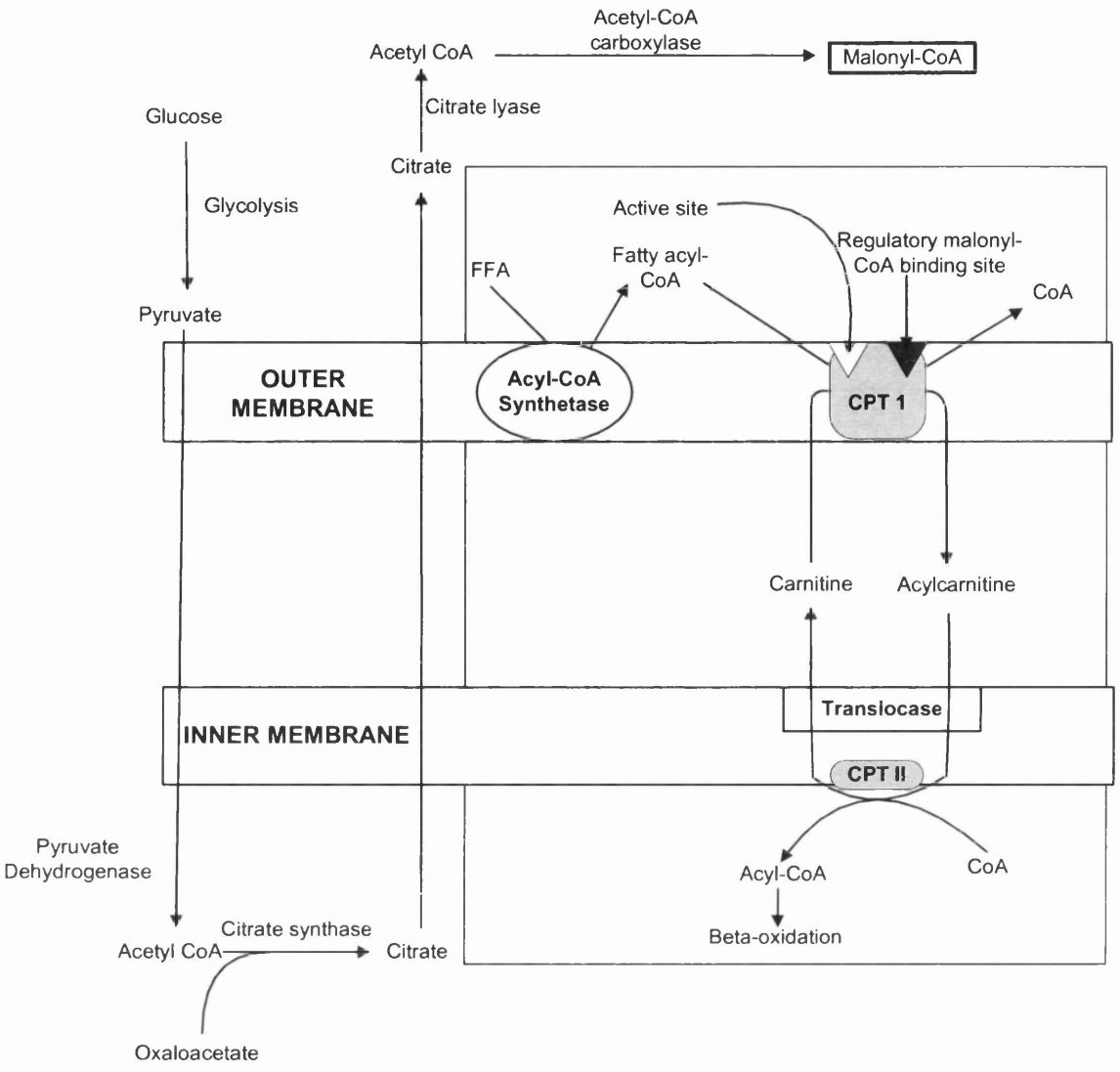


Figure 6.4: Schematic diagram of the formation of malonyl-CoA from glucose and the regulation of free fatty acid transport into the mitochondria (process within the red outline) by carnitine palmitoyltransferase 1 (CPT1) and 2 (CPT II) (adapted from Spriet, 2002).

