STRATEGIES TO MANAGE POST-EXERCISE GLYCAEMIA IN TYPE 1 DIABETES

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STRATEGIES TO MANAGE POST-EXERCISE GLYCAEMIA IN TYPE 1 DIABETES

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"…view the lengthening odds with disdain and bloody well get on with it. You know it's not easy, but it's a challenge. Those that win for a living like a challenge."

Sir Steve Redgrave CBE, 5 Time Consecutive Olympic Gold Medallist, Diabetic.

ABSTRACT

For patients with type 1 diabetes, a fear of hypoglycaemia and a concern over a loss of control with wider diabetes management are the most salient barriers to exercise participation and adherence. A large proportion of patients report a lack of advice for preventing post-exercise hypoglycaemia, and many feel largely uninformed about insulin administration and carbohydrate intake around aerobic-based exercise. Presently, recommendations within the literature are based predominantly on anecdotal and observational, but not empirical or interventional data. Therefore, this thesis aimed to develop a strategy that enables patients to effectively self-manage glycaemia following exercise, supported by evidence pertaining to the deeper physiological implications and consequences.

Study one (chapter 3) revealed that under conditions of reduced pre-exercise rapid-acting insulin dose, it is also necessary to reduce post-exercise rapid-acting insulin administration by 50% to prevent early-onset hypoglycaemia (≤ 8 hours post-exercise). Consequently, some patients experienced post-prandial hyperglycaemia with this intervention, although this was not associated with any other metabolic, counter-regulatory hormonal, or inflammatory disturbances. The results of study two (chapter 4) demonstrate that post-exercise meal composition, under conditions of reduced pre- and post-exercise rapid-acting insulin dose, carry important implications for post-prandial glycaemia. Specifically, consumption of low GI post-exercise carbohydrates normalise post-prandial hyperglycaemia, whilst protection from early onset hypoglycaemia is maintained. In addition, post-exercise meal composition heavily influences inflammatory markers; a high GI meal results in a pronounced inflammatory response, but a low GI meal completely prevented any rise in measured inflammatory markers. Lastly, study three (chapter 5) assessed the efficacy of a combined basal-bolus insulin reduction and low GI carbohydrate post-exercise feeding strategy. A 20% reduction in basal insulin provided full protection from hypoglycaemia for a total of 24 hours after exercise. Furthermore, ketonaemia did not increase to clinically meaningful levels, nor did inflammatory

markers rise above concentrations seen at rest or when exercising under usual basal dose. No other metabolic or counter-regulatory hormonal disturbances were observed following a combined dose reduction to basal-bolus insulin and low GI carbohydrate post-exercise feeding.

Collectively, this thesis has shown that acute prandial adjustments in rapid-acting insulin and carbohydrate feeding, in combination with alterations in basal dose, are effective for managing post-exercise glycaemia and protecting patients from hypoglycaemia for a total of 24 hours after exercise. Moreover, this strategy aims to maintain euglycaemia by reducing post-prandial hyperglycaemia. This is not associated with clinically significant rises in ketonaemia, nor does it induce inflammatory, counter-regulatory hormonal, or other metabolic disturbances. Clinicians are advised to tailor these recommendations to a patient's individual exercise preferences, fitness and exercise ability, level of diabetes management, and treatment regimen.

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List of abbreviations

Abbreviation	Unabridged
ANOVA	Analysis of variance
AUC	Area under the curve
BG	Blood glucose
β-OH	β -hydroxybutyrate
BM	Body mass
BMI	Body mass index
BPM	Beats per minute
CGM	Continuous glucose monitor
GI	Glycaemic index
GLP-1	Glucagon-like peptide 1
Hb	Haemoglobin
HbA_{1c}	Glycosylated haemoglobin
Hct	Haematocrit
HGI	High glycaemic index
HR	Heart rate
IL-6	Interleukin-6
IU	International unit
LGI	Low glycaemic index
NEFA	Non-Esterified Fatty Acid
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
TNF-α	Tumour necrosis factor alpha
VAS	Visual analogue scale

Publications and conference proceedings arising from this thesis

Academic peer-reviewed journal manuscripts

Campbell MD, Walker M, Trenell MI, Stevenson EJ, Turner D, Bracken RM, Shaw JA and West DJ (2014). "*A low glycemic index meal and bedtime snack prevents postprandial hyperglycemia and associated rises in inflammatory markers, providing protection from early but not late nocturnal, hypoglycemia following evening exercise in type 1 diabetes patients"*. Diabetes Care **37**(7): 1845-53. DOI: 10.2337/dc14-0186.

Campbell MD, Walker M, Trenell MI, Luzio SC, Dunseath G, Tuner D, Bracken RM, Bain SC, Russell M, Stevenson EJ and West DJ (2014). "*Metabolic implications when employing heavy pre- and post-exercise rapid-acting insulin reductions to prevent hypoglycaemia in type 1 diabetes patients: A randomised clinical trial"*. PLoS ONE **9**(5):e97143. DOI: 10.1371/journal.pone.0097143.

Campbell MD, Walker M, Trenell MI, Jakovljevic DG, Stevenson EJ, Bracken RM, Bain SC and West DJ (2013). "*Large pre-and postexercise rapid-acting insulin reductions preserves glycemia and prevents early- but not late-onset hypoglycemia in patients with type 1 diabetes"*. Diabetes Care **36**(8): 2217-2224. DOI: 10.2337/dc12-2467.

Conference proceedings

Campbell MD, Walker M, Stevenson EJ, Cassidy S, Turner D, Bracken RM, Shaw JA and West DJ (2014). "*Low GI meals minimise post-prandial hyperglycaemia whilst protecting from early onset hypoglycaemia following evening exercise in T1DM"*. Proceedings of The European College of Sport Science Annual Conference, Amsterdam The Netherlands, 2-5 July 2014.

West DJ, Walker M, Stevenson EJ, Cassidy S, Gonzalez, JT, Turner D, Bracken RM, Shaw JA and **Campbell MD** (2014). "*Avoidance of post-prandial hyperglycaemia and associated rises*

in inflammatory markers with a low GI post-exercise meal in T1DM". Proceedings of The European College of Sport Science Annual Conference, Amsterdam The Netherlands, 2-5 July 2014.

Campbell MD, Walker M, Trenell MI, Stevenson EJ, Luzio SC, Dunseath G, Bain SC, Bracken RM, Turner D and West DJ (2014). "*Heavily reducing pre- and post-exercise rapid-acting insulin dose may cause hyperglycaemia, but does not augment ketonaemia or increase inflammatory cytokines in type 1 diabetes patients"*. Diabetic Medicine **31**(Suppl.1): 69. DOI: 10.1111/dme.12378_1. Proceedings of Diabetes UK Annual Professional Conference, Manchester UK, 5-7 March 2014.

Campbell MD, Walker M, Trenell MI, Luzio S, Dunseath G, Stevenson EJ, Bracken RM, Tuner D and West DJ (2013). "*Preventing hypoglycaemia by heavily reducing pre- and post-exercise rapid-acting insulin dose may cause hyperglycaemia, but not hyperketonaemia in type 1 diabetes patients"*. Diabetologia **56**(Suppl.1): 277. DOI: 10.1007/s00125-013-3012-z. Proceedings of The European Associate for the Study of Diabetes Annual Congress, Barcelona Spain, 23-27 September 2013.

Campbell MD, Walker M, Trenell MI, Stevenson EJ, Jakovljevic DG, Bracken RM, Bain SC, Turner D and West DJ (2013). "*The preservation of glycaemia through large pre- and post-exercise rapid-acting insulin reductions prevents early, but not late-onset hypoglycaemia in individuals with type 1 diabetes"*. Diabetic Medicine **30**(Suppl.1): 9. DOI: 10.1111/dme.12090_6. Proceedings of Diabetes UK Annual Professional Conference, Glasgow UK, 13-15 March 2013.

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Any ethical clearance from the research presented in this thesis has been approved. Approval has been sought and granted by local NHS Research Ethics Committee on 30th December 2011, 1st February 2013.

I declare that the word count of this thesis is 42,036.

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Date: 14/07/2014

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.0 Introduction

Somewhat simplistically, blood glucose concentrations are a function of the rate at which glucose enters the circulation (glucose appearance) and the rate at which glucose is removed from the circulation (glucose disappearance). If the rate of glucose disappearance exceeds the rate at which glucose appears, blood glucose concentrations will fall, and hypoglycaemia (blood glucose concentration <3.9 mmol.l^{-1}) will ensue (Cryer 1997). Although blood glucose naturally fluctuates during the day, concentrations are maintained within tight physiological limits (3.9-7.9 mmol.l^{-1}), achieved through the regulation of insulin release from the pancreatic β-cells. Defects in insulin secretion, insulin action, or both, consequently result in blood glucose excursions outside of these physiological ranges. The clinical classification of this is Diabetes Mellitus, a heterogeneous group of diseases with the common feature of glycaemic dysregulation. Whereas Type 2 is generally characterised by insulin resistance, Type 1 diabetes results from an absolute deficiency of insulin secretion due to an autoimmune destruction of the insulin secreting pancreatic β-cells. This is, at present, irreversible, and means that patients are ultimately dependent upon exogenous insulin replacement.

Adjunct to conventional insulin therapy, exercise may also be an important component of a patient's therapeutic regimen (Lehmann *et al.* 1997, Wiesinger *et al.* 2001, Stettler *et al.* 2006, Rachmiel *et al.* 2007, Gulve 2008, Maahs *et al.* 2009), providing numerous physiological benefits (Wasserman and Zinman 1994, Choi and Chisholm 1996, Praet *et al.* 2006, Manders *et al.* 2010, Maarbjerg *et al.* 2011, Van Dijk *et al.* 2012) associated with preventing and regressing diabetes related complications (Wasserman and Zinman 1994, Kulenovic *et al.* 2006, Fowler 2008). In spite of these benefits, exercise induces vast metabolic disturbances, often predisposing patients to hypoglycaemia (blood glucose ≤3.9 mmol.l^{-1}) for as long as 24 hours after exercise (Macdonald 1987, Steppel and Horton 2003, Tsalikian *et al.* 2005), and particularly at night (Mcmahon *et al.* 2007). Compared to their type 2 counterparts, patients with type 1 diabetes often want to exercise, but unfortunately have the highest risk of developing hypoglycaemia. Presently, incorporating exercise into the lives of type 1 diabetes

patients is confounded by a lack of evidence-based recommendations for self-managing post-exercise glycaemia. In light of this, there is a need to develop comprehensive strategies which enable type 1 diabetes patients to effectively manage glycaemia after exercise.

1.1 Type 1 diabetes mellitus

The immune system is equipped with T cells (or T lymphocytes), which, under normal circumstances play an integral role in cell-mediated immunity by controlling or eliminating autoantigens (Van Parijs and Abbas 1998, Morran *et al.* 2008). In type 1 diabetes, self-reactive T cells, specifically $CD4^+$ and $CD8^+$ T cells (Knip and Siljander 2008), destroy the insulin secreting pancreatic β-cells, whereby the immune system's ability to recognise self-pancreatic tissue is lost (Morran *et al.* 2008). The T cells expand and migrate from local draining lymph nodes and infiltrate the islets (Figure 1.0 A-C) causing chronic inflammation (insulitis), followed by the gradual deterioration of the β-cells and a reduction in insulin content (Figure 1.0 B) (Foulis *et al.* 1991). As the disease progresses the immunoreactivity spreads, such that by the time that symptoms present and diagnosis is made, 90-95% of β-cells have been destroyed (Daaboul and Schatz 2003, Atkinson 2005) (Figure 1.0 C). At this point endogenous insulin production is severely diminished or completely undetectable (Figure 1.0 C) (Daaboul and Schatz 2003). Patients are now in metabolic dysregualtion and require exogenous insulin therapy to control blood glucose concentrations.

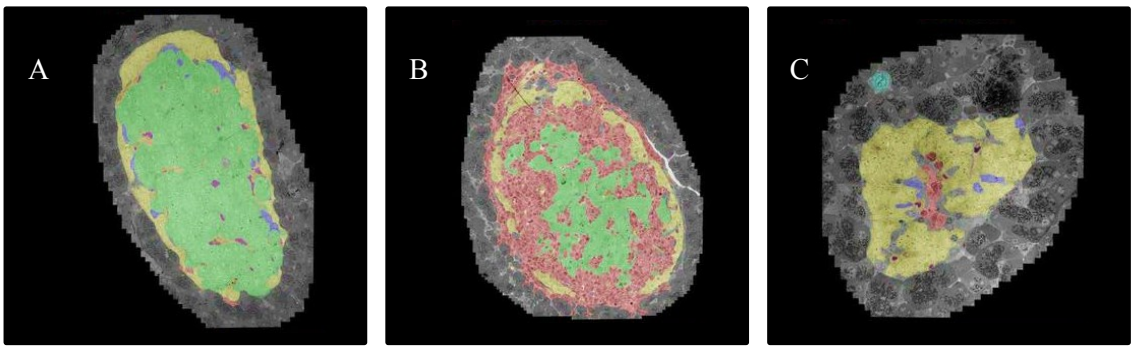

Figure 1.0 A-C. Islet of Langerhans during type 1 diabetes progression. **A** depicts healthy β-cells with abundant insulin content (β-cells, green) with α-cells (yellow), purple (ducts) and cyan (vasculature). **B** depict healthy β-cells as well as exhausted β-cells with empty granules, infiltration of leukocytes and β-cell phagocytosis. **C** end stage diabetes consists primarily of α-cells, with reduced leukocyte count. Images reproduced with permission from Ravelli (2013).

1.1.1 Aetiology of type 1 diabetes mellitus

Type 1 diabetes is a complex disease in which there is a strong genetic component, but also non-genetic factors including environmental exposure and stochastic events play part (Knip and Åkerblom 2009). To an extent, genetic risk can be estimated from family history and the presence of particular alleles of genes, so much so that the disease is partially predictable in genetically susceptible individuals more than any other common autoimmune disease (Anaya *et al.* 2006). Present in most patients are two characteristics, namely at least one susceptible human leukocyte antigen (HLA) class II haplotype (Ziegler and Nepom 2010), and islet autoantibodies (Ziegler *et al.* 1999). Indeed, the vast majority of genetic susceptibility is accounted for by loci within the HLA, however meta-analyses and genone-wide association studies indicate that over 40 HLA and non-HLA loci are involved (Ziegler and Nepom 2010). More than 85% of newly diagnosed patients have islet autoantibodies (Van Belle *et al.* 2011), the development of which is sequential (Ziegler *et al.* 1999) and arguably the most important change in the risk status of type 1 diabetes. Importantly, the appearance of autoantibodies at an early stage is positively related to the rate of disease progression (Hummel *et al.* 2004) (Figure 1.1), with a high risk of developing autoantibodies early in life (Bingley 1996). Interestingly, autoantibodies are unlikely to present in individuals younger than 6 months, and even in those with familial autoantibody appearance, concentrations do not peak until 18 months (Naserke *et al.* 1999, Hummel *et al.* 2004, Achenbach *et al.* 2006) suggesting that islet autoimmunity is triggered by events after birth and therefore influenced by environmental exposure.

The role of environmental exposure in the pathogenesis of type 1 diabetes is further supported by the rate at which the incidence of the disease is increasing, a rate at which genetic change alone cannot be solely accountable (Ma and Chan 2009, Patterson *et al.* 2009, Patterson *et al.* 2012, Roche *et al.* 2013). Within the literature, there are a number of environmental factors believed to be involved which could act *in utero* and thereafter, including: dietary factors (consumption of cereal proteins and cow milk proteins, low vitamin D and zinc) (Knip *et al.* 2010, Marjamäki *et al.* 2010, Samuelsson *et al.* 2011, Virtanen *et al.* 2012, Chiu and Beyan

2013, Atkinson *et al.* 2014), enteroviruses (Tracy *et al.* 2010, Atkinson *et al.* 2014, Dunne *et al.* 2014), changes in the composition of gut microbiota (Atkinson and Chervonsky 2012), seasonal changes (Svensson *et al.* 2009, Haynes *et al.* 2012; Dunne *et al.* 2014), the "hygiene hypothesis" (Okada *et al.* 2010, Virk *et al.* 2010), and even traumatic life events.

1.1.2 Clinical presentation of type 1 diabetes mellitus

Commonly, the disease occurs during childhood or adolescence and was therefore aptly named "*juvenile diabetes*"; it can in fact occur at any age. This is likely due to the variability in the progression of the disease, which is dependent upon the rate of β-cell deterioration (Von Herrath *et al.* 2007, American Diabetes Association 2011) although this tends to occur more rapidly in younger individuals. The pre-clinical phase, in which the disease starts and matures, typically occurs over a period of at least 2 years (Thrower and Bingley 2011). During this time there is gradual decline in stimulated C-peptide levels (a hallmark of endogenous insulin production) and a deterioration in glucose tolerance (Thrower and Bingley 2011), although patients are largely asymptomatic (Sosenko *et al.* 2006). Indeed, diagnosis is typically made when symptoms surface (hyperglycaemia and / or ketoacidosis) (Sosenko *et al.* 2006) at which point 90-95% of β-cells have been destroyed (Daaboul and Schatz 2003, Atkinson 2005). Although some residual β-cell function may be retained following diagnosis, and some insulin secretion may persist even in long-standing type 1 diabetes (Oram *et al.* 2014), usually ~99% of cells have been destroyed after a further 2 years (Meier *et al.* 2005). Ultimately, patients are dependent upon daily exogenous insulin administration and remain bound to this for the remainder of their lives.

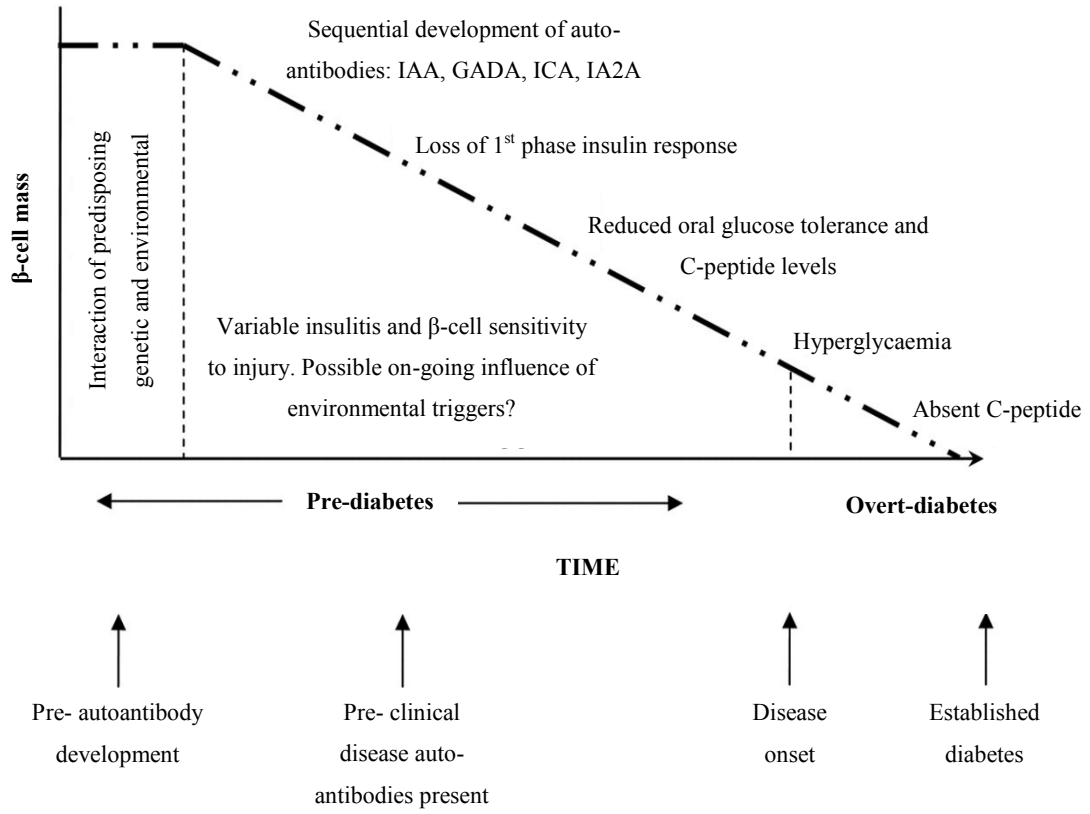

Figure 1.1. Clinical presentation of the development and progression of type 1 diabetes. Model adapted from Thrower and Bingley (2011).

1.1.3 Treatment of type 1 diabetes mellitus with exogenous insulin therapy

The purpose of insulin therapy is to mimic normal physiological insulin secretion by providing a basal insulin replacement together with insulin boluses to control post-prandial glucose excursions. Amino acid modification to the structure of the insulin molecule produces alterations in its biological properties (Drejer *et al.* 1991) which affect its absorption from the site of injection (Dimarchi *et al.* 1994, Lepore *et al.* 2000). Modern insulin analogues with improved pharmacodynamic and pharmacokinetic properties have allowed for an intensification in insulin therapy in recent years, with insulin regimens closer emulating physiological action-time profiles with lower within-subject variability (Heise *et al.* 2004), fewer glycaemic fluctuations (Gerich *et al.* 2006), and improved glycated haemoglobin (HbA_{1c}; gold standard marker of diabetes control) (Siebenhofer *et al.* 2006) than longer

established exogenous insulins (Ratner *et al.* 2000, Vague *et al.* 2003, Ashwell *et al.* 2006, Kølendorf *et al.* 2006).

In the UK, patients are predominantly treated with a basal-bolus regimen, injecting two types of insulin analogues (Mcintyre *et al.* 2010). The basal replacement aims to provide a continuous, reproducible and stable supply of insulin into the circulation to suppress excessive post-absorptive hepatic glucose production (Vajo and Duckworth 2000) and prevent excessive lipolysis (Rahn *et al.* 1994) and ketogenesis (Laffel 2000). This is achieved by injecting insulin, such as insulin *Glargine* (Lantus, Sanofi-Aventis), or *Detemir* (Levemir, Novo Nordisk), which is slowly absorbed from subcutaneous tissue and has a protracted action time-course. Although Glargine and Detemir are different chemical and structural entities (Bolli *et al.* 1999), a single dose carries similar metabolic effects over the initial 12 hours following administration (Porcellati *et al.* 2007), reaching a metabolic plateau 3-6 hours after injection (Heinemann *et al.* 2000, Lepore *et al.* 2000, Rave *et al.* 2003, Klein *et al.* 2007).