Within chapter 5, resting carbohydrate oxidation was lower and lipid oxidation higher under **30min**, in comparison with **120min**. The greater lipid oxidation under **30min** is likely related to lower blood glucose concentrations (Jenni et al., 2008). The lower blood glucose concentrations under **30min** are most likely due to the slow digestion of the LGI carbohydrate, which is the result of slower rates of hydrolysis within the gastrointestinal tract

(Lina, 2002). Therefore, after just 15-30 minutes, the slow digestion, and ultimately slow delivery of carbohydrate, resulted in a need for a greater contribution from lipids to meet energy demand. Specifically, less delivery of carbohydrate to the musculature would have resulted in less glycolytic flux, which would result in less citrate formation within the matrix of the mitochondria and subsequently result in a reduction in malonyl-CoA (Figure 6.4) and allow LCFA to enter the mitochondria for β-oxidation (Figure 6.4). Conversely, after 105-120 minutes greater carbohydrate availability would have directly suppressed lipid oxidation; through similar mechanisms involving the inhibition of LCFA transport into the mitochondria through increases in malonyl-CoA (Rasmussen et al., 2002). The increase in lipid oxidation prior to exercise under **30min**, may have contributed to a sparing of both endogenous and exogenous carbohydrate sources before exercise, increasing available carbohydrate reserves during exercise.

Based on the resting data, it seems that substrate oxidation is mainly influenced by blood glucose concentrations, as serum NEFA concentrations were not different across any of the studies. The rise in blood glucose associated with a reduced insulin dose suppresses any shift in fuel metabolism. Moreover, the digestion and ultimately the delivery of carbohydrate to resting musculature effects fuel oxidation more so than gross reductions in insulin dose or alterations in the glycaemic index of the ingested carbohydrate.

Exercise

Within chapter 3 the hyperglycaemia associated with reducing insulin dose suppressed any shifts in exercising fuel oxidation rates. Although prior research has demonstrated the role of insulin in promoting carbohydrate oxidation and suppressing lipid utilisation during exercise (Chokkalingham et al., 2007), this research did not address blood glucose concentrations. Within the research of Chokkalingham et al. (2007) reductions in insulin concentrations

provided a milieu for increased rates of lipid oxidation, however blood glucose was clamped at 8 $mmol.l^{-1}$ (Chokkalingam et al., 2007). Relating these data to that of this thesis, the hyperglycaemia associated with reducing insulin dose (13-15 $mmol.l^{-1}$), irrespective of condition, may have caused an equal but elevated carbohydrate oxidation rate (Jenni et al., 2008; Rasmussen et al., 2002; Figure 6.4) suppressing any shifts in the pattern of lipid oxidation, regardless of alterations to insulin concentrations. As previously described, hyperglycaemia and an increased glycolytic flux may have directly suppressed lipid oxidation through reducing mitochondrial fatty acid transport (Figure 6.4).

The importance of blood glucose concentrations on fuel oxidation was demonstrated further within chapter 4 where a low GI carbohydrate increased lipid oxidation and reduced carbohydrate oxidation towards the latter stages of exercise. These data are the first to demonstrate that a low GI carbohydrate improves fuel oxidation rates within T1DM individuals. These findings are similar to research examining the influence of the glycaemic index on exercising fuel metabolism within non-T1DM individuals (Demarco et al., 1999; Stevenson et al., 2006). The differences in substrate oxidation pattern are likely related to blood glucose concentrations during the exercise bout (Jenni et al., 2008). At the beginning of exercise blood glucose concentrations were hyperglycaemic under both conditions, however, were ~3 $mmol.l^{-1}$ lower under **LGI**, in comparison with **HGI**. The high blood glucose concentrations may have limited fuel use to predominantly carbohydrates under **HGI** (Jenni et al., 2008). Moreover, towards the later stages of running blood glucose concentrations would have been lower and more euglycaemic under **LGI** in comparison with **HGI**, removing the mass action effect of glucose, and reducing glycolytic flux and lessening its inhibitory effect on lipid metabolism (Coyle et al., 1997; Jenni et al., 2008). Reductions in glycolysis would result in a decreased formation of malonyl-CoA (Figure 6.4) and increase

LCFA transport into the mitochondria and ultimately reduce carbohydrate oxidation and concomitantly increase lipid oxidation towards the latter stages of exercise under **LGI**. The increase in lipid oxidation during exercise would have contributed to a sparing effect on carbohydrate reserves (Figure 6.1), potentially reducing the oxidation of blood glucose and explaining the lesser drop in concentrations experienced by 75% of participants under **LGI**.

Similar mechanisms are responsible for the changes in fuel oxidation demonstrated within chapter 5. During exercise, oxidation rates of carbohydrate and lipids were lower and greater, respectively, under **30min** in comparison with **120min**. The difference between these two trials is likely related to differences in the digestion of the LGI carbohydrate and ultimately its delivery to the active tissue. Under **120min**, two hours would have been sufficient time for complete digestion of the LGI carbohydrate, resulting in a greater availability of carbohydrate to the active tissue, which may have resulted in a promotion of carbohydrate oxidation and suppression of lipid metabolism due to greater glucose availability (Coyle et al., 1997; Spriet, 2002). Conversely, 30 minutes after ingestion would not have been sufficient time for complete digestion of the LGI carbohydrate, as demonstrated within blood glucose responses of chapter 4 and the lower pre-exercise concentrations within this study. As such under **30min** the delayed absorption, similar to a fasted state, would have resulted in a lesser glycolytic flux (Figure 6.4), and promoted lipid combustion. This improved fuel use may have contributed to a sparing effect on carbohydrate reserves, promoting the preservation of blood glucose in the post-exercise period.

Ultimately, the progressively lower blood glucose concentrations within chapters 4 and 5 (pre-exercise concentrations: chapter 3 **25%** Δ+9.0; chapter 4 **LGI** Δ+4.5; chapter 5 **30min** Δ+2.8 $mmol.l^{-1}$) resulted in an increased contribution from lipids to energy expenditure during exercise. A reduced reliance on carbohydrate for fuel helped preserve blood glucose

concentrations, as blood glucose oxidation and potentially disposal (Coyle et al., 1997) would have been less, evidenced by lower drops in blood glucose during exercise under **LGI** and **30min**, such that concentrations were more euglycaemic and similar to rest for 3 hours post-exercise under both conditions. Based on this thesis' data, improved fuel oxidation, through lower blood glucose concentrations, promotes a preservation of carbohydrate reserves which contributes to improving post-exercise glycaemia.

6.4 The effects of insulin reductions and carbohydrate consumption on ketogenesis

Low insulin concentrations with concomitant rises in counter-regulatory hormones create a milieu that promotes ketone body formation (Laffel, 1999). In addition, exercise has been demonstrated to induce hyperketonaemia (>1 $mmol.l^{-1}$) within non-T1DM individuals (Koeslag et al., 1980). Therefore, within the T1DM individual there is potential that reductions in insulin dose, exercise and concomitant increases in counter-regulatory hormones could create a milieu where ketogenesis could increase after exercise (Figure 1.8). However, data from this thesis refutes this hypothesis.

When fasting, ketones play a role in reducing glucose utilisation (Balasse and Fery, 1989); however, insulin restrains ketogenic enzyme activity and increases their uptake into extra-hepatic tissue (Laffel, 1999). Moreover, after feeding, an increase in glucose availability leading to an increased glycolytic flux results in an increased availability of oxaloacetate, which condenses with acetyl CoA, ultimately diverting acetyl CoA from ketone forming pathways (Laffel, 1999). Therefore, the consumption of a bolus of carbohydrates and administration of rapid-acting insulin at rest resulted in a transient reduction in β-hydroxybutyrate concentrations. Therefore, exercise took place under elevated blood glucose concentrations, and insulin concentrations still elevated above rest, which potentially created a restraining effect over ketogenesis.

With regards chapter 3, changes in insulin dose did not result in conditional changes in β-hydroxybutyrate concentrations. This is an important finding as reductions in insulin dose or omission of insulin dose is a significant factor in the development of diabetic ketoacidosis (Wallace and Matthews, 2004). Moreover, despite reduced insulin concentrations and an exercise induced increase in ketogenic hormones, such as adrenaline, β-hydroxybutyrate did

not reach concentrations anywhere near that which could be defined as hyperketonaemia (>1 $mmol.l^{-1}$), let alone reach ketoacidosis (>3 $mmol.l^{-1}$; Laffel, 1999), with peak concentrations reaching ~0.5 $mmol.l^{-1}$. A potential explanation for this may be related to unaltered basal insulin administration. The basal insulin dose has a primary role of restraining excessive hepatic glucose output (Barnett, 2003) and a consequence of this is a similar effect on ketogenesis. As basal insulin remained unaltered across trials, and studies, and with only small alterations in rapid-acting insulin dose, it suggests a milieu that differences in β-hydroxybutyrate synthesis could take place was not created.