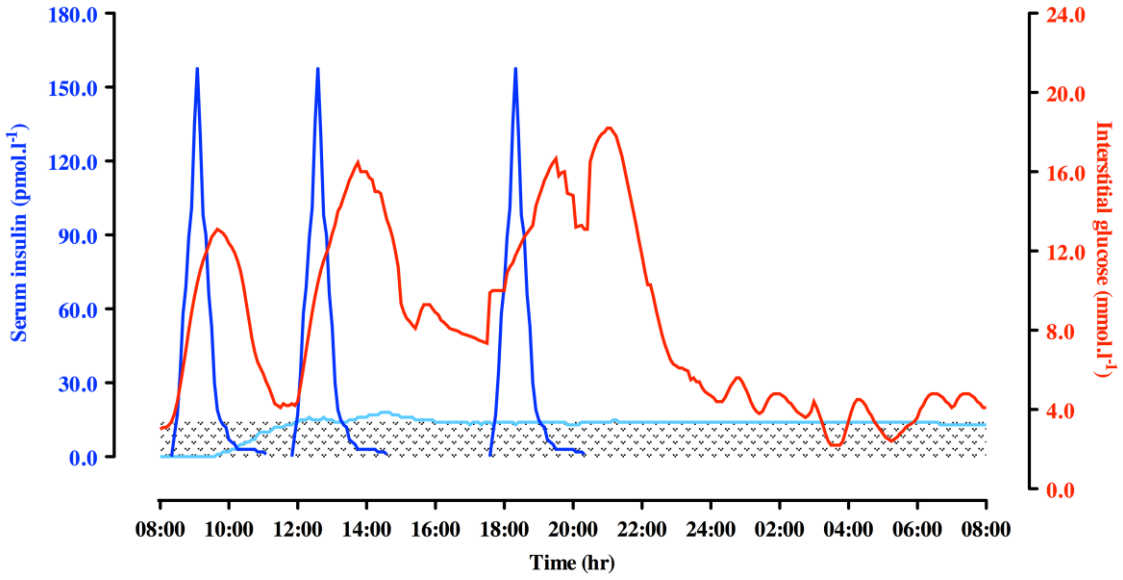

Figure 1.2. Representation of acute glycaemic control in a type 1 diabetes patient under treatment with a basal-bolus regimen. Light blue trace = basal insulin, dark blue trace = bolus insulin, red trace = glycaemia (interstitial glucose). CGM trace taken from an individual with type 1 diabetes during data collection.

Beyond this time, pharmacodynamic and pharmacokinetic properties differ between these two long-acting insulins, with Glargine remaining in a steady state activity close to 100% for 24

hours, and possibly longer (Porcellati *et al.* 2007), whereas Detemir exhibits a progressive decrease in activity to ~55% by 24 hours post-administration (Porcellati *et al.* 2007). Therefore, basal dose and timing is an important clinical consideration for patients.

Prandial insulin requirements are provided by injecting a bolus of a readily absorbed, rapid-acting insulin analogue (insulin *Lispro* or *Aspart*) that controls post-prandial glucose excursions. However, maintaining insulin levels at a concentration that keeps blood glucose close to euglycaemia without necessarily increasing the risk of hypoglycaemia is the most challenging aspect of insulin therapy. Bolus insulin titration is based on estimation of carbohydrate amount (DAFNE Study Group 2002; Mcintyre *et al.* 2010) with patients using an individualised carbohydrate-to-insulin ratio to determine meal-time insulin doses. However, this method does not take into consideration the *composition* of carbohydrate. As such, there is often a mismatch between the absorption of bolus insulin and the appearance of carbohydrate into the circulation. Therefore, patients still struggle to adequately control blood glucose and the risk of hypoglycaemia remains, despite the intensification of insulin therapy.

1.1.4 Epidemiology of type 1 diabetes mellitus

Today, an estimated 366 million people worldwide are affected by Diabetes (Diabetes UK 2012). Of those, ~40 million (10% to 20%) of patients have type 1 diabetes (Rewers 2012). With the prevalence in the UK expected to rise up to 1 million by 2015 (Quality of Outcomes Framework 2011). Type 1 diabetes is associated with higher relative morbidity and mortality rates and health care costs than type 2 diabetes. At present, it is estimated that diabetes care alone costs the National Health Service (NHS) an estimated £25 million per day, which equates to £1 million per hour, or £286 per minute. This excludes social service costs such as residential home help services and nursing care. Clearly, diabetes is a huge burden upon public spending.

1.1.5 Complications arising from type 1 diabetes mellitus: implications for glycaemic control

Diabetes is the fifth most common cause of mortality in the world (Roglic and Unwin 2010) accounting for an estimated 15% of all deaths occurring in England (National Diabetes Audit 2011). Life expectancy is reduced by more than 20 years in individuals with type 1 diabetes (Diabetes UK 2012), and patients are exposed to a host of co-morbidities. For example, within 20 years of diagnosis, almost all patients with type 1 diabetes have a degree of retinopathy, over 44% of diabetics die from cardiovascular disease, and 21% from renal disease (Diabetes UK 2012). Approximately 50% of patients have neuropathy, with one in twenty developing foot ulcers, of which one in ten ulcers require amputation of the foot or leg (Diabetes UK 2012); up to 70% of these patients die within five years as a result of diabetes (Bate and Jerums 2003). In addition, patients are susceptible to other autoimmune disorders such as Graves' disease, Hashimoto's thryoditis, and Addison's disease (Anaya *et al.* 2006), and depression and sexual dysfunction are twice as high in this population (Tagliabue *et al.* 2011, Morgan *et al.* 2014).

1.2 Exercise and type 1 diabetes

The American Diabetes Association (ADA) and Diabetes UK encourage patients to engage in physical activity of all levels, including leisure activities, recreational exercise and competitive sports. General guidelines advocate patients perform ~150 minutes per week of moderate intensity aerobic exercise, 90 minutes of vigorous aerobic exercise, or a combination of the two (Thompson *et al.* 2009). This roughly translates to 20-45 minutes of moderate-to-high intensity aerobic exercise 5-7 days per week (Thompson *et al.* 2009).

1.2.1 Benefits of exercise to type 1 diabetes patients

Regular exercise plays an important role in maintaining a "*healthy*" lifestyle as well as preventing and treating diseases. Indeed, there is a strong negative correlation between exercise and the risk of disease and premature death in both healthy individuals (Warburton *et*

al. 2006), and those with type 1 diabetes (Moy *et al.* 1993). This is because exercise is strongly associated with improvements in a range of health outcomes; to name but a few, exercise results in increased levels of aerobic fitness (Komatsu *et al.* 2005), increased lean mass (Wasserman and Zinman 1994, Choi and Chisholm 1996), reductions in blood pressure (Whelton *et al.* 2002), improved autonomic tone and augmented cardiac function (Davison *et al.* 2002, Petersen and Pedersen 2005), reduced blood coagulation (Kupchak *et al.* 2013), improved coronary blood flow (Sonnenschein *et al.* 2011), improvements in lipid profiles (Kelley *et al.* 2012), enhanced endothelial function (Sonnenschein *et al.* 2011, Dubé *et al.* 2012), and reduced systemic inflammation (Loimaala *et al.* 2003, Lucini *et al.* 2012). Potentially, these benefits may be of great importance to those with type 1 diabetes as macrovascular (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy) are a major cause of morbidity and mortality (Wasserman and Zinman 1994, Kulenovic *et al.* 2006, Fowler 2008). In addition, exercise has profound effects on insulin sensitivity and glucose metabolism (Praet *et al.* 2006, Manders *et al.* 2010, Van Dijk *et al.* 2012), with studies indicating less daily insulin requirements and a need to reduce the insulin-to-carbohydrate ratio (Ebeling *et al.* 1995, Fuchsjäger-Mayrl *et al.* 2002, Sideravičiūtė *et al.* 2006). Thus, exercise has the capacity to improve quality of life in patients, and potentially aid in diabetes management.

1.2.2 Exercise and glycaemic control

Unfortunately, the benefits of exercise on long-term glycaemic control are less clear. Two recent meta-analyses provide conflicting conclusions, either a beneficial effect (Tonoli *et al.* 2012), or no effect at all (Kennedy *et al.* 2013) (when measured by absolute change in HbA_{1c} values). In addition, a number of stand-alone studies demonstrate worsening glycaemic control with exercise training (Huttunen *et al.* 1989, Ebeling *et al.* 1995, Ramalho *et al.* 2006). This may, at least in part, be explained by variation in the exercise intervention employed in studies. Indeed, mixed study methodologies (type of exercise, diet, insulin administration, length of

intervention, patient characteristics) hampers the ability to draw conclusions from this body of evidence. However, when data are pooled, there is a trend, albeit not statistically significant, for a reduction in HbA_{1c} (%HbA_{1c}: ↓ 0.3, 95%CI = -0.59-0.09, p =0.144) when exercise of an aerobic nature is performed, and interventions are carried out over longer periods of time (Tonoli *et al.* 2012, Kennedy *et al.* 2013).

The majority of these studies report an increase in calorie intake. Although this may be due to changes in appetite (Dubé *et al.* 2014), most of the authors report additional carbohydrate consumption to avoid post-exercise hypoglycaemia (for review see Kennedy, Nirantharakumar *et al*. (2013)). This is unsurprising considering patients with type 1 diabetes experience severe blood glucose variability around the time of exercise (Gordin *et al.* 2008, Kapitza *et al.* 2010). Glycaemic variability occurs because of an inability to manage hypoglycaemia, a condition which is now considered an independent risk factor for diabetes related complications (Jaiswal *et al.* 2012). If exercise-induced hypoglycaemia can be managed, then euglycaemia would be more easily achievable, and patients would have near normal HbA_{1c} (Cryer 2010).

However, HbA_{1c} may not be the most appropriate marker of glycaemic control, especially in patients who lead an active lifestyle. HbA_{1c} is the gold standard for assessing long-term glycaemic control; the method uses the enzymatic glycation pathway by which circulating blood glucose is bound to haemoglobin. Erythrocytes, which are rich in haemoglobin, are present in the blood for ~100 days (Shemin and Rittenberg 1946), therefore the amount of glucose bound to haemoglobin offers a good clinical bio-marker of the average blood glucose concentration over a ~3 month period. However, this measure does not reflect daily glycaemic variability (Figure 1.3), specifically the severity of glycaemic excursions (Nalysnyk *et al.* 2010). This has prompted the use of a range of glycaemic variability indices (Rodbard 2009).

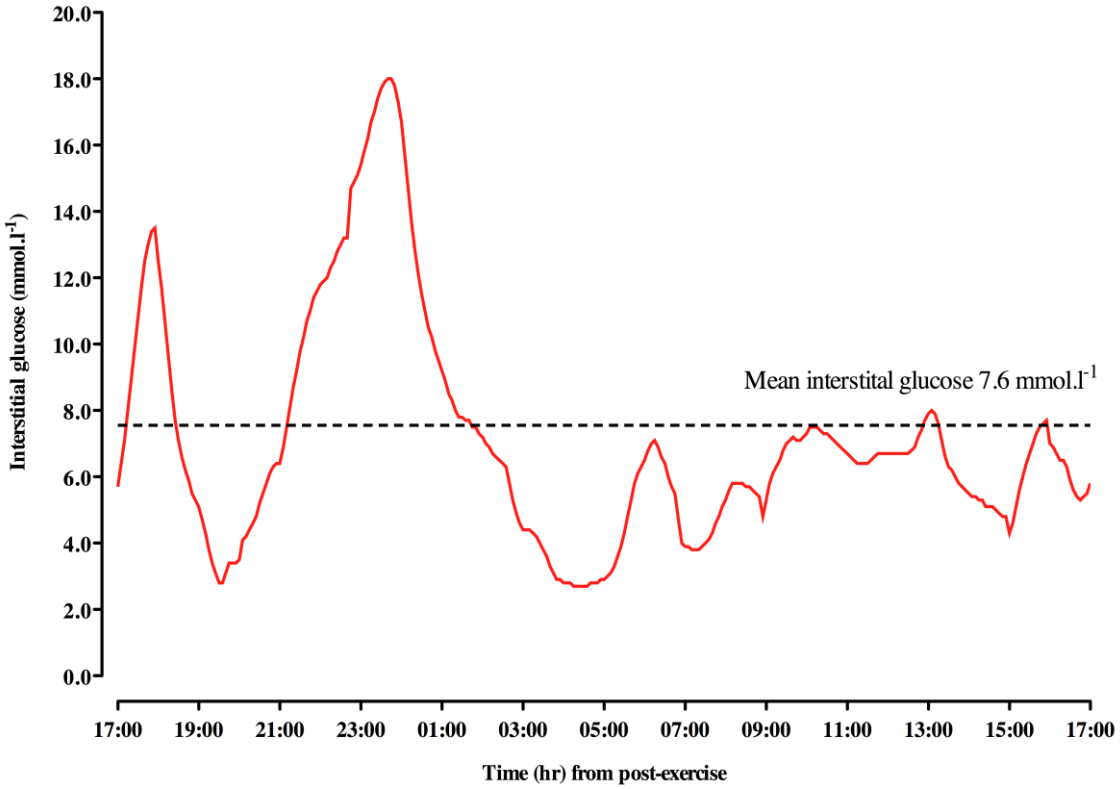

Figure 1.3. Representation of glycaemic variability over a 24 hour period in a type 1 diabetes patient. Red trace = interstitial glucose. CGM trace taken from a type 1 diabetes patient during data collection.

Glycaemic variability is suggested to exhibit a greater effect on oxidative stress (Monnier *et al.* 2006), and also acute and chronic changes in inflammation (Erbağci *et al.* 2001, De Rekeneire *et al.* 2006) opposed to exposure to chronically-sustained hyperglycaemia. This is important because changes in these parameters are heavily associated with vascular complications (Giugliano *et al.* 1996, Saraheimo *et al.* 2003, Devaraj *et al.* 2007, Wentholt *et al.* 2008). Although exercise training has been demonstrated to reduce systemic inflammation (Petersen and Pedersen 2005), and increase protection against oxidative stress (Cooper *et al.* 2002), somewhat paradoxically, these long-term adaptations occur despite opposing acute effects in which there is a pronounced increase in inflammatory cytokines early after exercise (Sprenger *et al.* 1992, Drenth *et al.* 1995, Nehlsen-Cannarella *et al.* 1997, Ostrowski *et al.* 1998, Ostrowski *et al.* 1999, Nemet *et al.* 2002, Turner *et al.* 2014). Thus, an inability to effectively manage post-exercise glycaemia may in fact offset a number of associated health benefits and precipitate deeper metabolic, hormonal and inflammatory disturbances. This may offer some

explanation towards the divergent opinion surrounding the efficacy of exercise on long-term glycaemic control and progression of diabetes complications in active type 1 diabetes patients (Rosa *et al.* 2010, Padgett *et al.* 2013).

1.3 Hypoglycaemia and exercise

The physical symptoms of hypoglycaemia (blood glucose <3.9 $mmol.l^{-1}$) range from unpleasant feelings such as anxiety, hunger, palpitations, tremor, and paraesthesia, to neurological impairments including changes in behaviour, cognitive dysfunction, seizures, coma and death. Hypoglycaemia, which is widely acknowledged as *the* limiting factor in the management of type 1 diabetes (Davis *et al.* 1997, Cryer 1999, Cryer 2008, Cryer 2010), is a frequent and dangerous occurrence which is long associated with exercise (Macdonald 1987, Malik and Taplin 2014). So much so, exercise is the most frequently identified specific cause of severe hypoglycaemia (Bhatia and Wolfsdorf 1991), that which is not only debilitating, but life threatening. In addition, the threat of hypoglycaemia is not only limited to during exercise, but may persist for many hours after (Macdonald 1987, Steppel and Horton 2003, Tsalikian *et al.* 2005, Mcmahon *et al.* 2007) particularly at night (Mcmahon *et al.* 2007, Taplin *et al.* 2010). Unsurprisingly, patients often avoid exercise due to a fear of hypoglycaemia (Dubé *et al.* 2006, Brazeau *et al.* 2008, Cryer 2008); two thirds of patients currently fail to achieve the minimal amount of exercise needed for good health (Plotnikoff *et al.* 2006). It is therefore important that patients feel empowered to exercise, and providing strategies that enable patients to engage in exercise without fear of hypoglycaemia may aid in this endeavour.

1.3.1 Physiological mechanisms preventing exercise-induced hypoglycaemia in individuals without diabetes

Skeletal muscles are tissues that convert chemical energy to mechanical work through muscular contractions. To perform muscular contractions, a vast increase in energy turnover is required, such that during exercise, the metabolic demands of skeletal muscle can increase 100-fold (Sahlin *et al.* 1998). Generally, this energetic challenge is met by the oxidation of

carbohydrates and lipids (Holloszy and Kohrt 1996, Richter *et al.* 2001, Kiens 2006), with carbohydrate, in the form of glucose derived from the circulation and intramuscular glycogen stores, becoming the predominant fuel source with increasing exercise intensity.

Exercise of a moderate intensity (~55-75% $\dot{V}O_{2max}$), involving the rhythmical contraction and relaxation of large muscle masses performed over a prolonged period of time is considered "aerobic" exercise. This is because fuels are hydrolysed and metabolised in the presence of oxygen in the mitochondria of muscle cells for subsequent resynthesis to adenosine triphosphate (ATP). Initially, aerobic exercise is fuelled predominantly by muscle glycogen before non-esterified fatty acids (NEFA) and blood glucose become the major fuel sources. The reliance upon NEFA and circulating blood glucose increases with exercise duration, as does gluconeogenesis and the breakdown of hepatic glycogen for uptake and use by the exercising musculature and maintenance of blood glucose. As exercise duration lengthens, relative exercise intensity increases despite a constant absolute workload. Thus, with prolonged continuous moderate-to-high intensity exercise, muscle glycogen is predominantly utilised (Jeukendrup 2014).

As muscle glycogen concentrations begin to deplete, overall contribution of carbohydrate to fuel metabolism is maintained by increasing glucose extraction from the circulation for uptake into musculature, via an increase in insulin-independent translocation of glucose transporter proteins (GLUT 4) to the surface of the muscle cell (Wojtaszewski *et al.* 2002). As the central nervous system relies heavily upon a continuous blood glucose supply to meet its energy requirements (Longo and Cryer 2013), it is important that blood glucose concentrations are maintained within normal physiological limits to ensure a constant supply. To do this, high levels of glucose production are necessary (Wasserman 2009). This is achieved by orchestrating a complex and well-coordinated neuroendocrine and autonomic nervous system response (Figure 1.4) that facilitates counteractive responses to decrements in blood glucose, and ultimately prevents hypoglycaemia.

In the post-absorptive state, liver release of glucose (hepatic output), through glycogenolysis and gluconeogenesis, is the primary means by which blood glucose is sustained during exercise (Wahren *et al.* 1971, Bergeron *et al.* 1999). Although the kidneys also produce glucose during exercise, renal output is negligible (Wahren *et al.* 1971) meaning maintenance of blood glucose is largely determined by the control of hepatic output which occurs through a change in the insulin-to-glucose output ratio (Battezzati *et al.* 2009). The normal physiological response to falling blood glucose includes a decrease in insulin concentrations, and an increase in glucagon, release of catecholamines from the adrenal medulla and sympathetic nerve fibres, cortisol from the adrenal cortex, and anterior pituitary release of growth hormone, which, collectively counteract falling blood glucose (Figure 1.4, Table 1.0) (Cryer and Gerich 1985, Cryer 2012). A reduction in insulin concentrations and an increase in counter-regulatory hormones also act to combat stimulation of muscle glucose uptake during exercise.

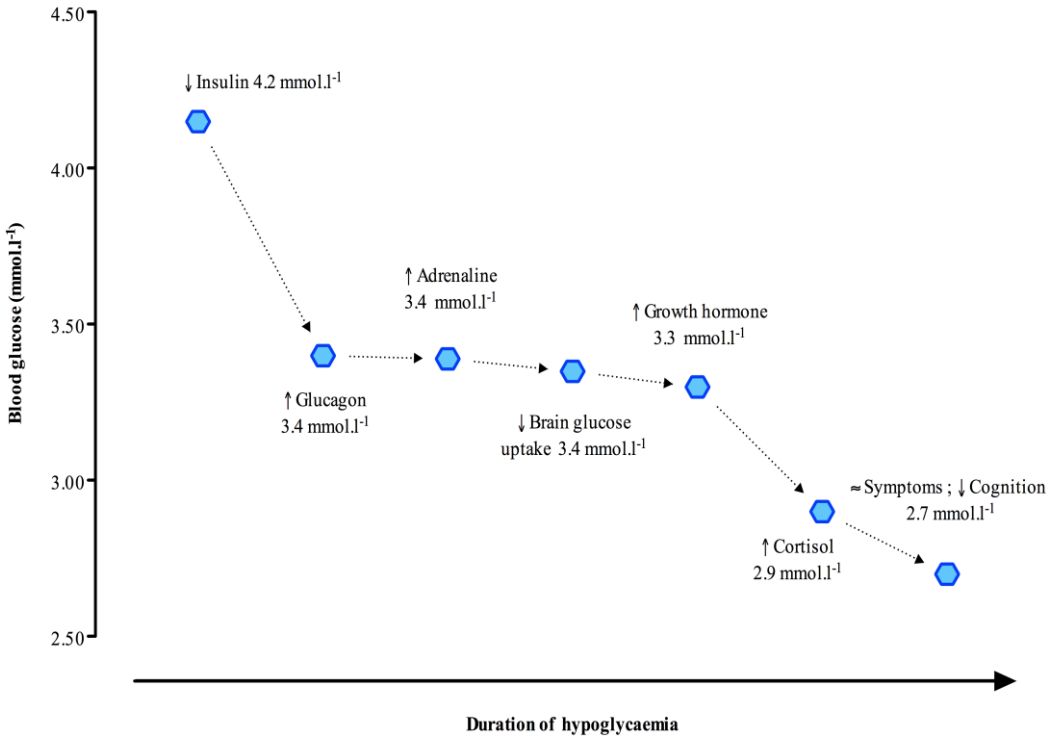

Figure 1.4 Normal physiological hierarchic counter-regulatory hormonal responses to falling blood glucose. Data taken from Cryer (Cryer 2002) and presented as mean. Note: Samples were taken from arterialised-venous blood.