The transient rise in β-hydroxybutyrate concentrations over the recovery period is likely due to a combination of factors. After an overnight fast, participants subsequently utilised ~2.6 MJ of energy during running, having only consumed ~1.1 – 1.3 MJ, across studies, this energy deficit created by the active musculature needed to be restored through utilisation of endogenous liver, muscle and adipose tissue energy stores. Furthermore, without feeding for the 3 hour recovery period the energy deficit would be magnified with time. Moreover, the increased insulin clearance after exercise (Tuominen et al., 1997) would have resulted in a transient reduction in insulin's restraint on enzymes involved in both hepatic glucose production and ketogenesis (Figure 6.1). β-hydroxybutyrate concentrations peaked 3 hours after exercise under all conditions (~0.3 - 0.5 $mmol.l^{-1}$, across studies). These values, alongside acid-base values, were within the normal physiological range.

Additionally, it should be noted that although insulin concentrations were declining, basal insulin dose remained unchanged. As previously mentioned, an important role of the basal dose is it to prevent unregulated ketogenesis (Barnett, 2003). Keeping this in mind, glucagon, a key stimulator of ketogenesis (Laffel, 1999), did not increase after exercise, moreover there were no conditional differences in other key hormones in ketogenesis, the catecholamines

(Laffel, 1999). Thus, a hormonal milieu which may have led to post-exercise hyperketonaemia or ketoacidosis was not created.

From a patient safety perspective, a heavy pre-exercise insulin reduction in combination with carbohydrate ingestion does not result any in adverse physiological changes. There is no increased risk of developing diabetic ketoacidosis, with ketone body formation and blood pH within clinically acceptable ranges for the duration of all trials within all studies. Employing our strategy of a 75% reduced rapid-acting insulin dose and a low glycaemic index carbohydrate, administered 30 minutes before exercise has the potential to induce post-prandial/pre-exercise hyperglycaemia, however this will dissipate with exercise.

6.5 General conclusions

The results of this thesis have demonstrated that:

1. The factors most likely to contribute to reducing hypoglycaemia after exercise are a 75% reduction to pre-exercise rapid-acting insulin dose, combined with a low glycaemic index carbohydrate, administered 30 minutes prior to exercise.

2. The reduced incidence of hypoglycaemia after exercise is achieved by alterations in blood glucose concentrations and substrate metabolism.

3. Results suggest no greater risk of developing ketoacidosis by engaging in these protocols. Indeed, by optimising these strategies there was an improvement in post-exercise glycaemia and a concomitant reduced incidence of hypoglycaemia in individuals with type 1 diabetes mellitus.

6.6 Limitations

A potential limitation is that within chapter 4 participants were exercising at a $\%VO_{2peak}$ that was greater than that of chapters 3 and 5. The preliminary testing for study 4 was identical to that of chapter 3 and 5, yet exercise intensity was ~10% above the intended intensity of 70% VO_{2peak}. This was surprising as in addition to an identical preliminary testing protocol, trial running velocity, average VO_2, average HR and average peak blood lactate concentrations were similar across conditions and chapters (Table 6.2). The unexpected greater $\%VO_{2peak}$ within chapter 4 may be related to the oxygen cell within the metamax-3b system. Within chapter 4, after completion of the preliminary testing, the oxygen cell within the Metamax-3b was replaced with a fresh cell. Potentially, the cell in place during the preliminary testing period was under estimating VO_2, relative to the new cell, which resulted in lower maximal values, thus explaining the greater $\%VO_{2peak}$ elicited during the trials when running at a velocity of 70% V_{max}.

Table 6.1: Summary of the average physiological and metabolic markers of exercise intensity across chapters 3 to 5.

	Chapter 3				Chapter 4		Chapter 5			
	Full	**75%**	**50%**	**25%**	**HGI**	**LGI**	**120min**	**90min**	**60min**	**30min**
Running Pace			8.5			8.3			8.5	
VO_2 ($l.min^{-1}$)	2.5	2.5	2.5	2.5	2.9	2.9	3.1	3.0	3.0	3.0
HR (bpm)	158	155	159	159	159	163	156	158	158	159
Peak blood lactate ($mmol.l^{-1}$)	4.2	4.3	4.1	5.4	4.4	4.5	3.0	3.9	3.2	2.9

Based on previous data collected by Rabasa-Lhoret et al. (2001) (e.g. blood glucose changes with exercise, Full Δ-3.36 ± 6.08 and 50% Δ-2.26 ± 4.32 $mmol.l^{-1}$) for a statistical power of 80% 33 subjects would have been required. Unfortunately, this sample size was beyond the pool of T1DM individuals available. As a result, data was underpowered with power ranging between 45 and 59 % across chapters 3 to 5. Furthermore, for 80% power sample sizes of 33, 16, and 34 would have been required across chapters 3 to 5. Although lacking statistical power, there was sufficient power to detect condition*time interactions, and a lack of statistical power does not detract the clinical meaningfulness of these findings.

The findings of this thesis are applicable to an exercise model that falls within the ACSM guidelines for T1DM individuals. However, as shown by Rabasa-Lhoret et al. (2001), the required insulin reduction varies with intensity and duration of exercise. Therefore, subtle alterations to the exercise intensity and duration could result in altered insulin, carbohydrate and timing requirements. Moreover, the modality of the exercise also effects blood glucose responses (i.e. continuous vs. intermittent; Guelfi et al., 2005b) with differences in gluco-regulatory hormonal responses being responsible. Again, these differences in blood glucose responses may result in different insulin, carbohydrate and timing requirements.

6.7 Directions for future research

Future research should aim to address:

- Pre-exercise insulin dose in combination with the glycaemic index and amount of carbohydrate (i.e. the glycaemic load) consumed prior to exercise at various intensities and duration.

- With modern basal insulin analogues having peakless 24 hour profiles and exercise increasing insulin sensitivity, there is a need to examine the efficacy of reducing basal insulin dose. Specifically examining the impact of reducing basal insulin dose on glycaemia and ketogenesis before, during and for 24 hours after exercise.

- After exercise there is an increased uptake of blood glucose to replenish depleted muscle glycogen stores. Moreover, it has been suggested that hepatic glucose output is unable to meet the augmented uptake of glucose by the exercised musculature and this mismatch in glucose production and uptake contributes to the development of late-onset hypoglycaemia. Therefore, methods to preserve/replenish muscle and liver glycogen during and after exercise need to be examined.

7.0 References

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Appendices

Appendix A

A1 - Local Research Ethics Committee Approval

A2 - Chapter 3 participant information sheet

A3 - Chapter 4 participant information sheet

A4 - Chapter 5 participant information sheet

Appendix B

Participant response form

Swansea University
Prifysgol Abertawe

Subject Contact Form

Thank you for showing an interest in this project. We look forward to meeting and working with you. Please fill in the details below so that we may contact you to organise the testing schedule.

PLEASE FILL IN BLOCK CAPITALS

Your Name:
_____

Date of Birth: _____ Age: (years)____(months)_____

Contactable Address:
_____
_____

Home Phone_____

Work Phone _____

Mobile Phone _____

E-mail _____

What types of exercise do you typically perform? _____

How long do your exercise sessions typically last? _____

How long have you been in regular training? _____

What insulins are you using? _____

Are you able to jog for 45 minutes? _____

Appendix C

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire.

Name _____
Address _____
Phone number home _____ work _____
Emergency contact name _____ phone _____
Date of birth _____

History ***Assess your health needs by marking all true statements.***

You have had:
- ☐ a heart attack
- ☐ heart surgery
- ☐ cardiac catheterization
- ☐ coronary angioplasty (PTCA)
- ☐ pacemaker/implantable cardiac defibrillator/rhythm disturbance
- ☐ heart valve disease
- ☐ heart failure
- ☐ heart transplantation
- ☐ congenital heart disease

If you marked any of the statements in this section, consult you a health provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

Symptoms and other health issues:
- ☐ You experience chest discomfort with exertion.
- ☐ You experience unreasonable breathlessness.
- ☐ You experience dizziness, fainting, blackouts.
- ☐ You take heart medications.
- ☐ You take prescription medication(s).
- ☐ You have musculoskeletal problems.
- ☐ You have concerns about the safety of exercise.
- ☐ You are pregnant.

Cardiovascular risk factors
- ☐ You are a man older than 45 years.
- ☐ You are a woman older than 55 years or you have had a hysterectomy or you are postmenopausal.
- ☐ You smoke.
- ☐ Your blood pressure is greater than 140/90.
- ☐ You don't know your blood pressure.
- ☐ You take blood pressure medication.
- ☐ Your blood cholesterol level is >240 mg/dL.
- ☐ You don't know your cholesterol level.
- ☐ You have a blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
- ☐ You are diabetic or take medicine to control your blood sugar
- ☐ You are physically inactive (i.e., you get less than 30 minutes of physical activity on at least 3 days per week).
- ☐ You are more than 20 pounds overweight.

If you marked two or more of the statements in this section, you should consult your healthcare provider before engaging in exercise. You might benefit by using a faciltiy with professionally qualified exercise staff to guide your exercise program.

☐ **None of the above is true.**

You should be able to exercise safely without consulting your healthcare provider in almost any facility that meets your exercise programme needs.

Declaration:

Please sign below to confirm that you have answered questions honestly and to the best of your ability.