1.3.2 Pathophysiological mechanisms of exercise-induced hypoglycaemia in type 1 diabetes

For patients with type 1 diabetes, circulating insulin concentrations are the result of the previously administered insulin dose, and are therefore unregulated and do not decrease in response to falling blood glucose concentrations or exercise. As such, patients are likely to be exercising under relatively hyperinsulinaemic conditions, with insulin levels well in excess of those seen in non-diabetic individuals (Chokkalingam *et al.* 2007). Additionally, exercise causes marked hypereamia (Sjøberg *et al.* 2011) and increases in temperature (Koivisto 1980). This results in an increased delivery of glucose to the working muscle (Wasserman *et al.* 2011, Richter and Hargreaves 2013) and an increased permeability of the muscle to glucose (Hamrin *et al.* 2011, Wasserman *et al.* 2011) which collectively enhance the capacity for glucose exchange (Hamrin *et al.* 2011). This heamo- and thermodynamic effect not only increases the delivery of glucose, but of all blood constituents including insulin, and causes an accelerated absorption of the previously administered dose from subcutaneous tissue (Koivisto and Felig 1978, Koivisto 1980, Lauritzen *et al.* 1980, Wojtaszewski *et al.* 2002). Furthermore, skeletal muscle, which represents approximately 40-45% of total body mass (Hargreaves and Hawley 2003), comprises the bulk of insulin-sensitive tissue (Galbo *et al.* 1975) and is a site of enhanced sensitivity to insulin, resulting from muscular contraction induced (and insulin independent) GLUT-4 recruitment (Galassetti *et al.* 2001). Therefore, patients are likely to experience an increase in potency of the previously administered insulin dose during exercise. Together, the superimposition of hyperinsulinaemia and muscle contraction exert a synergistic stimulatory effect on glucose uptake and carbohydrate metabolism (Galbo *et al.* 1975, Wasserman *et al.* 1991, Chokkalingam *et al.* 2007).

Insulin inhibits the counter-regulatory response to falling blood glucose even in healthy individuals (Hirsch *et al.* 1991) through suppression of both net hepatic glycogenolysis (through an increase in GSK3-mediated activation of glycogen synthase activity) and gluconeogenesis (by decreasing the delivery and extraction of gluconeogenic precursors and supressing lipolysis in adipose tissue) (DCCT Research Group 1994, Nathan *et al.* 2005),

although the former effect is more potent (Reichard *et al.* 1993, Fisher *et al.* 2002). Insulin reduces the liver's sensitivity to glucagon, and supresses glucagon release itself (Cooperberg and Cryer 2010). In non-diabetic individuals, glucagon exerts a rapid and potent increase in hepatic glucose production (potentially through an AMPK-mediated increase in the hepatic glycogen phosphorylase to glycogen synthase activity ratio), thus favouring an increase in net hepatic glycogenolysis (UKPDS Group 1998). Additionally, the hormone serves to increase gluconeogenesis through increasing hepatic gluconeogenesis precursor extraction and conversion to glucose (Wasserman *et al.* 1989, Davis *et al.* 2000). Although only a small increase in glucagon is needed to increase hepatic glucose output (Wasserman 1995), the progressive destruction of the pancreatic β-cells results in a concomitant and temporal disturbance of intra-islet signalling (Taborsky *et al.* 1998, Banarer *et al.* 2002, Raju and Cryer 2005, Briscoe *et al.* 2007) resulting in diminished α-cell function in response to decrements in glycaemia (Xu *et al.* 2006, Cooperberg and Cryer 2010). Hence, glucagon secretion in response to falling blood glucose concentrations is typically attenuated in type 1 diabetes.

When the intra-islet hormone response is disturbed, other counter-regulatory responses play a more important role to counteract hypoglycaemia (Gilbertson *et al.* 2001). Catecholamines (adrenaline and noradrenaline) increase during hypoglycaemia, and also exercise in direct response to intensity and duration (Cryer and Gerich 1985, Schwartz *et al.* 1987, Wasserman *et al.* 1989, Heller and Cryer 1991, Mitrakou *et al.* 1991, Mcaulay *et al.* 2001, Brand-Miller *et al.* 2003, Battezzati *et al.* 2009). These responses coincide with increased hepatic output, although a direct causal relationship is yet to be fully established. Catecholamines (although primarily adrenaline (Davey *et al.* 2013)) stimulate glycogen phosphorylase and activate hormone-sensitive lipase, which in turn, enhance hepatic glycogenolysis and lipolysis, respectively (Brand-Miller *et al.* 2003, Thomas *et al.* 2007, Nansel *et al.* 2008). Although the catecholamine response to exercise is intact in patients (Petersen *et al.* 2004), the impact of acute hormonal responses on glycaemia are likely to be short lasting (Yardley *et al.* 2013). Moreover, catecholamine responses during hypoglycaemia are often attenuated in type 1

diabetes patients (Amiel *et al.* 1988, Dagago-Jack *et al.* 1993, Cryer 2006, Cryer 2008, Cryer 2009, 2010, Parillo *et al.* 2011) and under hyperinsulinemic conditions are likely to be reduced even further (Hirsch *et al.* 1991).

Growth hormone which is secreted from the anterior pituitary gland, and cortisol from the adrenal cortex increase glucose production and reduce glucose utilisation by insulin sensitive tissues (Davis *et al.* 2000, Khani and Tayek 2001, Jorgensen *et al.* 2004). Both are increased during exercise and hypoglycaemia, but play only minor roles in regulating glucose homeostasis (Parillo and Riccardi 1995). Therefore, increases in the appearance of these hormones are not necessarily related to declines in glycaemia *per se* (Galbo *et al.* 1975, Jenkins *et al.* 1981, Bantle *et al.* 2008) and are heavily influenced by sex, maturation and circadian rhythm (Knutsson *et al.* 1997).

Table 1.0 Counter-regulatory hormones affecting blood glucose homeostasis

Hormone	**Origin**	**Action**	**Healthy control**	**Type 1 diabetes**
Glucagon	Pancreatic α-cell	↑ glycogenolysis ↑ gluconeogenesis	✓	×
Adrenaline	Adrenal medulla and sympathetic nerve fibres	↑ glycogenolysis ↑ lipolysis	✓	≠
Noradrenaline	Adrenal medulla and sympathetic nerve fibres	↑ lipolysis	✓	≠
Cortisol	Adrenal cortex	↑ gluconeogenesis ↑ lipolysis	✓	≠
Growth hormone	Anterior pituitary gland	↑ lipolysis	✓	≠

Note: ✓ = present or functioning, × = absent or dysfunctioning, ≠ = attenuated. Information taken from Cryer and Gerich (1985) and Mitrakou, Ryan *et al* (1991).

Many other hormones are released during hypoglycaemia (Cryer 1997), potentially counter-regulatory neurotransmitters (sympathetic neural noradrenaline and parasympathetic neural acetylcholine) (Cryer 1997, Chu *et al.* 1998) as well as an array of neuropeptides (Cryer 2012). In addition glucose auto-regulation, a phenomenon in which ambient glucose levels,

independent of hormonal and neural regulation, increases hepatic glucose production, may play a role (Cryer 2012). However, these mechanisms are only triggered at very low circulating glucose concentrations (Cryer 2012) and with a lack of conclusive evidence, it is yet to be established whether these mechanisms are active, ineffective or redundant in type 1 diabetes (Cryer 2002).

Another important consideration is exposure to antecedent hypoglycaemia, sleep or indeed prior exercise (Cryer *et al.* 2003, Cryer 2009, Cryer 2012, Longo and Cryer 2013). Hypoglycaemia, exercise and sleep have all been shown to blunt the counter-regulatory hormone response to subsequent hypoglycaemia (Davis *et al.* 2000, Galassetti *et al.* 2001, Galassetti *et al.* 2003, Sandoval *et al.* 2006). Unfortunately, the exact mechanisms are yet to be described within the literature, however, clinical presentation is quite clear. An attenuation of autonomic, sympathetic neural and adrenomedullary responses following exposure to hypoglycaemia, exercise and / or sleep cause a loss of symptomatic awareness for falling blood glucose concentrations (Cryer *et al.* 2003, Cryer 2009, Cryer 2012, Longo and Cryer 2013). The glycaemic threshold for counter-regulatory hormone release is lowered, and behavioural defences, such as carbohydrate ingestion prompted by symptoms such as hunger, tiredness and irritability (Mcaulay *et al.* 2001), are compromised; the clinical classification of this has been aptly coined "*hypoglycaemia unawareness*" (Heller and Cryer 1991). Those patients with hypoglycaemia unawareness face a 25-fold greater risk of severe hypoglycaemia, and it is likely to develop in those who are regularly active (Cryer 2010).

1.3.3 Post-exercise hypoglycaemia in type 1 diabetes

In type 1 diabetes, the post-exercise period is characterised by an increase in glucose requirements to maintain euglycaemia. Indeed, research indicates that the requirement to maintain glucose in the period after exercise is increased in a biphasic manner, early after exercise and again 7-11 hours later (Mcmahon *et al.* 2007), meaning the threat of developing hypoglycaemia exists not only during exercise, but remains for many hours after (Macdonald

1987). Following exercise, the glucose disappearance is increased because of two phenomena: (1) the residual effect of previously contracted musculature, independent of insulin action, and (2) increased sensitivity towards insulin (Garetto *et al.* 1984, Richter *et al.* 1984). Contraction-stimulated glucose uptake usually reverses completely within the first few hours after exercise, whereas increased insulin sensitivity persists for longer, and in some situations has been observed to last as long as 48 hours (Mikines *et al.* 1988, Cartee *et al.* 1989). Current opinion suggests that increased glucose uptake and enhanced insulin sensitivity following exercise occur to replenish depleted glycogen stores induced by exercise (Jentjens and Jeukendrup 2003). Glycogen restoration is a high metabolic priority (Jentjens and Jeukendrup 2003), and in a state of defective hormonal counter-regulation it is a challenge for patients to maintain euglycaemia during this time.

Mechanisms of glycogen replenishment have received notable attention within the literature (Maæhlum *et al.* 1977, Garetto *et al.* 1984, Blom *et al.* 1987, Ivy *et al.* 1988, Price *et al.* 1994, Aulin *et al.* 2000), mainly because restoration rates are strongly associated with post-exercise recovery and subsequent exercise performance (Jentjens and Jeukendrup 2003). Muscular contraction and insulin increase glycogen synthase activity (Danforth 1965, Friedman *et al.* 1991, Ivy 1991, Nielsen *et al.* 2004), more so when muscle glycogen concentrations are low or depleted (Zachwieja *et al.* 1991, Montell *et al.* 1999, Nielsen *et al.* 2004). Initially following exercise, there is a rapid phase of muscle glycogen synthesis which lasts for ~60 minutes, and occurs independently from the actions of insulin. Increased membrane GLUT4 protein expression is enhanced during this time, which leads to an increased permeability of the muscle cell membrane to glucose (Lund *et al.* 1995, Kuo *et al.* 1999, Richter and Hargreaves 2013). During this time, hyperaemia-induced increased glucose supply to the muscle and an enhanced capacity to convert glucose to glycogen promotes the rapid restoration of muscle glycogen. Following this period, insulin sensitivity is increased. Increased insulin sensitivity has been associated with increased activity of the serine/threorine kinase (PKB/Akt), although this is not the only mechanism suggested to play a part (Gao *et al.* 1994, Goodyear and Kahn

1998, Hansen *et al.* 1998, Wojtaszewski *et al.* 2000, Fisher *et al.* 2002, Thong *et al.* 2003) and the exact physiology behind this remains to be fully elucidated. Irrelevant of the exact mechanisms at play, patients are likely to experience an increase in the potency of administered insulin in the post-exercise period.

1.4 Influence of exercise type on blood glucose in type 1 diabetes patients

Prolonged continuous aerobic exercise generally has glucose lowering effects and therefore carries a risk of hypoglycaemia in patients with type 1 diabetes (Macdonald 1987, Tuominen *et al.* 1995, Riddell *et al.* 1999, Rabasa-Lhoret *et al.* 2001, Francescato *et al.* 2004, Tansey *et al.* 2006, West *et al.* 2010). However, not all forms of exercise acutely lower blood glucose, meaning some types of exercise may confer a lower risk of hypoglycaemia (Fahey *et al.* 2012). High-intensity exercise (such as sprinting) often results in an acute increase in blood glucose concentrations in patients with type 1 diabetes (Marliss and Vranic 2002, Fahey *et al.* 2012). This form of exercise induces a substantial increase in catecholamine release ($\geq +\Delta 500\%$ from rest (Fahey *et al.* 2012, Davey *et al.* 2013, Davey *et al.* 2014)) which can increase hepatic glucose output (Kjaer *et al.* 1986) at a greater rate than glucose clearance (Sigal *et al.* 1996). Exercise in intermittent form, short bursts of high intensity exercise interspersed with moderate intensity aerobic exercise, has been demonstrated to reduce the risk of hypoglycaemia early after exercise (Guelfi *et al.* 2005, Bussau *et al.* 2006, Bussau *et al.* 2007, Guelfi *et al.* 2007, Maran *et al.* 2010).

From a practical perspective the vast majority of studies suffer from short observation periods, which means it is difficult to assess the effectiveness of manipulating exercise for the prevention of late-onset hypoglycaemia (Guelfi *et al.* 2005, Bussau *et al.* 2006, Bussau *et al.* 2007, Guelfi *et al.* 2007, Maran *et al.* 2010, Campbell *et al.* 2014). Indeed, much of the work in this area was designed to simulate the demands of team games such as soccer, rugby and hockey (Guelfi *et al.* 2005, Guelfi *et al.* 2007, Maran *et al.* 2010, Campbell *et al.* 2014), rather than strategies to prevent hypoglycaemia *per se*. Much of this existing literature has focused

predominantly upon cycling (Guelfi *et al.* 2005, Bussau *et al.* 2006, Bussau *et al.* 2007, Guelfi *et al.* 2007, Iscoe and Riddell 2011, Fahey *et al.* 2012, Davey *et al.* 2013, Davey *et al.* 2013), however cycling fails to adequately replicate the physiological demands of games-type activities, in which repeated changes in speed *and* direction are a major component. Cycling involves primarily concentric muscle actions (Bijker *et al.* 2002) meaning that the muscle shortens as it contracts, whereas in the majority of intermittent game-type activities, which typically involve running, a significant proportion of eccentric muscle action occurs, where the muscle lengthens during the contraction phase. This is a particularly important consideration in type 1 diabetes, as eccentric muscle actions have the potential to down-regulate the insulin receptor, thus hindering insulin action and glucose uptake following exercise (Asp *et al.* 1995). Moreover, weight bearing exercise, which typically requires greater muscle mass involvement (running versus cycling), has a greater energy demand (Robertson *et al.* 2009). Thus, the ecological validity of these studies is somewhat questionable, and the impact of exercise modality on late-onset hypoglycaemia has been under researched.

A recent investigation which I conducted outside of this series of studies (2014) aimed to address the limitations of these former studies, by comparing the 24 hour glycaemic responses to continuous versus intermittent running exercise, which was designed to closely simulate games-play. The results from this study indicate that the preservation in blood glucose early after exercise is only marginally greater following intermittent running, compared to continuous running exercise at a similar intensity (matched $\%\dot{V}O_{2peak}$). Moreover, there was an equal incidence of late-onset hypoglycaemia in this dataset, indicating that falls in glycaemia are likely to occur irrelevant of exercise modality.

Recently, resistance exercise in type 1 diabetes has received notable attention within the literature because this form of exercise also elicits similar hormonal and metabolic responses to that of intermittent and high-intensity running or cycling (Kraemer and Ratamess 2005). Resistance exercise results in a lesser decline in blood glucose immediately after exercise (Yardley *et al.* 2010), however, the risk of late-onset hypoglycaemia remains (Yardley *et al.*

2013), irrelevant of exercise intensity (Silveira *et al.* 2014) or duration (Turner *et al.* 2013, Turner *et al.* 2014), whether this is incorporated into aerobic exercise or not (Yardley *et al.* 2010), and regardless of the order exercise is performed (Yardley *et al.* 2012).

Manipulating an acute hormonal response through altering exercise type, is likely to carry only short lasting effects on glycaemia (Yardley *et al.* 2013). Thus, it would appear that manipulating exercise modality alone is not a completely protective strategy against exercise-induced hypoglycaemia. Moreover, the majority of studies utilising high-intensity work in intermittent (Guelfi *et al.* 2005, Guelfi *et al.* 2005, Bussau *et al.* 2006, Bussau *et al.* 2007, Guelfi *et al.* 2007, Maran *et al.* 2010, Iscoe and Riddell 2011, Fahey *et al.* 2012, Davey *et al.* 2013) or resistance form (Yardley *et al.* 2010, Yardley *et al.* 2012, Turner *et al.* 2013, Yardley *et al.* 2013, Silveira *et al.* 2014), have typically recruited patients young in age (mean ~26 years, range 18-30 years), in good glycaemic control (HbA_{1c} ~7.4%), and are already regularly engaged in exercise; three studies include competitive athletes in their cohort (Iscoe and Riddell 2011, Yardley *et al.* 2012, Yardley *et al.* 2013). Results in these studies, may not necessarily be generalised to the wider population of type 1 diabetes patients, and may be inappropriate or even unachievable for many individuals considering not all studies demonstrate good adherence rates for novices (~62% adherence rate to exercise in type 1 diabetes (Plotnikoff *et al.* 2006)). Eccentric-based intermittent shuttle running exercise can induce severe muscle soreness and muscular dysfunction (Bailey *et al.* 2007), and has been observed as a primary mechanism of injury (Hawkins *et al.* 2001, Woods *et al.* 2004); the frequency of speed changes places greater emphasis on the acceleration and deceleration phases of the running cycle applying more eccentric load than conventional cycling based sprinting protocols (Greig and Siegler 2009). An increased risk of muscle soreness, fatigue, and injury are likely to deter older patients or those unaccustomed to such movement patterns. Additionally, research suggests that the performance of prolonged lower-intensity exercise confers similar gains in cardiovascular fitness (Wenger and Bell 1986) and carries greater long-term adherence rates (Perri *et al.* 2002) compared to shorter-duration, higher intensity

training. In addition, aerobic exercises which are weight-baring, such as running and jogging, are likely to offer similar improvements in bone mineral density (Welsh and Rutherford 1996, Kelley *et al.* 2001) and deliver greater improvements in stability and maintenance of gait in older individuals (Sauvage Jr *et al.* 1992).

1.5 Influence of exercise on markers of inflammation

Type 1 diabetes is, by large, an inflammatory disease (Rosa *et al*. 2010). However, diabetes-related inflammation is complex and multifactorial. Whilst low grade systemic inflammation is present due to hyperactivation of specific leukocyte subtypes pertinent to autoimmune events, diabetes control also acutely and chronically influences inflammatory exacerbations (Schram et al. 2003;Yamagishi and Imaizumi 2005). In healthy individuals free of diabetes, regular exercise training results in a chronic reduction in systemic inflammation (Robertson *et al*. 2008). Somewhat paradoxically however, this occurs following acute exacerbations in inflammatory status, with increased pro-inflammatory cytokines such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-α) (Ludbrook 1998; Galassetti et al. 2006; Pedersen et al. 2007). Importantly, hypoinsulinaemia and/or hyperglycaemia can acutely increase these inflammatory markers. Under such conditions, inflammation may be compounded by the performance of exercise Galassetti *et al.* 2005; this may result in a dampening or even loss of the long-term anti-inflammatory effects of exercise (Cooper *et al*. 2004). Thus, exercise, hyperglycaemia, and insulin administration all have the capacity to heavily influence inflammation status in type 1 diabetes patients. This is important because a change in inflammation status is good proxy marker for the development of long term diabetes related complications (Yamagishi and Imaizumi 2005).

1.6 Strategies for preventing hypoglycaemia during and after aerobic exercise

1.6.1 Carbohydrate intake for type 1 diabetes patients

1.6.1.1 Daily macronutrient recommendations

The provision of exogenous fuels for exercise is important not only for performance (Wright *et al.* 1991, Below *et al.* 1995), but also for the avoidance of hypoglycaemia in type 1 diabetes (Gallen 2003, Hibbert-Jones and Regan 2005, De Feo *et al.* 2006, Maahs *et al.* 2009, Perry and Gallen 2009). Thus, it is important that energy and macronutrient needs, especially carbohydrate, are met to maximise training effects and avoid hypoglycaemia. Despite this, there are no specific guidelines pertaining to macronutrient intake for regularly exercising type 1 diabetes patients. Instead, recommendations have been taken from research investigating athletic performance in non-diabetic individuals, and then tailored using anecdotal evidence and experience. A number of review articles suggest active type 1 diabetes patients should consume ~5g carbohydrate.kg^{-1} BM daily (Gallen 2003, Lumb and Gallen 2009, Perry and Gallen 2009, Robertson *et al.* 2009) constituting ~60-80% of total daily energy intake (Burke 2006, Riddell and Perkins 2006, Inge and Sutherland 2007, Sedlock 2008), with 5-10% protein, and less than 30% fat (Inge and Sutherland 2007). If patients maintain their usual insulin-to-carbohydrate ratio however (i.e. increasing total daily insulin administration to match increased carbohydrate ingestion), then simply consuming large quantities of carbohydrate is unlikely to negate hypoglycaemia. Moreover, increased carbohydrate consumption may neutralise any gains from the glycaemic lowering effects of exercise (Mckewen *et al.* 1999), and disrupt energy balance. Patients are therefore recommended to adopt a two-point strategy before engaging in exercise: 1) ingest carbohydrate (Ramires *et al.* 1997, Gallen 2003, Steppel and Horton 2003, De Feo *et al.* 2006, Riddell and Iscoe 2006, Perry and Gallen 2009), and 2) reduce the amount of rapid-acting insulin administered with it (Rabasa-Lhoret *et al.* 2001, West *et al.* 2010, West *et al.* 2011, West *et al.* 2011).