Signature _____ Date _____

Appendix D

Informed Consent for venesection

1. EXPLANATION OF VENESECTION BLOOD SAMPLING

Venesection includes the following venous blood sampling techniques: (i) venepuncture and (ii) cannulation. Following location of a suitable vein, the puncture site will be cleaned with an alcohol pad and allowed to dry. A tourniquet will be applied then the needle is used to enter the vein. During venepuncture the blood will be drawn directly into blood containers for future analysis. After blood collections have been completed the needle will be taken out of the vein and a dressing will be applied. During cannulation a thin flexible plastic tube will be advanced into the vein and this remains in the vein during the entire sampling period. Following each blood sample the cannula will be 'flushed' with saline. Following the period of blood collection the cannula will be removed from the vein and a dressing will be applied. After analysis the remaining blood will be disposed as clinical waste.

2. RISKS AND DISCOMFORTS

Even though the volume of blood taken is small (less than 30 ml per sample) there exists the possibility that during or immediately following this procedure you may feel light headed or faint. There is an extremely small risk that this procedure could result in an air or plastic embolism, but good practice minimises this risk. In addition, every effort will be made to minimise the risks of contaminating the wound by using sterile disposable equipment and standardised procedures for collection and disposal of biohazard wastes.

3. RESPONSIBILITIES OF THE PARTICIPANTS

Please advise a member of staff if you have experienced difficulties when blood has been taken in the past. You are responsible to fully disclose appropriate information when requested by those undertaking the assessment.

4. INQUIRIES

Any questions about the procedures used during the measurement process are encouraged. If you do have any questions, please ask for further explanations.

5. FREEDOM OF CONSENT

Your permission to perform this procedure is voluntary. You are free to deny consent or stop the assessment at any point.

I have read this form and I understand the procedures that will be perform. I consent to participate in this _____ procedure.

Questions:

Response:

Signature of subject

Signature of witness Date / /

Appendix E

Chapter 3 informed consent

Swansea University
Prifysgol Abertawe

Study Number: Patient Identification Number:

CONSENT FORM

Title of Project: 'The cardiorespiratory and metabolic effects of reductions in insulin dose prior to performing an acute bout of aerobic exercise'

Name of Researcher: Dr. Richard Bracken

		Please initial box:
1	I confirm that I have read and understand the information sheet dated..... for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
3	I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from Swansea University, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
4	I agree to take part in the above study	☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature
Researcher	Date	Signature

Appendix F

Chapter 4 informed consent

Swansea University
Prifysgol Abertawe

Study Number: Patient Identification Number:

CONSENT FORM

Title of Project: 'The cardiorespiratory and metabolic responses to aerobic exercise following alterations to the type of pre-exercise carbohydrate food'

Name of Researcher: Dr. Richard Bracken

		Please initial box:
1	I confirm that I have read and understand the information sheet dated..... for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
3	I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from Swansea University, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
4	I agree to take part in the above study	☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature
Researcher	Date	Signature

Appendix G

Chapter 5 informed consent

Swansea University
Prifysgol Abertawe

Study Number: Patient Identification Number:

CONSENT FORM

Title of Project: 'The cardiorespiratory and metabolic responses to alterations in the timing of insulin administration and carbohydrate feeding on the post-exercise glucose concentrations in type 1 diabetes patients'

Name of Researcher: Dr. Richard Bracken

		Please initial box:
1	I confirm that I have read and understand the information sheet dated..... for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
3	I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from Swansea University, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
4	I agree to my GP being informed of my participation in the study.	☐
5	I agree to take part in the above study	☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature
Researcher	Date	Signature

Appendix H

Anti-Oxidant

For chapters 3 to 5, 30 ml of anti-oxidant was required for the preservation of plasma adrenaline and noradrenaline.

1.83 g of glutathione, 2.28 g of EGTA and 20 ml of distilled water were added to a beaker and left to mix on an unheated stirrer (FB70806 Fisherbrand unheated stirrer, Fisher Scientific, UK). Once fully dissolved a pH meter (InoLab pH 720, WTW, GmbH, Germany) was placed within the beaker and either 0.5 - 1 ml of HCl or NaCl was added at a time until a pH of 7.0 was achieved. Once pH was stable at 7.0, the 30 ml volume was attained through the addition of distilled water.

e.g. 1.83 g glutathione + 2.28 g EGTA
20 ml of distilled H_20
3 ml NaCl
0.5 ml HCl
6.5 ml distilled H_20

Appendix I

GEM 3000: Overview of Operation

The GEM 3000 central component is a sensor card which provides a low volume, gas tight chamber in which the blood sample is presented to the sensors. Sensors specific for the analysis of pH, Hct, Glucose and Lactate, in addition to a reference electrode, are key parts of the chamber, with chemically sensitive membranes permanently attached to the chamber body (Figure below). When the cartridge is installed, the chamber is maintained at 37 ± 0.3 in a thermal block, and provides the electrical interface to the sensors.