1.6.1.2 Carbohydrate requirements for exercise

Recommendations for carbohydrate ingestion for exercise have been based upon exercise duration (Gallen 2003, Perry and Gallen 2009) as well as patient characteristics such as body mass (Ramires *et al.* 1997, Riddell and Iscoe 2006) or blood glucose concentrations prior to exercise (Steppel and Horton 2003, De Feo *et al.* 2006). Unsurprisingly, the amount of

carbohydrate recommended for patients to ingest varies greatly (Hernandez *et al.* 2000, Dubé *et al.* 2005, Iafusco 2006), although ~60g carbohydrate per hour of moderate-to-high intensity exercise is generally advised (Gallen *et al.* 2011). This is based on the assumption that glucose disposal from the circulation occurs at a rate of ~1g glucose.kg.h^{-1} in type 1 diabetes patients (Francescato *et al.* 2004). This amount of carbohydrate can be consumed either as a single bolus before exercise, or split into equal portions and consumed throughout (Hernandez *et al.* 2000, Dubé *et al.* 2005, Iafusco 2006). However, consuming carbohydrates throughout exercise is often impractical and may cause gastric distress. Moreover, ingesting large quantities of carbohydrate as a single bolus (~1-2g carbohydrate.kg^{-1} BM) (Robertson *et al.* 2009) will require administration of insulin to avoid post-prandial hyperglycaemia. If insulin adjustments are not made with the carbohydrate ingested before exercise, then hypoglycaemia will ensue (Rabasa-Lhoret *et al.* 2001). In one study, where pre-exercise insulin dose was not adjusted, administering additional carbohydrate (15 grams of carbohydrates, ~60 kcal) in frequent amounts was insufficient to restore euglycaemia (Tansey *et al.* 2006), and in another study, patients required an additional 70g (~280 kcal) of carbohydrate throughout exercise to prevent hypoglycaemia (Riddell *et al.* 1999).

The composition of the carbohydrate is also important. Meals containing identical macronutrient compositions are digested and absorbed at varying rates producing a range of glycaemic responses in type 1 diabetes patients (Parillo *et al.* 2011). Whereas the type, amount and concentration of carbohydrates determine the rate of gastric emptying, meal viscosity, osmolality, temperature, pH, ambient blood glucose concentrations as well as long term glycaemic control are also influential (Davis *et al.* 1990, Schvarcz *et al.* 1993, Murray *et al.* 1997, Schvarcz *et al.* 1997, Maughan and Leiper 1999, Ma *et al.* 2009, Jeukendrup and Moseley 2010). Beyond gastric emptying, it is the type of carbohydrate that largely influences subsequent absorption and systemic appearance. This is predicted, at least in part, using the glycaemic index (GI), a method originally developed to assist diabetes patients in controlling glycaemia, but now predominantly used as a tool in exercise nutrition to aid in carbohydrate

selection for sport performance. This method which expresses time-course changes in blood glucose resulting from the consumption of specific carbohydrate-containing foods, relative to that of a standardised reference food of equivalent available carbohydrate content (Jenkins *et al.* 1981). Carbohydrate foodstuffs with a low GI elicit a more gradual rise and decline in blood glucose in comparison to their high GI equivalents. Studies in both healthy individuals (Demarco *et al.* 1999, Kawai *et al.* 2003, Achten *et al.* 2007, Kawamura 2007, Stevenson *et al.* 2006) and those with type 1 diabetes (Nansel *et al.* 2008, Rovner *et al.* 2009, Parillo *et al.* 2011) demonstrate that low GI diets impart more favourable post-prandial blood glucose profiles. In the longer term, this is associated with a reduction in HbA_{1c}, less daily insulin requirements, and a reduced risk of hypoglycaemia in type 1 diabetes patients (Gilbertson *et al.* 2001, Brand-Miller *et al.* 2003, Nansel *et al.* 2008). Although, some factors such as cooking methods, taste preferences, and the co-ingestion of other foodstuffs may partially limit the translation of GI into clinical practice.

GI also has important implications for pre-exercise blood glucose concentrations. In one study, where pre-exercise insulin dose was not adjusted, 86% of patients developed hypoglycaemia when blood glucose concentrations before exercise were less than 6.6 $mmol.l^{-1}$ (Tansey *et al.* 2006). Typically, patients attempt to increase blood glucose before exercise, often via ingestion of high GI carbohydrate-based beverages that result in pre-exercise hyperglycaemia. However, Jenni et al (2008) observed that under hyperglycaemic conditions, exercising carbohydrate oxidation rates were significantly greater, compared to when exercise is performed under euglycaemic conditions. Increased glucose availability, thus increased glycolytic flux and ultimately greater rates of carbohydrate oxidation directly supresses lipid metabolism (Coyle *et al.* 1997). Importantly, increased combustion of exogenous carbohydrate occurs without sparing of muscle or hepatic glycogen stores in type 1 diabetes (Chokkalingam *et al.* 2007). Based on the premise that GI heavily influences pre-exercise blood glucose concentrations (Parillo *et al.* 2011) and thus the rate of glucose availability, potentially, manipulating the GI of foods consumed before exercise could alter exercising substrate

metabolism. Data in individuals without diabetes (Coyle *et al.* 1997, Demarco *et al.* 1999, Stevenson *et al.* 2006, Achten *et al.* 2007), and in those with type 1 (West *et al.* 2011) indicate that compared to high GI carbohydrate ingestion, carbohydrate utilisation is decreased and lipid oxidation increased during exercise when low GI carbohydrates are consumed before exercise. Thus the ingestion of low GI carbohydrates before exercise, may spare endogenous and exogenous carbohydrate utilisation, resulting in a greater preservation of blood glucose, and potentially lower rates of post-exercise hypoglycaemia.

1.6.2 Pre-exercise reductions in insulin-to-carbohydrate ratio

Hypoglycaemia *during* exercise is largely attributed to iatrogenic causes, whereby patients exercise during times of peak insulin absorption (Tuominen *et al.* 1995). Typically, patients are recommended to abstain from exercise during times of peak-absorption, because of increased risk of hypoglycaemia (Dandona *et al.* 1980, Tuominen *et al.* 1995); for rapid-acting insulin this is generally ~60 minutes following administration (Plank *et al.* 2002). This means delaying exercise for ~90-120 minutes. However, recent data indicates that when only small amounts of rapid-acting insulin is administered, exercise can be performed within 30-60 minutes following administration without increasing the risk of post-exercise hypoglycaemia (West *et al.* 2011). However, insufficient insulin administration, or indeed an omission of insulin, will induce hyperglycaemia and potentially raise ketone concentrations (Laffel 2000, Wallace and Matthews 2004). Exposure to severe hyperglycaemia before exercise may negate any beneficial glycaemic-lowering effect of the exercise and induce metabolic and / or inflammatory disturbances (Laffel 2000, Devaraj *et al.* 2005, Galassetti *et al.* 2006, Rosa *et al.* 2008, 2010, Rosa *et al.* 2011). Thus, an appropriate dose-reduction strategy is important.

Recommendations for reducing the amount of insulin administered before exercise vary within the literature, ranging from 10 up to a 90% reduction in dose (Campaigne *et al.* 1987, Rabasa-Lhoret *et al.* 2001, Mauvais-Jarvis *et al.* 2003, Grimm 2005, De Feo *et al.* 2006, West *et al.* 2010), although much of this variation can be attributed to differences in insulin type and

exercise intensity (Table 1.1). With current insulin regimens, patients are recommended to reduce the amount of rapid-acting insulin administered before exercise (Rabasa-Lhoret *et al.* 2001), with current opinion suggesting a reduction in dose by 75% when undertaking exercise of a moderate-to-high intensity (75% $\dot{V}O_{2max}$) (Rabasa-Lhoret *et al.* 2001, West *et al.* 2010, West *et al.* 2011). Importantly, when a large reduction is applied to rapid-acting insulin, patients are able to inject as little as 30 minutes before exercise (West *et al.* 2011) without increased risk of hypoglycaemia. Moreover, West and colleagues (2011) demonstrated that the composition of the pre-exercise meal consumed with this rapid-acting insulin reduction, does not seem to influence risk of hypoglycaemia during or immediately after exercise. It is noteworthy that in this study the authors used a carbohydrate beverage (10% solution) as the pre-exercise meal, which although not reported, may have caused gastro-intestinal discomfort in some patients, especially if ingested as little as 30 minutes before exercise (Peters *et al.* 1995). As it is important to integrate these strategies into a patient's habitual diet, incorporating the pre-exercise carbohydrate bolus into a meal format would make this strategy more applicable to patients normal exercising patterns.

Despite the importance of reducing pre-exercise rapid-acting insulin dose, current recommendations alone are inadequate at fully protecting patients from exercise-induced hypoglycaemia. Beyond 180 minutes post-exercise, patients are susceptible to hypoglycaemia irrelevant of pre-exercise rapid-acting insulin dose, meal composition, or insulin / meal timing (West *et al.* 2010, West *et al.* 2011, West *et al.* 2011).

Table 1.1 Summary of literature investigating pre-exercise reductions in insulin-to-carbohydrate ratio

Reference	Design	Main outcomes and Implications
Campaigne *et al*. (1987)	9 T1DM males treated with bi-daily pre-mixed NPH and Soluble insulin. **50%** reduction of NPH vs. **50%** reduction of soluble insulin vs. **Full** dose with 45 minutes of cycling at 60%$\dot{V}O_{2max}$	Under treatment with NPH and Soluble Human insulin the risk of early and late-onset hypoglycaemia remains, particularly at night.
Rabasa-Lhoret *et al*. (2001)	8 T1DM males treated with Basal Ultralente and bolus Lispro. **Full** dose vs. **50%** reduction vs. **75%** reduction before 60 minutes cycling at 25%$\dot{V}O_{2max}$, 30 or 60 minutes at 50% $\dot{V}O_{2max}$, and 30 minutes at 75%$\dot{V}O_{2max}$	Increased risk of hypoglycaemia during exercise, irrelevant of exercise intensity when a reduction is not performed. A **75%** reduction is needed when exercise of a moderate intensity is performed. Hypoglycaemia occurred over the course of 18 hours post-exercise.
Mauvais-Jarvis *et al*. (2003)	12 T1DM males treated on NPH and Regular insulin either bi-daily (n=6) or tri-daily (n=6). **50%** reduction for bi-daily patients, **90%** reduction for tri-daily patients with 60 minutes cycling at 70%$\dot{V}O_{2max}$	Increased risk (66%) of hypoglycaemia when reductions are not employed. Reductions serve to maintain glycaemia during exercise and in early recovery. Implications late after exercise were not reported.
West *at al*. (2010)	1 female and 6 T1DM males treated on basal Glargine, and bolus Aspart or Lispro. **Full** dose vs. **75%** reduction vs. **50%** reduction vs. **25%** reduction 120 minutes before 45 minutes of treadmill running at 70%$\dot{V}O_{2max}$	Hypoglycaemia was noted immediately after exercise when insulin was not reduced. Patients under all reduction trials were protected from hypoglycaemia for 180 minutes post-exercise.
West *et al*. (2011)	1 female and 7 T1DM males treated on basal Glargine or Detemir, and bolus Aspart or Lispro. Low vs. high GI pre-exercise meal with 75% reduced rapid-acting insulin dose, 120 minutes prior to 45 minutes of treadmill running at 70%$\dot{V}O_{2max}$	Low GI pre-exercise meal improved glycaemia before and after exercise, but did not influence risk of early onset hypoglycaemia. Implications late after exercise were not reported.
West *et al*. (2011)	7 T1DM males treated on basal Glargine and bolus Aspart or Lispro. Low GI meal and 75% rapid-acting insulin reduction taken 120, 90, 60, or 30 minutes prior to 45 minutes of treadmill running at 70%$\dot{V}O_{2max}$	No cases of hypoglycaemia during exercise, or for 180 minutes after exercise, despite insulin administration 30 minutes before exercise.

1.6.3 Post-exercise strategies for preventing hypoglycaemia

Existing strategies have focused on actions taken before and during exercise, yet the real challenge is managing glycaemia after exercise. Indeed, risk of late-onset post-exercise hypoglycaemia still remains (West *et al.* 2010). Despite this, there is a lack of available information for managing post-exercise glycaemia. There are currently no standard guidelines regarding carbohydrate amount, type, or timing of feeding for minimising post-exercise hypoglycaemia, with most advice centred on fluid replacement for avoidance of hyperglycaemia-induced dehydration (Sigal *et al.* 2006). However, carbohydrate consumption early after exercise is important, as studies using euglycaemic clamp techniques demonstrate an increased requirement for exogenous carbohydrate to maintain blood glucose concentrations early after exercise (Mcmahon *et al.* 2007). In addition, carbohydrate ingestion soon after exercise is also important for replenishing muscle and / or hepatic glycogen stores (Jentjens and Jeukendrup 2003) and delaying this could increase the risk of hypoglycaemia later in the day (Mcmahon *et al.* 2007, Gallen *et al.* 2011). Robertson *et al*. (2009) also recommends adjusting the insulin dose administered with this post-exercise meal to compensate for increased insulin sensitivity. However there is a lack of empirical evidence to support this at present, especially when following current recommendations for reducing pre-exercise rapid-acting insulin dose. Studies investigating alterations in pre-exercise insulin administration and carbohydrate consumption are limited by the extended observation period in which food was withheld after exercise (West *et al.* 2010, West *et al.* 2011, West *et al.* 2011).

1.6.3.1 Post-exercise reductions in rapid-acting insulin dose

Clearly, current pre-exercise recommendations carry only short-lasting protective effects from hypoglycaemia, yet there is no information for patients regarding insulin dosage with the meal after exercise. It is unknown whether administering unchanged insulin with the meal consumed after exercise, when employing pre-exercise insulin dose reductions, would increase the risk of

early post-exercise hypoglycaemia, whether reductions in dose could be a preventative action, or what the glycaemic implications are for late-onset hypoglycaemia. Conversely, reducing post-exercise rapid-acting insulin, under conditions of a pre-exercise reduction, may induce hyperglycaemia. Furthermore, it would be reasonable to speculate that low levels of circulating insulin and elevated concentrations of post-exercise counter-regulatory hormones could precipitate a metabolic milieu promoting increased lipolysis (Khani and Tayek 2001) and ketogenesis (Laffel 2000). Collectively and also independently, hyperglycaemia (Targher *et al.* 2001, Esposito *et al.* 2002, De Rekeneire *et al.* 2006, Rosa *et al.* 2008), lipid oxidation (Febbraio and Pedersen 2005), hyperketonaemia (Stouthard *et al.* 1995, Karavanaki *et al.* 2011, Karavanaki *et al.* 2012) and under-insulinisation (Rosa, Flores et al. 2008) could exacerbate inflammatory disturbances already evident following acute exercise (Sprenger *et al.* 1992, Drenth *et al.* 1995, Nehlsen-Cannarella *et al.* 1997, Ostrowski *et al.* 1999, Pedersen and Hoffman-Goetz 2000, Nemet *et al.* 2002). At present, the deeper metabolic, hormonal and inflammatory consequences of reducing pre- and post-exercise rapid-acting insulin dose are also unknown.

1.6.3.2 Post-exercise feeding

As discussed previously, the composition of carbohydrates is a particularly important consideration for type 1 diabetes patients. Carbohydrates with a high GI promote accelerated muscle glycogen restoration compared to lower GI equivalents (Jentjens and Jeukendrup 2003, Jensen and Richter 2012). Therefore, manipulating post-exercise carbohydrate composition could have implications for reducing post-exercise hypoglycaemia. However, under conditions of reduced pre- and post-exercise rapid-acting insulin dose, this may induce hyperglycaemia (Nansel *et al.* 2008, Rovner *et al.* 2009, Parillo *et al.* 2011) and increase ketoneamia (Koeslag *et al.* 1980, Laffel 2000, Wallace and Matthews 2004). On the contrary, glycaemic excursions resulting from a reduced pre- and post-exercise insulin-to-carbohydrate ratio may be avoided by simply consuming carbohydrates with a low GI (Qi *et al.* 2006, Nansel *et al.* 2008). However, a slower delivery of carbohydrate to exercised muscle tissue following low GI

ingestion, may result in reduced rates of muscle glycogen replenishment (Jentjens and Jeukendrup 2003), which could increase late-onset hypoglycaemia (Macdonald 1987, Riddell and Perkins 2006). At present there are no recommendations regarding the composition of the meal consumed after exercise for the avoidance of post-exercise hypoglycaemia

1.7 Influence of evening-time exercise

Many individuals prefer to exercise in the evening due to study or work commitments or even for social reasons. Research indicates that patients experience a delayed risk of hypoglycaemia 7-11 hours after exercise (Mcmahon *et al.* 2007), with exercise in the evening associated with a greater risk of post-exercise hypoglycaemia (Mcmahon *et al.* 2007, Davey *et al.* 2013), as these falls in blood glucose are likely to occur nocturnally (Taplin *et al.* 2010). Current recommendations for the avoidance of nocturnal hypoglycaemia following evening exercise consist of consuming a bedtime snack (Hernandez *et al.* 2000) to supply adequate carbohydrate availability during the night. Current opinion suggests a snack equating to ~0.4 g.carbohydrate.kg^{-1} BM, but there is little information pertaining to the composition of this snack, nor whether a snack is required at all if adjustments in post-exercise rapid-acting insulin and meal composition are made.

1.8 Application of basal insulin reductions

Although alterations in basal insulin administration are promoted in both clinical practice and within the literature (Tsalikian *et al.* 2005, Robertson *et al.* 2009), reductions in basal dose have been trialled predominantly in individuals treated with continuous subcutaneous insulin infusion therapy (CSII) (Edelmann *et al.* 1986, Sonnenberg *et al.* 1990, Admon *et al.* 2005, Tsalikian *et al.* 2006). This form of treatment involves continuous infusion of rapid-acting insulin delivered subcutaneously at a variable rate controlled via an electronically controlled pump. CSII adjustment strategies have focused on a reduction or suspension in the insulin infusion rate before and during exercise (Table 1.2), whereby 50% - 100% reductions have been shown to reduce rates of hypoglycaemia. However, when insulin infusion rates are

returned to full following exercise, it would seem patients are exposed to hypoglycaemia later in the day (Table 1.2), suggesting it may be necessary to reduce basal insulin rates over a longer period of time. In the UK, patients are predominantly treated using a basal-bolus regimen, which is associated with greater rates of post-exercise hypoglycaemia than CSII (Yardley *et al.* 2013). The basal component consists of a slowly-absorbed long-acting insulin analogue (insulin glargine [Lantus], Sanofi-Aventis; determir [Levemir], Novo Nordisk) that is self-administered once or twice per day. This is a far less flexible method of insulin delivery than CSII, meaning, manipulation of self-administered basal dose is likely to carry long lasting effects.

Interestingly, late falls in glycaemia following exercise typically coincide with glucose nadirs occurring 4-14 hours after administration of basal insulin on non-exercise days (Ashwell *et al.* 2006, Thomas *et al.* 2007). Potentially, reducing basal insulin over the course of an exercise day could be a strategy to combat late-onset hypoglycaemia, whereby hepatic glucose output is increased to supplement greater rates of glucose uptake and counteract anticipated glucose nadirs following exercise.

However, there is currently no investigative data exploring the glycaemic effects of reducing basal insulin as part of a basal-bolus regimen. In addition, there is no information pertaining to the glycaemic effects of applying a basal dose reduction under conditions of acute prandial adjustments in rapid-acting insulin and carbohydrate intake. Reducing basal dose under such conditions could expose patients to severe and / or prolonged hyperglycaemia. Moreover, considering basal insulin is important for restricting ketogenesis (Mcgarry and Foster 1980, Nosadini *et al.* 1994, Laffel 2000, Keller *et al.* 2009), such a strategy may precipitate a metabolic state of increased ketonaemia (Nosadini *et al.* 1994, Keller *et al.* 2009), which could augment an increased inflammatory response (Jain *et al.* 2003). There is a need for research to confirm or refute this, so that current clinical recommendations for reducing basal dose can be validated for use in conjunction with acute prandial adjustments.

Table 1.2 Summary of literature investigating alterations to basal insulin

Reference	Design	Main outcomes and Implications
Edelmann *et al.* (1986)	7 T1DM males performed 45 minutes of cycling at 60%$\dot{V}O_{2max}$ 30 minutes after insulin infusion was suspended, or during full insulin infusion. In the suspension trial, insulin infusion rate remained discontinued for a further 95 minutes	Despite a suspension in insulin infusion rate 30 minute before exercise, throughout exercise, and for a further 95 minutes afterwards, hypoglycaemia was still encountered by patients
Sonnenberg *et al.* (1990)	7 T1DM males treated on Actrpid (purified porcine regular insulin) performed 60 minutes of low-to-moderate intensity (80 watts) cycling 90 minutes after a carbohydrate meal and either a fully or 50% reduced bolus insulin. Basal insulin infusion was maintained or reduced by 25%, 50% or 100% during exercise	Hypoglycaemia during exercise was preventable when the pre-meal insulin bolus was reduced by 50% in concert with a reduction in basal insulin infusion rate by25%. Larger reductions were associated with hyperglycaemia. Late-onset hypoglycaemia was not reported
Admon *et al.* (2005)	6 female and 4 T1DM males treated with rapid-acting insulin Lispro performed 40-45 minutes of cycling exercise at 60%$\dot{V}O_{2max}$ during either a suspension in insulin infusion, or a reduction in infusion rate by 50%. A carbohydrate bolus were provided before and after exercise	This was the first study to utilise rapid-acting insulin analogues. No advantage found under either condition, but late-onset hypoglycaemia was more common than hypoglycaemia induced during exercise
Tsalikian *et al.* (2006	49 children with T1DM performed four 15 minute treadmill walking / jogging intervals at a heart rate of 140 bpm interspersed with three 5 minute rest breaks over 75 minutes. Basal insulin was either suspended during exercise, or continued. Glycaemia was monitored for 45 minutes after exercise	Suspending insulin infusion was associated with a reduction in the rate of hypoglycaemia from 43% to 16%. However, high rates of hyperglycaemia were evident. Late onset hypoglycaemia was not reported

1.9 Summary of literature

Current guidelines for preventing exercise-induced hypoglycaemia are not fully protective, leaving patients exposed to falls in glycaemia late after exercise. Moreover, if exercise is performed in the evening, hypoglycaemia is likely to occur nocturnally. There are at present, few guidelines which inform patients how to manage blood glucose after exercise, and these are based largely on opinion and experience rather than to empirical evidence.

1.10 Summary of experimental aims

The work in this thesis adds to the existing literature by investigating strategies to manage post-exercise glycaemia, and determine the effects of these strategies on wider markers of diabetes management in type 1 diabetes patients. Specifically, this thesis has examined:

1. The acute and 24 hour glycaemic effects of reducing post-exercise rapid-acting insulin dose whilst employing current recommendations for reducing pre-exercise rapid-acting insulin dose.

2. The acute metabolic, inflammatory, and counter-regulatory hormonal effects of reducing post-exercise rapid-acting insulin dose under conditions of reduced pre-exercise rapid-acting insulin dose.

3. The acute and 24 hour glycaemic effects of manipulating the glycaemic index of carbohydrates consumed following evening exercise, under conditions of reduced pre- and post-exercise rapid-acting insulin dose.

4. The metabolic, inflammatory, and counter-regulatory hormonal effects of manipulating the glycaemic index of post-exercise carbohydrates consumed following evening exercise, under conditions of reduced pre- and post-exercise rapid-acting insulin dose.

5. The appetite responses following the manipulation of the glycaemic index of carbohydrates consumed following evening exercise, under conditions of reduced pre- and post-exercise rapid-acting insulin dose.

6. The acute and 24 hour glycaemic effects of a combined basal-bolus insulin reduction and carbohydrate feeding strategy for evening exercise.

7. The metabolic, counter-regulatory hormonal, and inflammatory responses following a combined basal-bolus insulin reduction and carbohydrate feeding strategy for evening exercise.

CHAPTER 2

GENERAL METHODOLOGY

2.0 Project approval

Favourable ethical opinion was received from Sunderland National Health Service (NHS) Research Ethics Committee, and project approval was gained from Newcastle upon Tyne Hospitals NHS Foundation Trust Research and Development for carrying out this series of studies (Appendix A1-3). All patients provided written informed consent (Appendix A4).

2.1 Participants

2.1.1 Recruitment of patients

Type 1 diabetes volunteers were sought from Newcastle upon Tyne and the surrounding areas. Potential participants were approached in clinic (Newcastle Primary Care Trust Diabetes Centre) by their Diabetes Specialist Nurse / Clinician, or recruited through The Diabetes Research Network following a database search for eligible patients. In addition, advertisements were placed in local newspapers, on Northumbria University's webpage, on regional and national Diabetes UK and JDRF websites, and a Facebook® networking page was created to increase awareness. Interested participants were issued a study information pack. Those who were willing to participate were subsequently screened against inclusion / exclusion criteria (Table 2.0), and invited to attend an interest meeting to discuss the study further.

2.1.2 Health screening

Patients attended the Newcastle NIHR Clinical Research Facility exercise laboratory for a pre-participation health screening between the hours of 09:00AM and 17:00PM. The screening visit was conducted in accordance with the American College of Sports Medicine's (ACSM) guidelines for exercise testing (Thompson *et al.* 2009), and administered by a certified ACSM Clinical Exercise Specialist. Patients completed a medical history and physical examination, including an assessment of hypoglycaemia awareness (Clarke *et al.* 1995) (Appendix B) before a resting and exercise stress test was conducted.

Table 2.0 Inclusion / exclusion criteria for patients across all experimental chapters

Males aged 18-50 years
Regularly and consistently physically active (aerobic-based physical activities ≥ 3 per week)
Free of diabetes complications (excluding background diabetic retinopathy), including impaired awareness to hypoglycaemia
Diagnosed for of minimum of one year
HbA1c < 10% / 86 mmol/mol
Treated with a basal-bolus regimen composed of either insulin Glargine or Detemir, and Aspart of Lispro (stable for ≥ 6 month)
Receiving no other medication
Free of muscular-skeletal or orthopaedic contraindications
No abnormalities in cardiac function (assessed using a resting and exercise electrocardiogram stress test)

2.1.2.1 Resting and exercise electrocardiogram

A modified Mason-Likar 12 lead ECG configuration was used to assess real-time cardiac function during rest and throughout the exercise stress test. Blood pressure was measured at rest, during the last minute of each stage of exercise, and for 15 minutes after the cessation of exercise. The exercise test was terminated if a patient demonstrated an abnormal response to exercise. Contraindications to exercise were derived from criteria established by ACSM (Thompson *et al.* 2009). Clinical cover and an on-call physician were available during all testing procedures in the event of an adverse reaction to exercise.

2.1.2.2 Referral from unsuccessful screening

If a participant failed the screening process or showed resting or exercise-induced cardiac abnormalities they were referred to their general physician. A screening report was provided, and the patient was advised to avoid exercise until cleared to do so. All patients who

participated in this series of studies demonstrated a normal cardiopulmonary response to exercise.

2.2 Experimental procedures

2.2.1 Preliminary testing

Patients arrived to the laboratory in a fed and hydrated state to perform an incremental exercise test to determine peak oxygen uptake ($\dot{V}O_{2peak}$) and peak heart rate (HR_{peak}); peak cardio-respiratory parameters of patients participating in chapters 3-5 are provided in Table 2.1. Individual peak cardiorespiratory parameters of patients are presented in respective chapters. To avoid time of peak circulating insulin concentrations, visits were organised such that exercise commenced a minimum of 3 hours following prandial insulin administration (Homko *et al.* 2003). Having established stature and mass (Seca 220, Seca, Germany), capillary blood glucose concentrations were taken (Glucomen Lx+, A. Menarini diagnostics, UK). Participants with a blood glucose concentration < 6.5 $mmol.l^{-1}$ (De Feo *et al.* 2006) consumed a 20g carbohydrate-based hypertonic drink (117ml Lucozade®, GlaxoSmithKline, UK). Patients commenced exercise when blood glucose was ≥ 6.5 $mmol.l^{-1}$ for a minimum of 15 minutes.

Following completion of a standardised warm-up (3 minutes at 6 $km.hr^{-1}$), patients performed a continuous incremental running protocol on a motorised treadmill (Woodway ELG, Woodway Inc, USA), as per West et al (2010). The protocol comprised of a series of 3 minute stages of steady-state exercise, starting at a speed of 8 $km.hr^{-1}$ and increasing by 1 $km.hr^{-1}$ per stage. A gradient was set at 1% to reflect the running cost of outdoor running (Jones and Doust 1996). Expired air was measured using an online gas analyser (Metalyser 3B, Cortex, Germany), and HR via online telemetry (RS400, Polar, Polar Global, Finland). Strong verbal encouragement was given throughout. The test was terminated upon volitional exhaustion. It was considered a maximal effort if patients met any two of the following criteria: a respiratory exchange ratio (RER) of 1.12 or greater, ≥ 90% age predicted maximum HR (220-age), a rating of perceived exertion (RPE) of 18 and / or a distinct plateau in oxygen consumption

(Day *et al.* 2003, Midgley *et al.* 2007). Patients were monitored closely during and after exercise by observation for symptoms of hypoglycaemia (pallor, confusion, or presyncope). Patients were discharged following completion of the exercise test if blood glucose was ≥ 6.5 $mmol.l^{-1}$ for a minimum of 15 minutes.

2.2.1.1 Quantification of peak aerobic capacity and trial running speed

The peak rate of O_2 consumption ($\dot{V}O_{2peak}$) was defined as the greatest volume of O_2 attained during the last stage of the test. The speed at which this occurred was defined as absolute $v\dot{V}O_{2peak}$. A linear regression equation was used plotting $\dot{V}O_2$ against running speed ($km.h^{-1}$) to determine relative $v\dot{V}O_{2peak}$. This value represents the estimated speed at which $\dot{V}O_{2peak}$ would be attained if treadmill speed was not limited by the protocol stage speed (Jones and Doust 1996, Bishop *et al.* 1998). This value was then used to calculate a treadmill running speed which would elicit 70% $\dot{V}O_{2peak}$:

$\alpha + (\beta * \dot{V}O_2) = \text{Relative } v\dot{V}O_{2peak}$

Note: α = the slope of the regression line, β = the point of intercept of the regression line and γ axis.

Trial velocity = Relative $v\dot{V}O_{2peak}$ * 0.7.

Table 2.1 Patient peak cardiorespiratory characteristics across experimental chapters

	Chapter 3	**Chapter 4**	**Chapter 5**
$\dot{V}O_{2peak}$ ($l.min^{-1}$)	3.5 ± 0.5	3.4 ± 0.6	3.6 ± 0.9
$\dot{V}O_{2peak}$ ($ml.kg.min^{-1}$)	53.1 ± 1.1	51.9 ± 1.2	51.3 ± 2.1
$v\dot{V}O_{2peak}$ ($km.hr^{-1}$)	13.2 ± 0.5	14.4 ± 0.3	14.1 ± 0.4
HR_{peak} (bpm)	199 ± 2	192 ± 2	201 ± 2

Note: Data presented as mean ± SEM.

2.2.2 General study design

All studies were a randomised, counterbalanced, cross-over design. Randomisation and counterbalancing was determined via simulated computer programme. Prior to main trials, patients recorded and replicated their diets and activity patterns for a total of 24 hours prior to their laboratory visit. During this time glycaemia was monitored using subcutaneous continuous interstitial glucose monitoring (CGM). All main trials were conducted at the Newcastle NIHR Clinical Research Facility, separated by a minimum of 7 days. Glycaemia was further monitored following discharge from the laboratory, so that glycaemic responses were captured for a minimum of 24 hours after the performance of exercise. Diet and activity were recorded during this time.

2.2.3 Continuous interstitial glucose monitoring systems

Patients were fitted with CGM (Figure 2.2) a minimum of 48 hours before attending the laboratory on each occasion, so that data during the 24 hours prior to laboratory attendance was not influenced by the initialisation period of the sensor. Across chapters, patients wore either an iPro2 (chapter 3; Medtronic Diabetes, Medtronic Minimed, USA) CGM which was blinded, or a Paradigm Veo (chapter 4 and 5; Medtronic Diabetes, Medtronic Minimed, USA) CGM which was unblinded and reported interstitial glucose in real-time. Enlite sensors (Enlite, Medtronic Diabetes, Medtronic Minimed, USA) were used for both CGM. The sensor is a glucose-oxidase based platinum electrode, which, enclosed in a waterproof casing, is secured by applying a dressing. Enzyme-mediated oxidation of glucose in the interstitial fluid generates an electrical current that is transmitted to the CGM. Sensor readings are acquired every 10 seconds, and averaged values are reported every 5 minutes.

The sensor was inserted into the subcutaneous tissue of the anterior-superior abdomen (Figure 2.2) to minimise the physiological time-lag between blood and interstitial concentrations (Keenan *et al.* 2012). The insertion site was taken as equidistant between the most medial portion of the iliac crest and navel which was marked with indelible ink, so that initial

placement was replicated on subsequent insertions. Sensor fit was checked upon arrival and discharge from the laboratory. During CGM wear, patients were provided with a glucose testing meter (GlucoMen Lx+, A. Menarini diagnostics, UK; see 2.4) and lancets to obtain blood glucose values for calibration purposes. Data was captured for a total of 24 hours after exercise, at which point the CGM and sensor was removed. A new sensor was used for each experimental trial. Reliability and validity were established for both CGM devices and the glucose testing meter against venous blood glucose (Appendix C); measures were comparable to findings in previous literature (Kubiak *et al.* 2004).

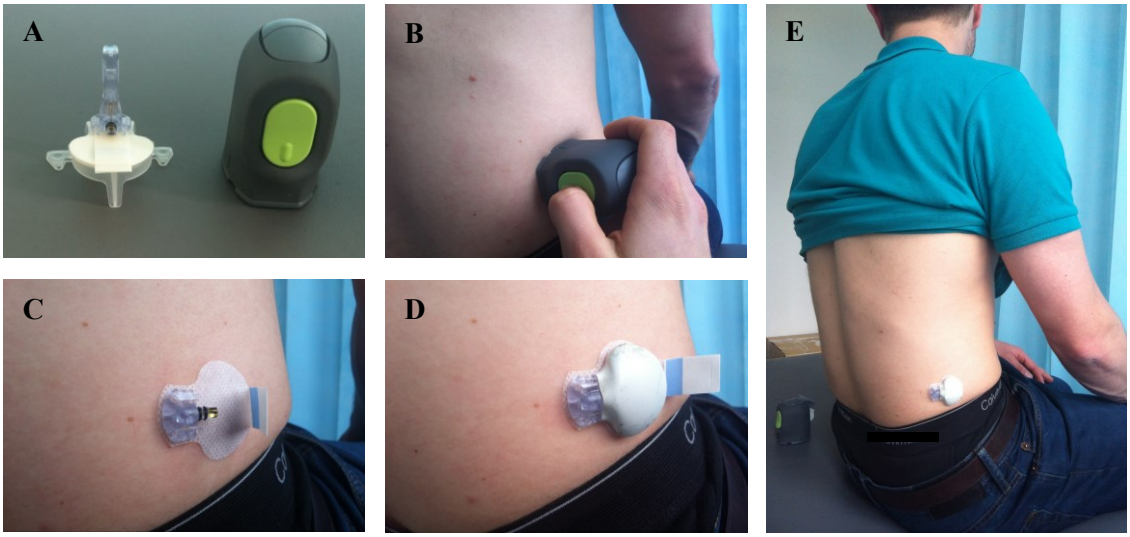

Figure 2.0 Insertion and fitting of the Enlite sensor and Medtronic transmitter. **A** Enlight sensor in protective casing (left) and automated insertion device (right). **B** Enlight sensor inserted into anterior-superior abdomen on a site with sufficient adiposity to minimise discomfort. Insertion site was replicated between trials. **C** Removal of insertion device leaving imbedded sensor in subcutaneous tissue. **D** Fitting of either Ipro2, or CGM transmitter. **E** Fully fitted sensor and transmitter.

2.2.3.1 The Medtronic iPro2 CGM

Patients used the iPro2 CGM during study 1. The iPro2 consists of a recording device that is attached to the indwelling sensor (Figure 2.0). The sensor outputs are stored in the device's memory for download and analysis after removal. Real-time interstitial glucose readings are not available to patients, therefore ensuring blinded continuous interstitial glucose readings.

For calibration purposes, patients were required to record a minimum of 4 blood glucose readings per day, which were retrospectively entered into the CGM software (CareLink, Medtronic Diabetes, Medtronic Minimed, USA) for automatic calibration through modified linear regression. Following processing, an average interstitial glucose value was attained for each 5 minute period over the duration of wear. Raw data was transferred to Microsoft® excel (Microsoft, USA) for analysis.

2.2.3.2 The Medtronic Paradigm Veo Real Time CGM

Real-time CGM (Paradigm Veo; Figure 2.1) was used during studies 2 and 3 as a safety precaution to falling blood glucose concentrations at night, a concern raised by the Research Ethics Committee. Glucose alerts were set at ≤3.5 and ≥16 $mmol.l^{-1}$ during the pre-trial period, the upper alert was discontinued once patients left the laboratory after experimental trials. The Paradigm Veo system is primarily used for subcutaneous insulin infusion with an in-built CGM. Patients did not use the insulin infusion facility, and maintained their usual basal-bolus regimen. A transmitter is attached to the sensor which signals the sensor readings to a portable device that displays interstitial glucose concentrations on-screen (Figure 2.1). For calibration purposes, patients were required to input blood glucose values 4 times per day. Patients were informed to take calibration blood glucose values at standardised times which were replicated between trials; alarms were set to remind patients.

Figure 2.1 Medtronic Paradigm Veo Real Time CGM. Receiver, transmitter and sensor.

2.2.4 Diet and activity replication

In the 24 hours preceding their arrival to the laboratory, patients were required to replicate their diet and eating patterns, which were assessed using weighed dietary recording sheets (Appendix D). Patients were required to weigh individual foodstuffs consumed during this time and describe items in as much detail as possible, retaining packaging and nutritional information. Patients were required to note down meal times and additional carbohydrate intake to correct falling blood glucose concentrations. In addition, patients recorded their insulin regimen noting injection time and dose of basal and bolus insulin administration, detailing additional rapid-acting insulin units administered to correct high blood glucose concentrations. Online programs and nutrition analysis software (Microdiet, Downlee systems LTD, UK) were used to determine the composition and nutritional content of individual foods. Raw data was transferred to Microsoft® excel (Microsoft, USA) for dietary analysis. During this time patients were instructed to avoid caffeine, and strenuous activity. Activity patterns were recorded using a pedometer (Walking style pro, Omron,Omron Healthcare Europe B.V., Hoofddrop, The Netherlands) adjusted for individual stride length. Pedometer placement was standardised (attached onto the belt loop of trousers). The pedometer was tested for repeatability over different speeds (Appendix E).

2.2.5 Standardised meals

For chapter 4A-C and chapter 5A-B patients consumed a total ~5.0 g.carbohydrate.kg^{-1}BM over the course of each trial day. This was estimated to provide enough carbohydrate to cover the cost of the exercise bout, providing a positive energy balance post-exercise. This was chosen so that an assessment of: GI (chapter 4A), and basal dose (chapter 5A), on post-prandial glycaemia could be made without being influenced by inadequate carbohydrate intake. This was achieved by providing patients with standardised meals. The combined macronutrient content for all meals collectively was: carbohydrate = ~77%, fat = ~12%, and protein = ~11%. Carbohydrate intake and macronutrient content aimed to match current

recommendations for exercising type 1 diabetes patients (Riddell and Perkins 2006, Perry and Gallen 2009). The glycaemic index (GI) was determined for all meals.

2.2.5.1 Glycaemic index testing

The GI of all test meals were calculated using the methods described by Wolever and Jenkins *et al* (1986) (refer to *Calculation of blood and interstitial glucose area under the curve*; see 2.6). Testing was conducted using non-diabetic controls, following the procedures outlined by Brouns et al (2005). In a randomised and counterbalanced fashion, either a test food or a standard was administered on each separate occasion, so that the test food and the standard were repeated three times by each participant. The standard comprised of a ~10% glucose solution dissolved in still water (75g Dextrose, 750ml water). The standard and test meal were both adjusted relative to body mass (1.0 g.carbohydrate.kg^{-1}BM). A baseline blood sample (1 ml venous blood) was taken, with further periodic sampling at 15 minute intervals up to 135 minutes. All blood samples were analysed for blood glucose using a Biosen (EKF Diagnostic GmbH, Germany; see 2.2.8.1). Following the baseline blood sample, participants consumed either the test food or standard within a 5 minute period.

The area under the glycaemic-response curve (see 2.6) for each food was expressed as a percentage of the mean response to the standard food for each participant, and then averaged to obtain the GI value for the food. If an individual's value was > 2 standard deviations from the mean, it was considered an outlier. If individuals demonstrated an unrepresentative response to a test food, their results were cross-checked with their response to the standard. If their response to the standard was normal, the test meal was repeated. If a participant's response to the standard was idiosyncratic, their data were removed from analysis. A GI value was obtained and classified as either low (<55), moderate (56-75), or high (>76) (Kirpitch and Maryniuk 2011). A summary of the composition of each standardised, experimental meal is provided in table 2.2.

2.2.5.2 Pre-laboratory standardised meals (Chapter 4 and Chapter 5)

In studies 2 and 3 patients received two standardised meals that were consumed on the day of the trial, and before arrival. The composition of the meals was based on the habitual dietary patterns of type 1 diabetes patients (assessed from weighed food diaries from chapter 3 and an online questionnaire). Both meals were tested for palatability. Participants were required to replicate eating times (breakfast ~08:00, lunch ~13:00).

2.2.5.3 Pre-trial meal 1: MEAL 1

The first meal was a cereal-based breakfast meal (frosted flakes, semi-skimmed milk, and peaches) equating to 1.3 g.carbohydrate.kg^{-1} BM (Table 2.2). Fibre content was negligible across studies.

2.2.5.4 Pre-trial meal 2: MEAL 2

The second meal was a pasta-based lunch (pasta, tomato-based sauce, cheddar cheese, olive oil) equating to 1.3 g.carbohydrate.kg^{-1} BM (Table 2.2). Fibre content was negligible across studies.

2.2.5.5 Laboratory test meals

Meals consumed in the laboratory were designed to cover the estimated energy cost of the exercise bout. Patients drank water *ad libitum*.

2.2.5.6 Pre-exercise meal: MEAL 3

The pre-exercise meal was consistent across all three studies. The pre-exercise meal was cereal-based (frosted flakes, semi-skimmed milk, and peaches) equating to 1.0 g.carbohydrate.kg^{-1}BM (Table 2.2). Fibre content was negligible across studies.

2.2.5.7 Post-exercise meal (Chapter 3): MEAL 4

The post-exercise meal for chapter 3 was a pasta-based lunch (pasta, tomato-based sauce, cheddar cheese, olive oil) equating to 1.0 g.carbohydrate.kg^{-1} BM (Table 2.2). Fibre content was negligible across studies.

2.2.5.8 Post-exercise meals (Chapter 4): MEAL 5 and 6

Each meal was designed to be isoenergetic and was matched for macronutrient content, equating to 1.0 g.carbohydrate.kg^{-1} BM, but differing in GI (low versus high).

The low GI meal consisted of basmati rice, tomato-based sauce, turkey breast and a isomaltulose orange flavoured drink [10% solution] (Table 2.2). Fibre content was negligible across studies. Food provided 29.8% of carbohydrates, and the drink 70.2%.

The high GI meal (jasmine rice, tomato-based sauce, turkey breast; maltodextrin orange flavoured drink [10% solution]) (Table 2.2). Fibre content was negligible across studies. Food provided 29.8% of carbohydrates, and the drink 70.2%.

2.2.5.8 Post-exercise meal (Chapter 5): MEAL 7

The low GI post-exercise meal from chapter 5 was adopted in this study. The meal (basmati rice, tomato-based sauce, turkey breast; isomaltulose orange flavoured drink [10% solution]) (Table 2.2). Fibre content was negligible across studies. Food provided 29.8% of carbohydrates, and the drink 70.2%.