Calibration and Control Checks

Within the cartridge are two solutions, A and B. These solutions purpose is for calibration and/or internal process control checks. The A and B solutions provide high and low concentrations for all parameters, except Hct, which calibrates at one level using the B solution. Prior to calibration, the A and B solutions are read as unknown solutions, and these values are recorded in the instruments database. During calibration, these values are adjusted for any slope or drift that may occur over time.

The third solution, C, is used to calibrate the low oxygen level. The C solution is also used for conditioning the glucose and lactate sensors, removing micro clots and cleaning the sample path (below).

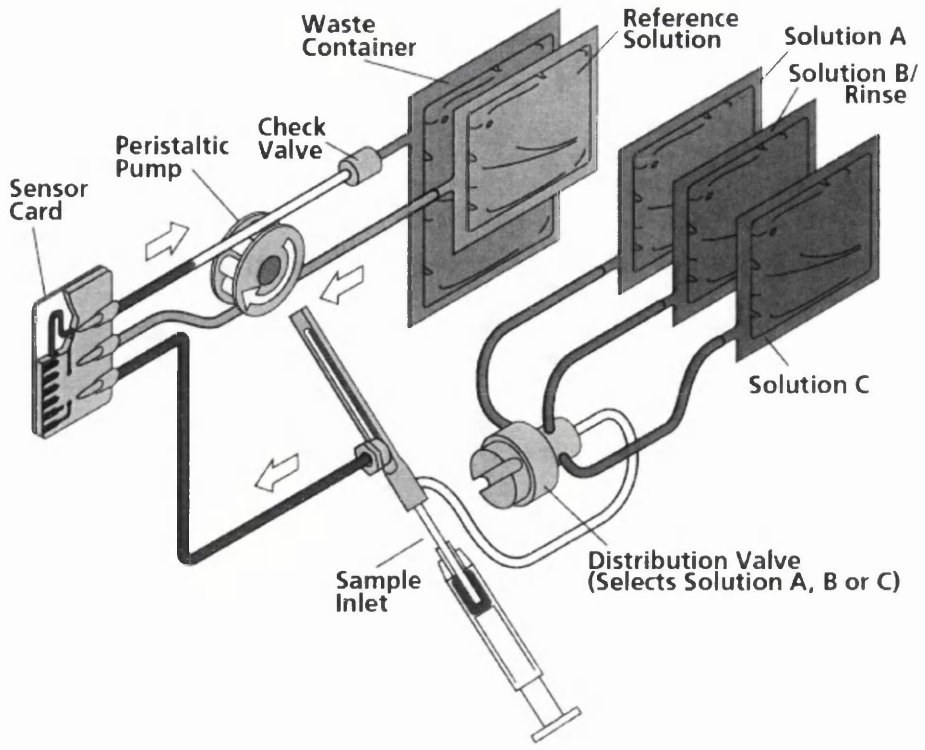

Figure: Internal components of the GEM 3000.

Electrochemical sensors: pH

The pH sensors are all based on the principle of ion-selective electrodes, where an electrical potential can be established across a membrane which is selectively permeable to a specific ion. The potential is described by the Nernst equation:

$$E = E' + (S \times \text{Log } C)$$

E = Electrode potential, E' is the standard potential for the membrane, S is the sensitivity (slope), and C is the ion activity. E' and S can be determined by the sensor responses to the calibration solutions, and the equation can be solved for the activity of the ion of interest. For pH, Log C is replaced by pH. The pH sensors are polyvinyl chloride (PVC) based on ion selective electrodes, consisting of an internal silver chloride (AgCl) reference electrode and an internal salt layer. Their potentials are measured against the card reference electrode.

Appendix J

Calculation of Plasma Volume Shifts

Plasma volume shifts were calculated via the method of Dill and Costill (1974), described below.

Where BV = blood volume, CV = cell volume, PV = plasma volume. $BV_{pre} = 100$

1) $BV_{pre} = BV_{post} \times (Hb_{post} / Hb_{post})$

2) $CV_{pre} = BV_{pre} X (Hct_{pre})$

3) $PV_{pre} = BV_{pre} - CV_{pre}$

Calculations of % changes in BV, CV and PV.