2.2.5.9 Bedtime snack (Chapter 4): MEAL 8 and 9

Each snack was designed to be isoenergetic and was matched for macronutrient content, equating to 0.4 g.carbohydrate.kg^{-1} BM as per current recommendations for patients exercising in the evening (Hernandez *et al.* 2000). However, each meal differed in GI (low versus high).

The low GI snack (burgen sliced bread [soya and linseed]; isomaltulose orange flavoured drink [10% solution]) (Table 2.2). Fibre content was negligible across studies. Food provided 47.3% of carbohydrates, and the drink 32.9%.

The high GI snack (white sliced bread; maltodextrin orange flavoured drink [10% solution]) was calculated to have a GI of 86. (Table 2.2). Fibre content was negligible across studies. Food provided 47.3% of carbohydrates, and the drink 52.7%.

2.2.5.10 Bedtime snacks (Chapter 5): MEAL10

The bedtime snack in chapter 5 was adopted from the low GI snack in chapter 4 (burgen sliced bread [soya and linseed]; isomaltulose orange flavoured drink [10% solution]) (Table 2.2). Fibre content was negligible across studies. Food provided 47.3% of carbohydrates, and the drink 52.7%.

2.2.5.11 Subsequent morning meal: MEAL 11

The subsequent morning breakfast meal for chapter 5 was a cereal-based meal (frosted flakes, semi-skimmed milk, and peaches) equating to 1.0 g.carbohydrate.kg^{-1} (Table 2.2). Fibre content was negligible across studies.

Table 2.2 Macronutrient composition of experimental meals

Meal	Meal code	Macronutrient composition (%)			Glycaemic index (GI)	Ingredients and manufacturer
		CHO	FAT	PRO		
Pre-trial meal 1	MEAL 1	87.1	2.8	10.1	57	Milk (semi-skimmed; Tesco), Frosted flakes (sugar coated corn flakes, Tesco), Peaches (peach halves, Tesco).
Pre-trial meal 2	MEAL 2	60.3	21.8	17.9	57	Pasta (penne pasta, Tesco), Tomato-based sauce (bolognaise sauce, Tesco), Cheese (Cheddar, Tesco), Olive oil (refined virgin olive oil, Tesco).
Pre-exercise meal	MEAL 3	87.1	2.8	10.1	57	Milk (semi-skimmed; Tesco), Frosted flakes (sugar coated corn flakes, Tesco), Peaches (peach halves, Tesco).
Post-exercise meal (chapter 3)	MEAL 4	60.3	21.8	17.9	57	Pasta (penne pasta, Tesco), Tomato-based sauce (bolognaise sauce, Tesco), Cheese (Cheddar, Tesco), Olive oil (refined virgin olive oil, Tesco).
LGI Post-exercise meal (chapter 4)	MEAL 5	85.8	2.4	11.8	37	Basmati rice (Tesco), Tomato-based sauce (bolognaise sauce, Tesco), Turkey breast (Turkey breast pieces, Tesco), Isomaltulose powder (supplied by Beneo™).
HGI Post-exercise meal (chapter 4)	MEAL 6	85.8	2.4	11.8	92	Jasmine rice (Tesco), Tomato-based sauce (bolognaise sauce, Tesco), Turkey breast (Turkey breast pieces, Tesco), Dextrose powder (supplied by Beneo™).
Post-exercise meal (chapter 5)	MEAL 7	85.8	2.4	11.8	37	Basmati rice (Tesco), Tomato-based sauce (bolognaise sauce, Tesco), Turkey breast (Turkey breast pieces, Tesco), Isomaltulose powder (supplied by Beneo™).
LGI Bedtime snack (chapter 4)	MEAL 8	86.8	2.5	9.5	86	Burgan bread (sliced soya and linseed burgan loaf, Tesco), Isomaltulose powder (supplied by Beneo™).
HGI Bedtime snack (chapter 4)	MEAL 9	86.8	0.3	12.9	86	White bread (sliced white loaf, Tesco), Dextrose powder (supplied by Beneo™).
Bedtime snack (chapter 5)	MEAL 10	86.8	2.5	9.5	86	Burgan bread (sliced soya and linseed burgan loaf, Tesco), Isomaltulose powder (supplied by Beneo™).
Subsequent morning meal	MEAL 11	87.1	2.8	10.1	57	Milk (semi-skimmed; Tesco), Frosted flakes (sugar coated corn flakes, Tesco), Peaches (peach halves, Tesco).

Note: Cooking methods and times were standardised.

2.2.6 Self-administered insulin

2.2.6.1 Insulin regimen of patients

The insulin regimen of patients across studies is presented in Table 2.2. All patients were treated on a stable basal-bolus regimen for a minimum of 6 months prior to enlistment. The basal component consisted of insulin Detemir (Levemir, NovoNordisk, Denmark) or Glargine (Lantus, Sanofi Aventis, France), and the bolus component consisted of rapid-acting insulin Lispro (Humalog, Lilly, USA) or Aspart (Novorapid, NovoNordisk, Denmark). Although insulin Glargine and Detemir carry different pharmacodynamic properties (Porcellati *et al.* 2007), they both exhibit a peak-less action-time profile over a 24 hour period (Gulve 2008), are unaffected by exercise (Peter *et al.* 2005), and are promoted as equal in clinic, despite different metabolic effects in the 12 hours after administration (Heller *et al.* 2009). Insulin Lispro and Aspart have been demonstrated to have similar action-time profiles (Plank *et al.* 2002), and metabolic effects (Homko *et al.* 2003).

In chapter 3, all patients treated with insulin Detemir (n=3) administered bi-daily, injecting on a morning and on an evening / before bed. All patients treated with insulin Glargine (n=8) administered once daily, with 50% administering on a morning and 50% administering on an evening / before bed. In chapter 4, all patients were treated on insulin Glargine and Aspart only, administering insulin Glargine once daily either on a morning (50%) or on an evening / before bed (50%). In chapter 5, patients were treated on insulin Glargine (n=8) or Detemir (n=2), and Aspart only, administering insulin Glargine once daily either on a morning (50%) or on an evening / before bed (50%). Patients treated with insulin Detemir administered bi-daily.

Table 2.3 Patients' insulin regimen across experimental chapters

		Patient ID											
Chapter	**Insulin IU**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Mean±SEM**
3 A-B	**Basal**	38^{G}_{M}	20^{D}_{B}	22^{G}_{M}	26^{D}_{B}	34^{G}_{E}	18^{D}_{B}	20^{G}_{E}	31^{G}_{M}	24^{G}_{M}	30^{G}_{E}	20^{G}_{E}	26 ± 2
	Bolus	1^{L}	1^{A}	1^{A}	1^{A}	1^{A}	0.8^{A}	1^{A}	1^{A}	1^{A}	1^{A}	1^{A}	1.0 ± 0.0
4 A-B	**Basal**	20^{G}_{E}	30^{G}_{M}	38^{G}_{M}	20^{G}_{E}	24^{G}_{E}	26^{G}_{M}	14^{G}_{M}	20^{G}_{E}	34^{G}_{M}	43^{G}_{E}	-	27 ± 3
	Bolus	1^{A}	1^{A}	1^{A}	0.8^{A}	1^{A}	1^{A}	1^{A}	1.3^{A}	0.8^{A}	1^{A}	-	1.0 ± 0.0
5 A-B	**Basal**	26^{G}_{M}	30^{G}_{M}	38^{D}_{B}	20^{G}_{E}	26^{G}_{M}	14^{G}_{M}	44^{D}_{B}	31^{G}_{E}	43^{G}_{E}	52^{G}_{E}	-	32 ± 4
	Bolus	1^{A}	1^{A}	1^{A}	0.8^{A}	1^{A}	0.5^{A}	1.5^{A}	1^{A}	1^{A}	1^{A}	-	1.0 ± 0.0

Note: G = Glargine, D = Detemir, A = Aspart, L = Lispro, M = once daily (morning), E = once daily evening , B = bi-daily; bolus insulin calculated per 10g CHO. Patients administered bolus insulin based on their individual carbohydrate-insulin ratio.

2.2.6.2 Bolus dose administration

Patients self-administered all rapid-acting insulin into abdominal sites to avoid exercising musculature, injecting on the contralateral site of the previously administered dose at each injection time. In an attempt to minimise the influence of injection location on insulin absorption kinetics, the site of bolus injection was standardised using prominent anatomical landmarks (equidistant from the most medial portion of iliac crest and navel) which was marked with indelible ink. Particular care was taken to avoid potential sites of insulin lipoatrophy (Kiivisto and Felig 1980, Young *et al.* 1984). Bolus dose was calculated using the carbohydrate counting method of rapid-acting insulin units per 10g carbohydrate consumed. Across chapters patients administered a 25% (75% reduced) dose of rapid-acting insulin with the pre-exercise meal, as per current recommendations (West *et al.* 2010, West *et al.* 2011).

2.2.7 Exercise protocol

Patients performed 45 minutes of treadmill running at a speed calculated to elicit 70% $\dot{V}O_{2peak}$ (see 2.2.3.1) on each visit across chapters. The exercise intensity and duration was chosen such

that the bout of exercise fell within the recommendations of the ACSM for exercising type 1 diabetes patients (see 1.2).

2.2.8 Cannulation and blood sampling

All participants were instructed to arrive to the laboratory in a hydrated state to aid in vein palpability and blood draws. In a seated or supine position, a 20-gauge cannula (Vasofix®, B.Braun Melsungen AG, Germany) was inserted into the antecubital vein of the non-dominant arm and secured with a dressing (Tegadrern™ I.V., 3M Health Care , Germany). For comfort and ease of use, a Stylet (Madrin/Stylet Introcan®, B Braun, Germany) was used to keep the cannula patent during exercise and rest periods. Periodic infusion of saline during rest was used to flush the cannula at regular intervals.

Blood was sampled via the cannula by connecting a multi-sample leur encased in a sampling barrel to the leur connection. A total 12 ml of whole blood was collected using 2 vacutainers®, a Lithium-Heparin tube (6 ml), and a serum separation tube (6 ml) during each sample point. Two ml of blood were used to determine blood glucose and lactate concentrations (see 2.2.8.1) and determine plasma volume (see 2.2.8.2) before both vacutainers were held on ice and centrifuged for 15 minutes at 3000 $rev.min^{-1}$ at 4˚C; samples were centrifuged within 5 minutes from being drawn. Plasma and serum were extracted, and stored in 5 ml aliquot tubes at -80˚C. On one visit, an additional 5 ml of whole blood was sampled at rest using a K^+ EDTA vacuainer®. This was analysed for glycosylated haemoglobin (HbA_{1c}) via standard routine of the Clinical Biochemistry Department of the Royal Victoria Infirmary, Newcastle-upon-Tyne.

2.2.9 Quantification of blood, serum and plasma analytes

2.2.9.1 Blood glucose and lactate - Biosen C_line blood glucose and lactate analyser

The Biosen C_line (EKF Diagnostic GmbH, Germany; Figure 2.2) system is designed to quantitatively determine blood glucose and lactate concentrations. Concentrations are

determined by comparison to a calibration standard (a known concentration of a glucose and lactate), which are run through two measurement channels for each metabolite. The system operates using microchip sensors which transforms the sample concentration (glucose and lactate) into an evaluable electrical signal, producing an on-screen reading. The blood sample is collected via an 20 µL end-to-end capillary tube which is sodium heparinised for anticoagulation, then hemolysed in a 1 mL micro test tube. The tube is then placed into a sample tray which is automatically recognized and processed. Tests of reliability were conducted on blood glucose samples ranging from hypoglycaemia to severe hyperglycaemia (Appendix F), and blood lactate over a range of concentrations (Appendix G).

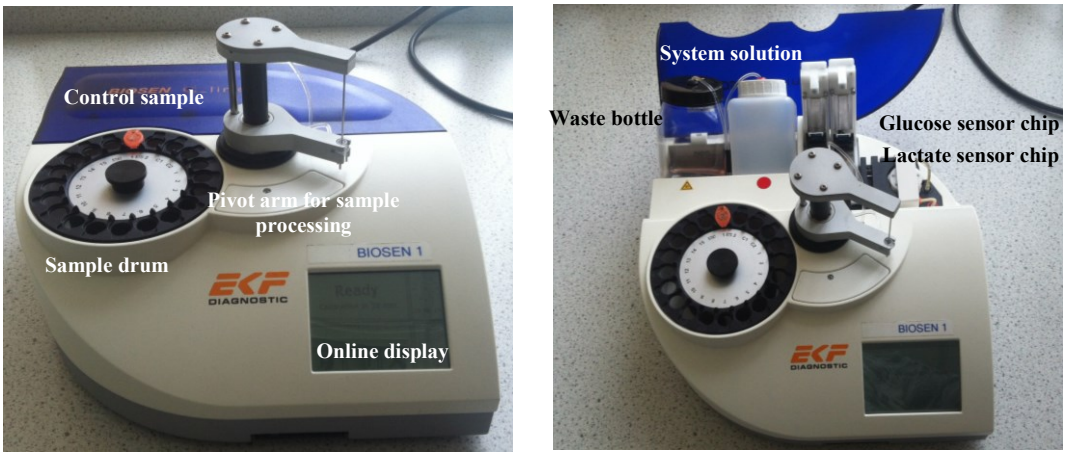

Figure 2.2 Biosen C_Line, determination of blood glucose and lactate.

2.2.9.2 Haematocrit, haemoglobin and the calculation of plasma volume

Haematocrit and haemoglobin were determined using the HemoCue Hb 201^{+} (EKF Diagnostic GmbH, Germany). Whole blood is collected in a microcuvettte that is analysed using a modified azidemethemoglobin reaction. Erythrocyte membranes are disintegrated by sodium deoxycholate that release haemoglobin, and sodium nitrate converts the haemoglobin iron from the ferrous to the ferric state to form methemoglobin, this then combines with azide to form azidemthemoglobin. Once a steady state has been reached following the reaction, haematocrit and haemoglobin concentrations are displayed on screen. Plasma volume, and

changes in plasma volume were determined using the methods of Dill and Costill (1974) (Appendix H).

2.3 Determination of hypoglycaemia and hyperglycaemia

Hypoglycaemia was defined as a blood or interstitial glucose concentration of ≤ 3.9 mmol.l^{-1}, and hyperglycaemia defined at glucose ≥ 8.0 mmol.l^{-1}. A duration of 10 minutes at or below each threshold, which equated to three consecutive interstitial glucose readings, were required for the determination of hypo- or hyperglycaemia. If patients demonstrated a symptomatic hypoglycaemic episode within 24 hours prior to a main experimental trial, their visit was rescheduled. Patients were monitored closely during the post-exercise period by observation for symptoms of hypoglycaemia (pallor, confusion, or presyncope). If blood glucose dropped < 3.5 mmol.l^{-1} (Rabasa-Lhoret *et al.* 2001) patients received a bolus of carbohydrate (20 g carbohydrate; Lucozade$_{®}$, GlaxoSmithKline, UK).

2.4 Self-recorded capillary blood glucose and ketone measurements

Patients used the Glucomen LX capillary blood glucose and ketone meter (GlucoMen Lx+, A. Menarini diagnostics, UK). Tests of reliability were conducted on capillary blood glucose (ranging from hypoglycaemia to severe hyperglycaemia) and a range of blood ketone samples (Appendix C).

2.5 Serum and plasma analytes

All biochemical analysis was conducted first hand, and in-house. All samples were thawed at room temperature, and placed on a vortex before analysis. All reagents were prepared to manufacturer's specifications. All assays composed of standards and quality controls (QC) in duplicate, with samples in singlet. For calculation of concentrations, a standard curve was constructed and results from QCs and samples were calculated from the curve using a computer software package (Multicalc®; PerkinElmer, Wallac OY; Finland). The standard curve was calculated by plotting the mean absorbance for each standard on the linear y-axis

against the concentrations on a logarithmic x-axis, a line of best fit was applied to the curve, and sample concentrations determined from the mean absorbance for the standard curve: whereby percentage absorbance = (B – blank OD)/(B_o – blank OD), B = OD of sample or standard, and B_o = OD of zero standard (total binding). Concentrations were presented as per standard convention. Optical density was determined using a plate illuminometer (Microplate illuminometer LB 96P, EG&G Berthold, Germany). The coefficient of variance for all assays conducted was < 10%, as determined from standards on each plate. A summary of the assays used to determine hormones, metabolites and cytokines measured across studies is provided in Appendix I. Concentrations derived as a molarity ($g.mol^{-1}$) then converted to mass ($pg.ml^{-1}$) by multiplication of the molecular mass of the peptide by the peptide molarity; the reverse was performed for concentrations derived in molecular mass and typically presented as a molarity (Appendix J).

2.5.1 Insulin

The Invitron insulin assay (Invitron Ltd, Monmouth, UK) was used to analysis serum insulin. The insulin assay is a two-site immunoassay, which employs a solid base phase insulin antibody immobilised on microtitre wells, and a soluble insulin antibody labelled with a chemiluminescent acridinium ester. Labelled insulin antibody is added to each well followed by either a standard, QC or sample. Following an incubation period and a wash, optical density was determined. Concentrations were established in $pmol.l^{-1}$.

The Invitron assay is 100% cross-reactive with insulin Lispro, Aspart, and Glargine, and 300% cross reactive with Determir (Pennartz *et al.* 2011). Therefore, all patients treated with insulin Determir were excluded from the analysis of serum insulin. In addition, the insulin assay is also 100% cross reactive with human insulin, however, all patients in this series of studies had long-standing type 1 diabetes and were solely dependent upon exogenous insulin administration, thus, the influence of any residual β-cell function was considered negligible (Wang *et al.* 2012). When basal dose was maintained (chapters 3 and 4), changes in insulin

concentrations detected by this assay were considered to be due to the appearance / disappearance of rapid-acting insulin analogues. Conversely, where bolus administration was matched in trials (chapter 5), changes were considered to be due to the appearance / disappearance of insulin Glargine. The coefficient of variance for all insulin assays conducted was 7.4 ± 1.1%, as determined from standards on each plate.

2.5.2 Glucagon

Plasma glucagon was analysed using a competitive enzyme immunoassay (Glucagon EIA RAB0202; Sigma Aldrich, MD, USA). The microplate kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-glucagon antibody, both biotinylated glucagon peptide and peptide standard or targeted peptide in samples interacts competitively with the glucagon antibody. Uncompleted, or bound, biotinylated glucagon peptide then interacts with streptavidin-horseradish peroxidase (SA-HRP), which catalyses a colour development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-HRP complex and inversely proportional to the amount of glucagon peptide in the standard or samples. This is due to the competitive binding to glucagon antibody between biotinylated glucagon peptide and peptides in standard or samples. The coefficient of variance for all glucagon assays conducted was 6.9 ± 2.1%, as determined from standards on each plate.

2.5.3 Catecholamines (Adrenaline and Noradrenaline)

Plasma adrenaline and noradrenaline were analysed using the CatCombi enzyme-linked immunosorbent assay (ELISA) kit (IBL, Europe Ltd). The test consists of 2 stages, an extraction phase and an assay phase. The extraction phase consists of an extraction plate to which standards, QC or samples are added. Deionised water is added to all wells to correct for differences in volume. The extraction plate then undergoes a number of incubations and washes, in which an acetylation reaction occurs producing an “extracted sample”. The samples, standards ad QCs are then ready for assay. Adrenaline and noradrenaline anti-serum

is added before a further incubation. Optical density was immediately determined. Concentrations were derived as a mass ($pg.ml^{-1}$) then converted to molarity ($nmol^{-1}$) by division of the molecular mass of the peptide by the peptide molarity (Appendix J). The coefficient of variance for all insulin assays conducted was 9.1 ± 0.8%, as determined from standards on each plate.

2.5.4 Cortisol

Serum cortisol concentrations were determined using the Parameter™ Cortisol Assay (R&D Systems, Minneapolis, USA). For this assay, serum samples require a 20-fold dilution; therefore, 20µl of the serum sample was mixed with 380µl of calibrator diluent. The assay uses a cortisol conjugate (horseradish peroxidase with red dye and preservatives). To which a primary antibody solution (mouse monoclonal antibody to cortisol in buffer with blue dye with preservatives) is added followed by a series of incubation periods and washes before two colour regents (stabilised hydrogen peroxide and stabilised chromogen (tetramethylbenzidine)) are added. Following a further incubation period optical density is determined. The concentrations read from the standard curve were multiplied by the dilution factor to account for dilution. Concentrations were derived as a mass ($pg.ml^{-1}$) then converted to molarity ($nmol.l^{-1}$) by division of the molecular mass of the peptide by the peptide molarity (Appendix J). The coefficient of variance for all insulin assays conducted was 9.0 ± 0.7%, as determined from standards on each plate.

2.5.5 Non-esterified fatty acids (NEFA)

The enzymatic non-esterified fatty acids (NEFA) assay (NEFA, Randox Laboratories, UK) was used in accordance with the Randox Daytona Plus (Randox Daytona Plus, Randox Laboratories, UK) for the determination of NEFA concentrations. The assay uses direct photometry to measure a coloured endpoint from the following reaction:

$$\text{1) NEFA + ATP + COA} \xrightarrow{\text{Acyl CoA Synthetase}} \text{Acyl CoA + AMP + PPi}$$

$$\text{2) Acyl CoA + AMP + PPi} \xrightarrow{\text{Acyl CoA Oxidase}} \text{2,3,-trans-Enoyl-CoA +} H_2O_2$$

$$\text{2) 2,3,-trans-Enoyl-CoA +} H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Purple adduct + } 4H_2O$$

Concentrations are automatically determined using Randox Daytona Plus computer software (Randox Daytona Plus, Randox Laboratories, UK), and derived in $mmol.l^{-1}$. The coefficient of variance for all insulin assays conducted was 8.3 ± 1.2 %, as determined from standards on each plate.

2.5.6 β-Hydroxybutyrate

Serum β-hydroxybutyrate was chosen as the primary marker of ketone body formation. β-hydroxybutyrate is the most abundant ketone body and is more sensitive to changes in acid-base balance than acetoacetate (3:1 or greater) (Laffel 2000). As ketoacidosis normalises, there is a coincidental conversion of β-hydroxybutyrate to acetoacetate, driven by an oxidized state in the hepatocytes, meaning that whilst acetoacetate levels plateau, β-hydroxybutyrate and overall ketone body levels are decreasing (Davidson 1998). In addition, urinary ketone tests which utilise acetoacetate are unreliable for monitoring recovery due to differing and unpredictable reabsorption rates of ketone-bodies in the kidneys (Sulway and Malins 1970). Moreover, acetoacetate is likely to be detected in urine long after blood concentrations have returned to normal levels (Sulway and Malins 1970).

The enzymatic β-hydroxybutyrate assay (β-hydroxybutyrate, Randox Laboratories, UK) was used in accordance with the ILab300 Plus (ILab 300 Plus Chemistry Analyser, Instrumentation Laboratory, Werfen Group, Spain) for the determination of β-hydroxybutyrate concentrations. The assay uses direct photometry to measure a coloured endpoint from the following reaction:

$$\beta\text{-3-Hydroxybutyrate} + NAD^{+} \xrightarrow[\text{Dehydrogenase}]{3-\text{Hydroxybutyrate}} \text{Acetoacetate} + NADA + H^{+}$$

Concentrations are automatically determined using ILab300 Plus computer software (ILab 300 Plus Chemistry Analyser, Instrumentation Laboratory, Werfen Group, Spain), and derived in $mmol.l^{-1}$. The coefficient of variance for all insulin assays conducted was 8.8 ± 1.1%, as determined from standards on each plate.

2.5.7 Markers of Inflammation

Several studies report chronically, and permanently, elevated levels of inflammatory cytokines at rest in type 1 diabetes patients (Erbağci *et al.* 2001, Targher *et al.* 2001, Galassetti *et al.* 2006). Elevations are influenced by metabolic alterations such as high insulin concentrations, hyperglycaemia and also glycaemic fluctuations, as well as exercise (Brownlee 2001, Esposito *et al.* 2002, Fishel *et al.* 2005, Pedersen and Febbraio 2005). These metabolic disturbances may induce inappropriately elevated levels of inflammatory cytokines, which could potentially carry negative implications on the onset and progression of diabetic complications (Mohamed-Ali *et al.* 2001). Interlukin-6 (IL-6) and Tumour Necrosis Factor alpha (TNF-α) were chosen because amongst all other cytokines, they consistently display the most robust and greatest quantitative changes (Nemet *et al.* 2002, Galassetti *et al.* 2006, Rosa *et al.* 2011).

2.5.7.1 Interlukin-6 (IL-6)

IL-6 was determined via immunoassay (Quantikine HS ELISA Human IL-6 Immunoassay, R&D Systems, Minneapolis, USA). A monoclonal antibody specific for IL-6 is pre-coated onto a microplate. Standards and samples are pipetted into the wells and IL-6 which is present is bound by the immobilised antibody. A wash removes unbound substances before an enzyme-linked polyclonal antibody specific for IL-6 is added. A further wash removes any unbound antibody-enzyme reagent, before a substrate solution is added. Following an incubation period, an amplifier solution is added to the wells. Colour develops in proportion to

the amount of IL-6 bound in the initial step. The colour development is stopped and optical density is determined. Concentrations are derived and presented as $pg.ml^{-1}$. The coefficient of variance for all insulin assays conducted was 4.3 ± 3.2%, as determined from standards on each plate.

2.5.7.2 Tumour Necrosis factor alpha (TNF-α)

TNF-α was determined via immunoassay (ELISA Human TNF-α Immunoassay, R&D Systems, Minneapolis, USA). A monoclonal antibody specific for TNF-α is pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF-α present is bound by the immobilised antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of TNF-α bound in the initial step. The colour development is stopped and optical density is determined. Concentrations are derived and presented as $pg.ml^{-1}$. The coefficient of variance for all insulin assays conducted was 4.9 ± 2.3%, as determined from standards on each plate.

2.5.8 Glucagon-Like Peptide-1 total (GLP-1)

Total GLP-1 was determined via immunoassay (GLP-1 active ELISA, IBL International GmbH, Hamburg, Germany). Streptavidin is pre-coated onto a microplate. Standards and samples are pipetted into the wells. Subsequently, a mixture of biotinylated GLP-1 specific antibody and a horseradish peroxidate conjugated GLP-1 specific antibody is added. Following the first incubation, a "sandwich" immunocomplex of Streptavidin – Biotin-antibody – GLP-1 – HRP conjugated antibody is formed. After washing away any unbound substances, a substrate solution was added which acts in a timed reaction. The colour development is stopped and the optical density is determined. Concentrations are derived and presented as $pmol.l^{-1}$. The coefficient of variance for all insulin assays conducted was 9.3 ± 0.5%, as determined from standards on each plate.

2.6 Calculation of blood and interstitial glucose area under the curve

Area under the curve (AUC) was calculated using the methods described by Wolever and Jenkins (1986) and was time-averaged. In order to account for both an increase and decrease above or below baseline concentrations, respectively, the total area under the response curve was taken. The formula is as follows:

Total AUC = A*t* + B*t* + C*t*

A*t* = [((B – A)/2) + A) * Time (min)] +

B*t* = [((C – B)/2) + B) * Time (min)] +

C*t* = [((D – C)/2) + C) * Time (min)] +

Time average AUC = Total AUC ($mmol.l^{-1}.hour^{-1}$) / (total hours * 60)

Note: Capital letters correspond to respective time points, e.g. A = time point 1. *t* = time between respective time points.

2.7 Calculation of glycaemic variability

Several measures were chosen to characterise multiple aspects of glycaemic variability. Measures include: median, mean and standard deviation, percentage coefficient of variation (CV%), mean amplitude of glycaemic excursions (MAGE), mean of daily differences (MODD), and continuous overall net glycaemic action (CONGA), weighted average of glucose values (M_R), and J index, as per current recommendations (Rodbard 2009) (Table 2.6).

Table 2.4 Measures of glycaemic variability

Measure	Description	Calculation
Median	Identifies the most common value within a data set	The middle values from a data set arranged in numerical order
Mean	Describes the average value over a set period of time for a certain number of individuals	The sum of each value in the data set divided by the number of values in the data set
SD	Simplest tool for assessment of glycaemic variability (Garg *et al.* 2006). Provides information for both minor and major fluctuations but does not distinguish them (Rodbard 2009, 2009)	The sum of each value in the data set subtracted from the mean of all values in the data set, squared, and then divided by the number of values in the data set. The final value is produced from the square root of this
CV%	A description of the magnitude sample values and the variation within them whilst allowing for standardised comparison between patients with different levels of mean glycaemia	=100 X SD / Mean
MAGE	Most popular parameter for assessing glycaemic variability. Designed to assess major glucose changes and exclude minor changes (Molnar *et al.* 1970)	Average amplitude of peaks or nadirs with magnitude greater than 1 SD
MODD	Provides an estimation of inter-day glycaemic variability (Molnar *et al.* 1972)	Absolute mean difference between glucose values obtained at the same time on two consecutive days
CONGA	Assess glucose variability within a predetermined time window (Mcdonnell *et al.* 2005)	The SD of differences in data points measured at regular time intervals
M_R	Provides a measure of stability of glycaemia in comparison with an arbitrary assigned "ideal" glucose value (Wójcicki 1995)	$(1000 \times (LOG^{10}(glucose/100))^3 \times 18$
J index	A measure of quality of glycaemic control (Wojcicki 1995, Sun *et al.* 2010)	= 0.001 x (mean + SD)

Note: SD = standard deviation, CV% coefficient of variation, MAGE = mean amplitude of glycaemic excursions, MODD = mean of daily differences, CONGA = continuous overall net glycaemic action.

2.8 Gas analysis

Expired air was analysed using the Metalyser 3B (Metalyser 3B, Cortex, Germany). The system was turned on a minimum of 30 minutes before calibration to ensure the stabilisation of oxygen and carbon dioxide sensors. Calibration used gases of certified concentrations (17.1% O_2, 5.0% CO_2: BOC, Industrial Gases, Linde AG, Munich, Germany), and a 3 litre syringe (Series 5530, Hans-Rudolph Inc, Kansas City, Missouri, USA) to check sample volume. Expired air samples were collected using a facemask (model 7940, Hans-Rudolph Inc, Kansas City, Missouri, USA), which was connected directly to the breath-by-breath system. Ambient temperature, humidity and pressure were recorded, and expired gas samples corrected to

standard temperature and pressure. Metasoft software (Cortex Biophysik GmbH, Leipzig, Germany) was used to calculate the volume of oxygen and carbon dioxide. The software package uses the Haldane Transformation method.

2.8.1 Estimation of substrate oxidation rates and energy expenditure

Substrate oxidation rates during exercise were estimated from oxygen and carbon dioxide values using stoichiometric equations as described by Frayn (1983):

$$\text{Carbohydrate oxidation } (g.min^{-1}) = 4.55\ \dot{V}CO_2 - 3.21\ \dot{V}O_2$$

$$\text{Lipid oxidation } (g.min^{-1}) = 1.67\ \dot{V}O_2 - 1.67\ \dot{V}CO_2$$

Calculations for carbohydrate utalisation during rest were determined using updated methods of Frayn (1983) by (Jeukendrup and Wallis 2004). The calculations for carbohydrate during exercise are based upon the oxidation of blood glucose only. During exercise at ~70-80% $\dot{V}O_{2peak}$ in non-diabetic individuals, carbohydrate metabolism is met predominantly by oxidation of glucose (80%) and to a lesser extent muscle glycogen (20%) (Jeukendrup and Wallis 2004). Thus, it is possible that carbohydrate oxidation during exercise was over estimated by applying the equations of Frayn (1983). However, it is unknown whether assumptions of substrate utilisation in non-diabetic controls can be applied to individuals with type 1 diabetes. Furthermore, patients in this series of studies heavily reduced rapid-acting insulin dose prior to exercise. As such, patients were typically hyperglycaemic before and at least in part, during exercise, resulting in a greater availability of glucose for oxidation.

Correct calculation of lipid oxidation was important due to the influence of ketonaemia on RER (Frayn 1983). Differences in fat oxidation between equations in the literature are negligible, and ketogenesis was minimised before experimental trials by patients maintaining their usual basal insulin regimen. The equations for lipid oxidation during exercise followed those proposed by Frayn (1983), and those at rest based on the weighted-average of 99% of the

fatty acid composition of human adipose tissue (Péronnet and Massicotte 1991); 1.70 $\dot{V}O_2$ - 1.70 $\dot{V}CO_2$.

Calculations were based on negligible protein oxidation. Protein oxidation is minimal at rest and contributes least to total energy expenditure. In addition, protein oxidation stimulated above basal rates is unusual and only occurs when large quantities of protein are ingested (40 g) or exercise is vastly increased in duration (~6 hours). All studies in this thesis employed test-meals / drinks containing less than 40g protein.

In further support of these methods, recent studies in type 1 diabetes patients have followed these assumptions (West *et al.* 2010, West *et al.* 2011, West *et al.* 2011). The examination of indirect calorimetry was not underpinning to the outcome measures in any of the studies in this thesis.

2.9 Measurement of appetite sensations using Visual Analogue Scales (VAS)

Subjective appetite ratings were measured using previously validated Visual Analogue Scales (VAS) (Flint *et al.* 2000). Two commonly used scales were used in which patients answer a question relating to their perceived feelings of fullness or satiety (Appendix K). The questions asked were "*how full do you feel*?", and "*how satisfied do you feel*?". Patients marked a vertical line on a 100mm scale, which was anchored at either end by opposing extreme states to indicate how close to each extreme they felt. Previously made markings were not made visible to patients. Scales were analysed by measuring the distance in mm from the left-hand extreme of the scale to the point at which the participant had marked.

2.10 Sample size calculation

Sample size requirement was estimated using the methods described by Hopkins (2000). The calculation is provided below:

$$n = 8s^2 / d^2$$

Note: n is the sample size, s is the typical error in measurement and d is the meaningful effect size.

Considering large effect sizes are generally reported for changes in blood glucose concentrations following exercise and the manipulation of diet within patients with type 1 diabetes (Rabasa-Lhoret *et al.* 2001, West *et al.* 2010, West *et al.* 2011, West *et al.* 2011), the magnitude of d was derived from 0.8 of the between subject variation (Cohen 1988), which was calculated using mean baseline data collected during four repeated trials, presented in West et al. (2011). In this study, the mean resting blood glucose concentrations were 7.9 $mmol.l^{-1}$ with the between subject standard deviation of 2.8 $mmol.l^{-1}$. Therefore d was calculated at 2.2 $mmol.l^{-1}$. The typical error in measurement was derived at 3.1 $mmol.l^{-1}$. These data indicate a recommended sample size of 11 participants to detect 80% power (Hopkins 2000). However, due to strict inclusion criteria and the time commitment required to complete each study, it was not possible to recruit the number of patients required for 80% power in all studies (chapters 4 and 5). Therefore a sample of sufficient size to perform parametric statistics was attained.

2.11 Statistical analysis

All data is presented as mean ± SEM. Data described as a change from rest, or pre-meal were calculated by subtracting resting values (e.g. mean blood glucose) away from all subsequent sample time-points. A statistical software package (PASW; IBM PASW version 18; IBM, Armonk NY) was used to analyse all data with statistical significance set at $p \leq 0.05$. Data were tested from normality and parametricity prior to statistical analysis.

All variables were examined for interactions of time and condition using repeated measures ANOVA. Where significant *p*-values were identified for interaction effects (time*condition), *p* values, F value, and effect size (partial-eta^2) were reported. Significant within (time) effects were analysed using post-hoc Bonferroni adjusted pairwise comparisons. Significant between (condition) effects were analysed using a One-way repeated ANOVA or Bonferroni adjusted pairwise comparisons. Relationships were explored using Pearson's product moment correlation coefficient and reported with *r* and *p* values.

CHAPTER 3A

THE GLYCAEMIC EFFECTS OF REDUCING POST-EXERCISE RAPID-ACTING INSULIN IN TYPE 1 DIABETES

3.0 Introduction

Exercise-induced hypoglycaemia is a frequent (Briscoe *et al.* 2007) and dangerous (Cryer 2008) occurrence, which is widely feared by type 1 diabetes patients (Brazeau *et al.* 2008). Research suggests that falls in glycaemia during exercise occur due to a synergistic effect of both muscular contraction and unregulated circulating insulin promoting blood glucose uptake (Rabasa-Lhoret *et al.* 2001, Mauvais-Jarvis *et al.* 2003, West *et al.* 2010). To combat this, patients are recommended to reduce the dose of rapid-acting insulin which they administer with the meal before exercise (Rabasa-Lhoret *et al.* 2001, Mauvais-Jarvis *et al.* 2003, Grimm 2005, De Feo *et al.* 2006, West *et al.* 2010, West *et al.* 2011). However, this current strategy is not fully protective and patients may still be exposed to hypoglycaemia later in the day. This has, at least in part, been attributed to iatrogenic causes (West *et al.* 2010), whereby patients administer their usual doses of rapid-acting insulin in a heightened insulin-sensitive state.

During exercise at moderate to high-intensities, the predominant energy substrate is glucose derived from the circulation and intramuscular glycogen stores (Hargreaves and Richter 1988, Jentjens and Jeukendrup 2003, Jensen and Richter 2012). The replenishment of these stores is a high metabolic priority, and is accelerated early after exercise (Price *et al.* 1994). This is facilitated by increased glycogen synthesis (Mikines *et al.* 1988, Nielsen *et al.* 2004), and an up-regulation of insulin signalling pathways (Maarbjerg *et al.* 2011). Thus, early after exercise, patients are faced with a window of increased glucose uptake and an enhanced sensitivity to insulin. With this in mind, the dose of rapid-acting insulin administered with the meal during this time is of particular importance and may warrant adjustment (Robertson *et al.* 2009). In light of this, it may be intuitive to reduce the amount of rapid-acting insulin taken with the meal early after exercise. Although this has been previously proposed (Hiatt *et al.* 1994, Rabasa-Lhoret *et al.* 2001, Gallen 2003, Riddell and Perkins 2006), suggestions are largely anecdotal and there is little experimental data to support. Indeed, there is limited information examining adjustments in post-exercise rapid-acting insulin when following current recommendations for reducing pre-exercise dose. The subsequent glycaemic effects of

reducing pre and also post-exercise rapid-acting insulin dose are yet to be determined, and the implications of this for preventing hypoglycaemia early and also late after exercise are unknown.

Therefore, this study examined the glycaemic responses to reductions in post-exercise rapid-acting insulin dose, for 24 hours following running exercise in type 1 diabetes patients employing current pre-exercise rapid-acting insulin dose recommendations.

3.1 Methods

Patient demographic information is presented in Table 3.0. A schematic of the trial design is presented in Figure 3.0; this study was a randomised, counterbalanced, cross-over design. Patients arrived to the exercise laboratory on three separate mornings (~08:00 AM) having fasted overnight.. Following a resting sample, patients self-administered a 25% dose of rapid-acting insulin (1.8 ± 0.1 IU, see 2.2.6) into the abdomen (West *et al.* 2010). Patients consumed a pre-exercise carbohydrate meal equating to 1.0 g.carbohydrate.kg^{-1} BM (1.6 ± 0.04 MJ, see 2.2.5.6, Table 2.3, MEAL 3) within a 5 minute period. Patients remained at rest for 60 minutes following consumption of the pre-exercise carbohydrate bolus / rapid-acting insulin injection. Immediately after a blood draw at 60 minutes, patients commenced 45 minutes of treadmill running at a speed calculated to elicit 70% of their $\dot{V}O_{2peak}$. Immediately following exercise, a blood sample was taken, with further interval samples at 15, 30, and 60 minutes post-exercise. At 60 minutes, patients administered one of three rapid-acting insulin doses, either a **Full** (unchanged dose; 7.5 ± 0.3 IU), a **75%** dose (i.e. a 25% reduction: 5.6 ± 0.2 IU), or a **50%** (i.e. a 50% reduction: 3.7 ± 0.1 IU) dose (see 2.2.6) and consumed a standardised post-exercise meal equating to 1.0 g.carbohydrate.kg^{-1} BM (2.8 ± 0.04 MJ, see 2.2.5.7, Table 2.3, MEAL 4) within a 5 minute period. Patients remained rested for a further 180 minutes with periodic blood samples taken every 30 minutes (Figure 3.0). At 180 minutes, patients were discharged from the laboratory. CGM captured interstitial glucose responses for a further 21 hours (see 2.2.3.1) and patients self-recorded β-hydroxybutyrate (see 2.4).

Table 3.0 Patients demographic information

	Patient ID											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Mean±SEM**
Insulin (IU) Basal	38^{G}_{M}	20^{D}_{B}	22^{G}_{M}	26^{D}_{B}	34^{G}_{E}	18^{D}_{B}	20^{G}_{E}	31^{G}_{M}	24^{G}_{M}	30^{G}_{E}	20^{G}_{E}	26 ± 2
Bolus	1^{L}	1^{A}	1^{A}	1^{A}	1^{A}	0.8^{A}	1^{A}	1^{A}	1^{A}	1^{A}	1^{A}	1.0 ± 0.0
HbA1c (%)	7.9	7.7	7.8	7.8	6.3	7.7	7.9	8	7.9	7.2	7.7	7.7 ± 0.4
BMI ($kg.m^2$)	22.9	21.2	20.3	23.1	25.4	22.8	22.9	21.1	22.1	27.2	22.9	22.9 ± 0.7
Diabetes duration (years)	13	14	16	14	12	4	25	12	13	15	9	13 ± 2
$\dot{V}O_{2peak}$ ($ml.kg.min^{-1}$)	53.1	54.3	55.4	56.3	53.2	54.3	55.4	54.3	45.3	51.2	50.4	53.1 ± 1.1
Age (years)	29	24	26	24	23	34	27	19	26	21	19	24 ± 2

Note: G = Glargine, D = Detemir, A = Aspart, L = Lispro, M = once daily (morning), E = once daily evening , B = bi-daily; bolus insulin calculated per 10g CHO.

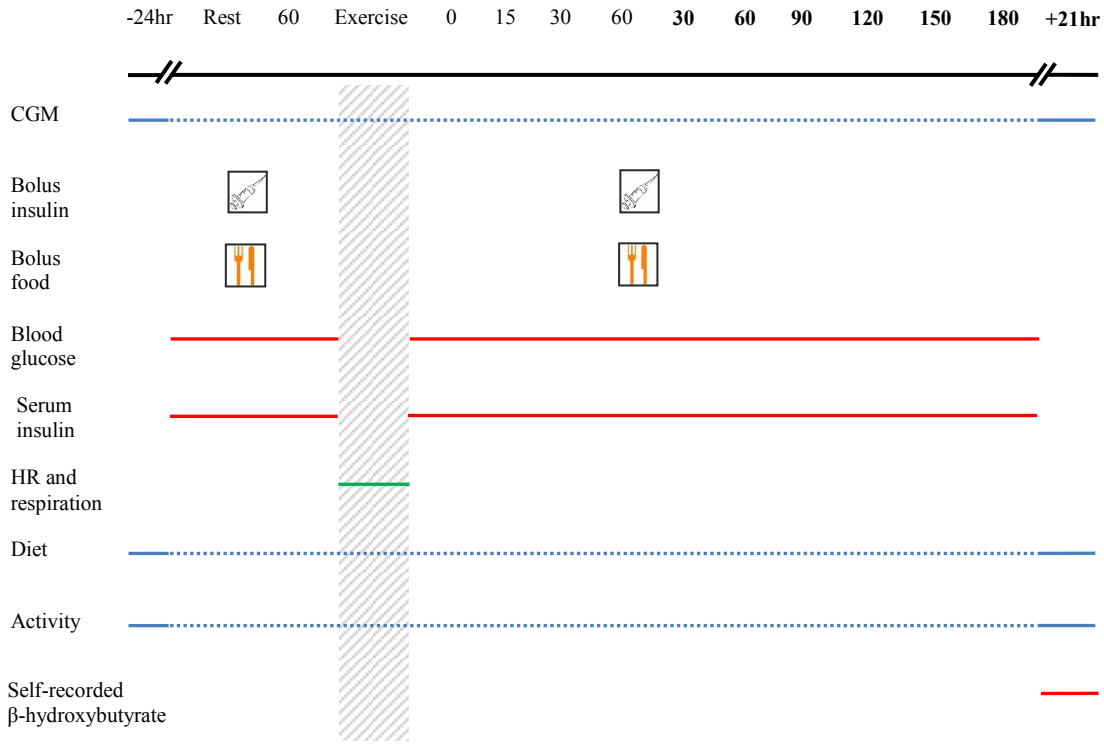

Figure 3.0. Schematic of experimental trial design. Note: Bold text indicates post-exercise intervention period.