1) $\Delta BV\% = 100 * (BV_{post} - BV_{pre}) / BV_{pre}$

2) $\Delta CV\% = 100 * (CV_{post} - CV_{pre}) / CV_{pre}$

3) $\Delta PV\% = 100 * (PV_{post} - PV_{pre}) / PV_{pre}$

Table 2.8: Percentage changes in plasma volume, pre to post-exercise, across chapters 3 to 5.

Chapter 3		Chapter 4		Chapter 5	
Trial	ΔPV%	Trial	ΔPV%	Trial	ΔPV%
Full	-6.7 ± 0.9	**LGI**	-8.0 ± 1.4	**120min**	6.5 ± 1.3
75%	-3.8 ± 1.4	**HGI**	-7.6 ± 1.2	**90min**	6.2 ± 2.2
50%	-3.5 ± 1.2			**60min**	4.0 ± 1.3
25%	-2.8 ± 1.0			**30min**	2.1 ± 0.7

Data presented as Mean ± SEM

Appendix K

Insulin and Exercise Regimen Questions

Name: [redacted] ***Date 07/04/08***

Age: [redacted]

What insulin(s) are you currently taking?
Brand names

Novorapid

Lantus

What typical doses do you usually administer?

Basal and short/rapid
Basal (before bed): 14 units

Rapid: Breakfast: 10 units

Lunch: 4-6 units

Dinner: 4-6 units

What time of day do you usually administer them?

Morning, midday, afternoon, prior to bed

See above!

How do you take them?

Insulin Pen, Type brand names etc

Novorapid Flexpen

Lantus Optiset

How long have you been on this regimen? 8 years

What regimen were you previously on?
Reasons for change

Novo Nordisk Penmix 30/70 for 9 years. Current regime was a little more flexible for my lifestyle.

How often do you exercise a week?

6 – 7 times a week for 1 -2 hours

What do you do with regards to insulin & carbohydrate before you exercise?

Food and drink consumed? How much?

I exercise at least 3 hours after last injection of rapid insulin (so that circulating insulin levels are lower) and prior to a main meal so that I can inject insulin soon after I have finished exercising in order to help with muscle glycogen repletion (to hopefully stop later onset hypos).

For prolonged high intensity exercise I usually take about 60g carbs (Lucozade Energy) prior to exercise which will last 1 – 1 ½ hours. I sip a sports drink (Lucozade Sport) during exercise giving me about another 15g of carbs.

For anaerobic exercise (i.e. weight lifting) with only a low intensity aerobic element a sports drink (about 30g carbs) will be enough assuming blood sugars are okay to begin with.
For steady state exercise (30-60 min) I usually eat a large portion of fruit and some weatabix to give me a slower release of carbohydrates and so that my sugars don't get too high,

Have you ever experienced a hypo during or after exercise?

How long after exercise did it occur?
I quite often experience nocturnal hypos which usually occur about 8 hours after high intensity exercise.

What did you eat or drink to clear it?

I usually drink lucozade energy – about 60g carbs.

Do you consume caffeine? E.g. coffee, Red Bull

How much/often?

I don't consume caffeine.

How does it affect your blood glucose and insulin regimen?

n/a

When was the last time your regimen was dramatically upset?

Date, Why?

Last week – blood sugars very high then very low for 24 hours. Possible over-correction of nocturnal hypo forcing sugars up in the morning. Extra insulin taken to combat high sugars which, when combined with exercise, lowers sugars dramatically etc. Often a weekly occurrence.

Appendix L

NAME: **DATE:** **TRIAL:**

Time	Blood Glucose	Food/Drink	Insulin & Dose	Comments
MORNING – B4 Breakfast				

Appendix M

NAME: Joe Bloggs **DATE**: 03.10.09 **TRIAL:** 1

Time	Blood Glucose	Food/Drink	Insulin & Dose	Comments
1:00pm	7.4	--		--
2:00pm	--	Chicken and pasta	NovoRapid 8 U	2 medium chicken breasts with 300 g of pasta
3:00pm	7.4	--	--	--
5:00pm	7.2	--	--	--
6:30pm		1 Jacket Potato, Salad, Orange Juice	NovoRapid 8 U	Medium sized potato, Small glass of Orange Juice, small mixed salad
7:00pm	9.0			
9:00pm	7.8			
11:00pm	7.2			
11:00pm	--	2 slices of toast	NovoRapid 6 U	Medium slice, white bread, with butter and jam
MORNING B4 BREAKFAST	8.2		Lantus 24 U	--