3.2 Results

3.2.1 Pre-laboratory phase

3.2.1.1 Pre-laboratory dietary intake, insulin administration and activity

Dietary intake was similar between conditions in the 24 hours before trials; there were no differences in total energy consumed (**Full** 9.4 ± 0.5, **75%** 9.6 ± 0.8, **50%** 8.9 ± 0.6 MJ; p = 0.750), with contribution from carbohydrate (**Full** 53.5, **75%** 49.7, **50%** 48.9 %; p = 0.844), fat (**Full** 29.7, **75%** 30.4, **50%** 33.2 %; p = 0.958), and protein (**Full** 17.8, **75%** 19.9, **50%** 17.9 %; p = 0.843) similar between conditions. There were no differences in the amount of carbohydrate ingested to correct blood glucose (**Full** 10 ± 7, **75%** 13 ± 5, **50%** 11 ± 8 g; p = 0.750) nor were there differences found in the total amount of rapid-acting insulin administrated (**Full** 24 ± 4, **75%** 27 ± 5, **50%** 24 ± 5 IU; p = 0.632) or in the carbohydrate-to-insulin ratio (p = 0.588) between conditions. There were no differences observed in activity patterns during the 24 hours prior to main trials with similar steps recorded (**Full** 5125 ± 75, **75%** 5211 ± 101, **50%** 5253 ± 92 steps; p = 0.693).

3.2.1.2 Pre-laboratory glycaemia

Patients displayed similar glycaemic control during the 24 hours prior to experimental trials with similar mean (**Full** 8.6 ± 0.4, **75%** 7.7 ± 0.5, **50%** 8.1 ± 0.6 $mmol.l^{-1}$; p = 0.451) and total interstitial glucose area under the curve (**Full** 12389 ± 705, **75%** 10986 ± 765, **50%** 11385 ± 791 $mmol.l^{-1}.min^{-1}$; p = 0.400) across trials with the majority of time in euglycaemic ranges (**Full** 949 ± 130, **75%** 1044 ± 101, **50%** 1053 ± 125 minutes; p = 0.451). Total time spent hypoglycaemic (**Full** 117 ± 43, **75%** 155 ± 49, **50%** 128 ± 58 minutes; p = 0.722) and hyperglycaemic (**Full** 375 ± 74, **75%** 242 ± 73, **50%** 245 ± 77 minutes; p = 0.575) were similar.

3.2.2 Laboratory phase

3.2.2.1 Serum insulin responses

The serum insulin responses are presented in Figure 3.1. There was a significant condition*time interaction ($F_{(22,154)} = 2.566$, $p < 0.001$, partial-eta$^2 = 0.391$), and a significant time ($F_{(11,77)} = 4.934$, $p = 0.046$, partial-eta$^2 = 0.552$) and condition effect ($F_{(2,14)} = 6.586$, $p = 0.020$, partial-eta$^2 = 0.622$) when examining insulin concentrations. No conditional differences were noted at rest (**Full** 67 ± 22, **75%** 69 ± 17, **50%** 66 ± 16 pmol.l^{-1}; $p = 0.989$; figure 3.1) or before the post-exercise meal (**Full** 68 ± 21, **75%** 73 ± 19, **50%** 64 ± 20 pmol.l^{-1}; $p = 0.648$; Figure 3.1). Following the post-exercise meal, serum insulin peaked at 60 minutes under all conditions, with concentrations greatest under **Full** and lowest under **50%** (**Full** 199 ± 47, **75%** 182 ± 38, **50%** 109 ± 28 mmol.l^{-1}; $p = 0.009$; Figure 3.1). Concentrations remained significantly greater under **Full** throughout the post-meal period ($p < 0.05$; Figure 3.1).

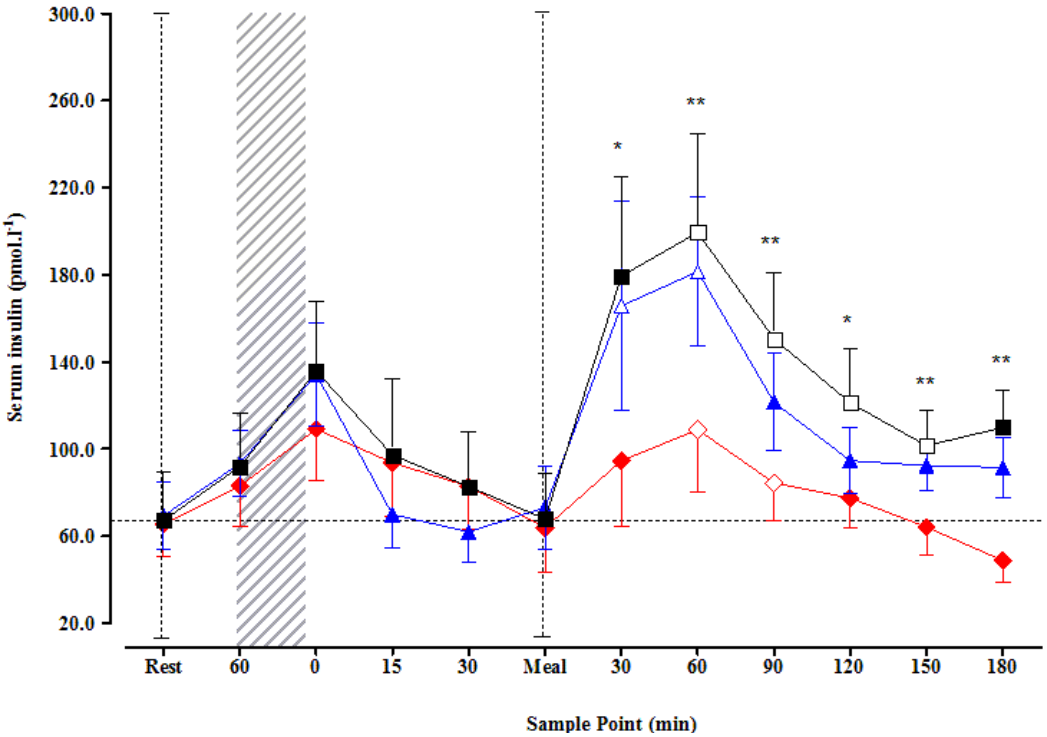

Figure 3.1. Time-course changes in serum insulin from rest. Data presented as mean ± SEM (n = 8). Black squares = **Full**, blue triangles = **75%**, red diamonds = **50%**. Transparent sample point within a condition indicates a significant difference from pre-meal concentrations ($p \leq 0.05$). * indicates significantly different from **Full** ($p \leq 0.05$). ** indicates significantly different from **Full** and **75%** ($p \leq 0.05$). Thatched area indicates exercise. Vertical dashed line break indicates carbohydrate meal and insulin administration. Note: Test meal and insulin were administered immediately following 60 minutes post-exercise sample point.

3.2.2.2 Blood glucose responses

The absolute blood glucose responses are presented in Figure 3.2. There was a significant condition*time interaction ($F_{(22,154)} = 4.085$, $p = 0.003$, partial-eta$^2 = 0.290$), and a significant time effect ($F_{(11,77)} = 18.030$, $p < 0.001$, partial-eta$^2 = 0.643$) for absolute blood glucose concentrations. There were no conditional differences at rest (**Full** 7.2 ± 0.9, **75%** 7.1 ± 0.7, **50%** 7.1 ± 0.6 mmol.l^{-1}; $p = 0.594$) or immediately before exercise (**Full** 13.9 ± 0.6, **75%** 15.0 ± 0.6, **50%** 13.1 ± 0.8 mmol.l^{-1}; $p = 0.352$).

3.2.2.3 Exercise and recovery period

On average, individuals ran at a velocity of 9.3 ± 0.3 km.h^{-1} and completed 7.0 ± 0.2 km. Patients exercised at a similar intensity (%$\dot{V}O_{2peak}$: **Full** 73.8 ± 0.0, **75%** 72.8 ± 0.0, **50%** 70.8 ± 0.0; $p = 0.575$; %HR_{peak}: **Full** 80 ± 2, **75%** 79 ± 3, **50%** 79 ± 1; $p = 0.631$), resulting in a similar decrease in blood glucose from pre-exercise concentrations (**Full** Δ-6.8 ± 0.0; **75%** Δ-6.9 ± 0.0; **50%** Δ-6.2 ± 0.0 mmol.l^{-1}, $p = 0.891$), such that immediately following the cessation of exercise glycaemia was similar to baseline under all conditions ($p > 0.05$; Figure 3.2). Blood glucose concentrations remained similar between conditions for 60 minutes post-exercise ($p > 0.05$; Figure 3.2) meaning concentrations immediately before the administration of the post-exercise meal were comparable (**Full** 7.6 ± 1.0, **75%** 8.20 ± 1.2, **50%** 8.5 ± 1.3 mmol.l^{-1}; $p = 0.822$). There were no incidences of hypoglycaemia during the exercise bout, or over the course of the 60 minute post-exercise recovery period.

3.2.2.4 Post-exercise intervention period

Following the consumption of the post-exercise meal, blood glucose declined under **Full** and **75%**, whereas glycaemia was preserved under **50%** (Figure 3.2). Following the post-exercise meal, 5 patients under **Full**, and 2 under **75%** experienced hypoglycaemia; whilst under **50%**, all patients remained protected. Furthermore, some participants under **Full** and **75%** experienced multiple bouts of hypoglycaemia, with total episodes across each trial greatest

under **Full** (**Full** n = 9 vs. **75%** n = 6, **50%** n = 0). On average, blood glucose concentrations were 2.8 ± 0.2 mmol.l^{-1} before receiving carbohydrate supplementation. Inversely, more patients experienced hyperglycaemia under **50%** compared to **75%** and **Full** (**50%** n = 9 vs. **75%** n = 5, **Full** n = 4).

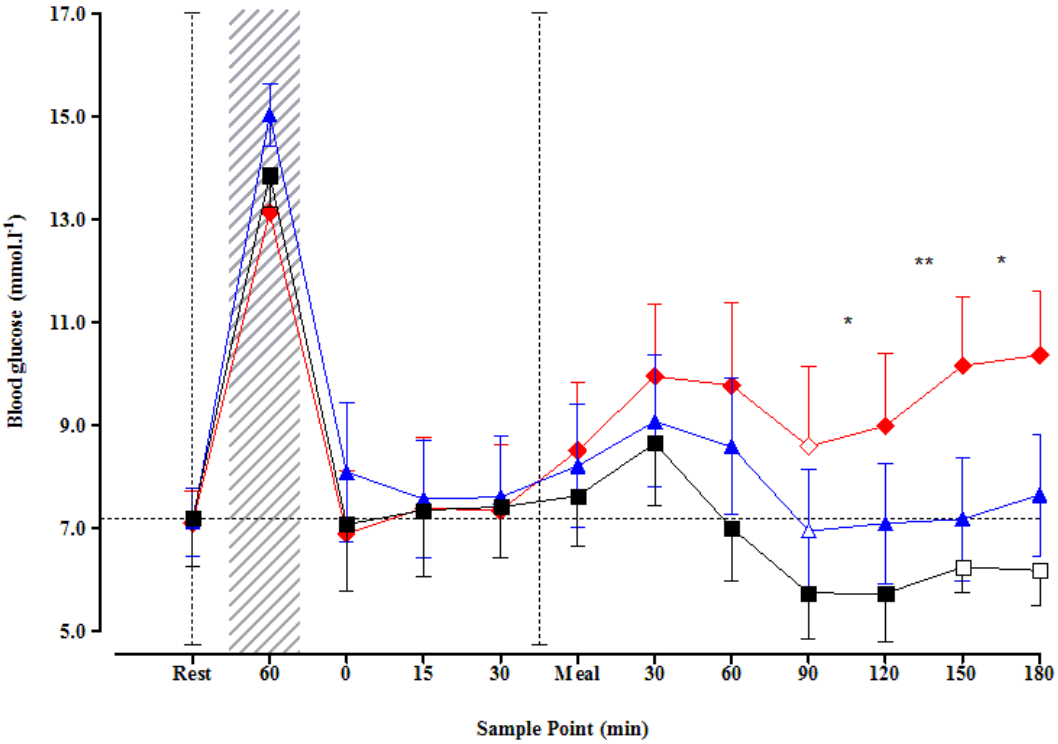

Figure 3.2. Time-course changes in blood glucose from rest. Data presented as mean ± SEM. Black squares = **Full**, blue triangles = **75%**, red diamonds = **50%**. Transparent sample point within a condition indicates a significant difference from pre-meal concentrations ($p \leq 0.05$). * indicates significantly different from **Full** ($p \leq 0.05$). ** indicates significantly different from **Full** and **75%** ($p \leq 0.05$). Thatched area indicates exercise. Vertical dashed line break indicates carbohydrate meal and insulin administration. Note: Test meal and insulin were administered immediately following 60 minutes post-exercise sample point.

3.2.3 Post-laboratory phase

3.2.3.1 Late evening glycaemia

There was a significant condition*time interaction ($F_{(2,33)} = 1.214$: $p = 0.046$, partial eta^2 = 0.148), and a significant time ($F_{(2,13)} = 6.583$: $p < 0.001$, partial eta^2 = 0.485) and condition

effect ($F_{(2,13)}$ = 4.213: p = 0.037, partial eta^2 = 0.376) and when examining the interstitial glucose concentrations during the post-laboratory period (Figure 3.3).

During the evening total interstitial glucose area under the curve was significantly greater under **50%** (2709 ± 245 mmol.l^{-1}.min^{-1}) compared to **Full** (1706 ± 247 mmol.l^{-1}.min^{-1}; p < 0.001) and **75%** (1860 ± 244 mmol.l^{-1}.min^{-1}; p < 0.001). Patients under **50%** remained protected for a further 4 hours, meaning that the first hypoglycaemic episode occurred at 8 hours post-exercise. No patient administered additional insulin to correct blood glucose concentrations during this time.

3.2.3.2 Nocturnal glycaemia

Beyond 8 hours post-exercise, declines in glycaemia were evident under all conditions and coincidental with sleep (Figure 3.3). Interstitial glucose nadir occurred during the night irrespective of condition (Figure 3.3) with similar total nocturnal interstitial glucose area under the curve (**Full** 3201 ± 401, **75%** 2519 ± 222, **50%** 3262 ± 330 mmol.l^{-1}.min^{-1}; p = 0.240). Across trials, 82% of all hypoglycaemic episodes occurred nocturnally (Figure 3.3, Table 3.1). Moreover, those patients experiencing a hypoglycaemic episode during their laboratory stay also developed nocturnal hypoglycaemia. However, fewer patients under **50**% experienced nocturnal hypoglycaemia (**50%** n = 2 vs. **Full** n = 6, **75%** n = 6; Figure 3.3, Table 3.1). In addition, total time spent in hypoglycaemic ranges across the whole post-laboratory period was significantly less under **50%** (**50%** 82 ± 23 vs. **Full** 113 ± 27, **75%** 126 ± 17, minutes; p = 0.042). Conversely, total time spent in hyperglycaemic ranges was significantly greater under **50**% (**50%** 418 ± 67 vs. **Full** 266 ± 62, **75%** 210 ± 54, minutes; p = 0.041). Patients on average corrected blood glucose at an interstitial glucose concentration of 4.5 ± 0.3 mmol.l^{-1}; however, this was typically performed following their CGM calibration routine. Morning glycaemia was also similar across trials (p > 0.05; Figure 3.3).

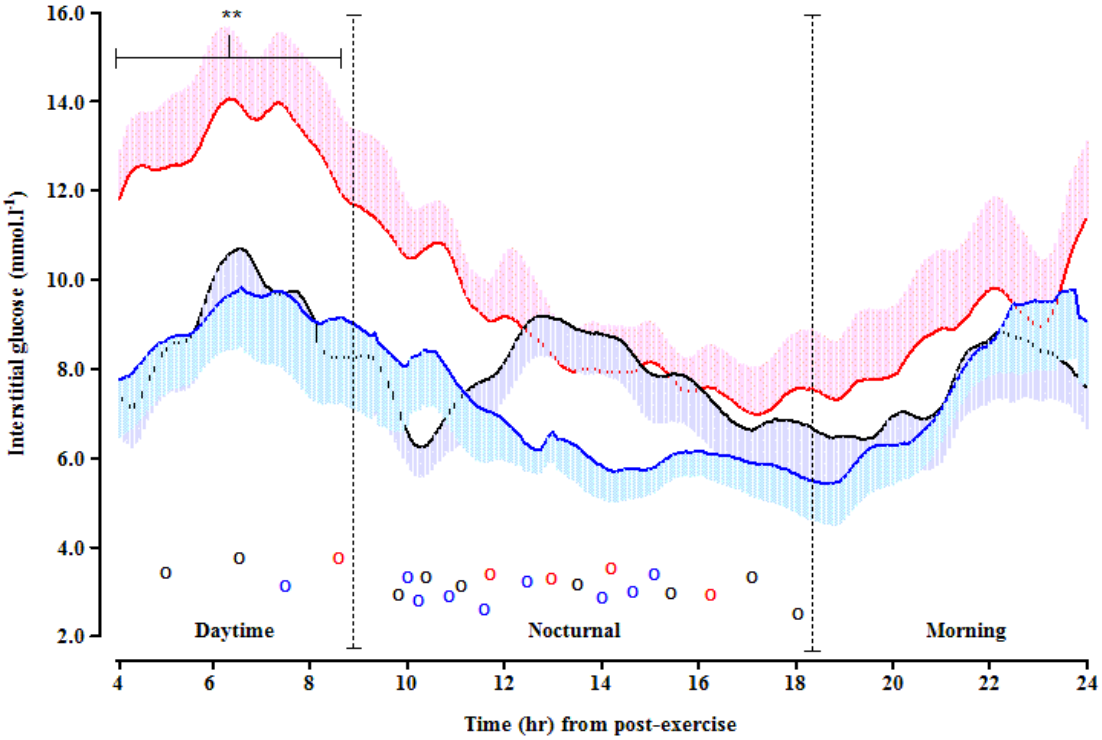

Figure 3.3. Time-course changes in interstitial glucose throughout the post-laboratory period. Data presented as mean ± SEM. Black solid trace = **Full**, blue dashed trace = **75%**, red broken trace = **50%**. ** indicates interstitial glucose area under the curve is significantly different from **Full** and **75%** ($p \leq 0.05$). Open circles represent hypoglycaemic episodes, as determined from CGM data. Vertical dashed line breaks indicate daytime, nocturnal, or morning periods.

Table 3.1. The total number of patients experiencing hypoglycaemia and the total number of hypoglycaemic episodes during the post-laboratory period.

		Full	75%	50%
Evening	**No. patients**	2	1	1
	Total episodes	2	1	1
Nocturnal	**No. patients**	4	5	2
	Total episodes	7	8	4
Morning	**No. patients**	0	0	0
	Total episodes	0	0	0
Total	**No. patients**	6	6	3
	Total episodes	9	9	5

3.2.3.3 Post-laboratory dietary intake, insulin administration, activity, and self-recorded blood β-hydroxybutyrate

Self-recorded β-hydroxybutryate concentrations were undetectable in all patients throughout the post-laboratory period. During this time, total energy consumed (**Full** 6.6 ± 1.0, **75%** 7.5 ± 1.2, **50%** 7.3 ± 0.6 MJ; $p = 0.836$), with contribution from carbohydrate (**Full** 55 ± 4, **75%** 47 ± 5, **50**% 48 ± 4 %; $p = 0.516$), fat (**Full** 30 ± 4, **75%** 31 ± 4, **50%** 34 ± 4 %; $p = 0.916$), and protein (**Full** 16 ± 2, **75%** 22 ± 6, **50%** 18 ± 2 %; $p = 0.827$) similar between conditions. Moreover, there were no differences in the total amount of insulin administered, or in the carbohydrate-to-insulin ratio ($p > 0.05$). No differences were observed in activity patterns for 24 hours after exercise, with similar steps recorded across conditions (**Full** 5341 ± 95, **75%** 4786 ± 87, **50%** 5543 ± 108 steps; $p = 0.877$).

3.3 Discussion

The aim of this study was to examine the glycaemic responses to reductions in post-exercise rapid-acting insulin dose following running exercise in type 1 diabetes patients adopting current recommendations for reducing pre-exercise rapid-acting insulin dose. This study demonstrates for the first time that despite a large reduction in pre-exercise rapid-acting insulin dose, it is important that *post-exercise* dose is also heavily reduced. Such a strategy is necessary so that glycaemia is preserved, and hypoglycaemia prevented early after exercise (≤ 8 hours). However, during this time patients may experience periods of post-prandial hyperglycaemia, and beyond this time, may still be at risk from late-onset hypoglycaemia.

All patients completed a bout of intensive aerobic exercise (~73% $\dot{V}O_{2peak}$), running at an average velocity of ~9 $km.h^{-1}$ and expending ~ 3.0 MJ (708 kcal). During exercise, patients exhibited a respiratory exchange ratio of ~0.97, which is typical of exercise of an intense nature (Marliss and Vranic 2002) and reflects the utilisation of carbohydrate as the primary fuel source. Despite this, there were no incidences of hypoglycaemia during exercise,

complimenting previous literature (Rabasa-Lhoret *et al.* 2001, Mauvais-Jarvis *et al.* 2003, West *et al.* 2010) and emphasising the importance of large reductions in rapid-acting insulin dose preceding exercise. Furthermore, patients were, on average within euglycaemic ranges for 60 minutes after exercise, suggesting that this strategy exposes patients to only transient hyperglycaemia prior to exercise (Figure 3.2).

As demonstrated in the present study, it is important that post-exercise rapid-acting insulin dose is also heavily reduced in order to minimise the risk of hypoglycaemia following the meal *after* exercise. Blood glucose was best preserved when reducing post-exercise rapid-acting insulin dose by **50%**, protecting all patients from hypoglycaemia ($\leq$ 3.9 mmol.l^{-1}) during post-meal period in the laboratory. Conversely, 45% of patients under **Full**, and 18% under **75%** required carbohydrate supplementation. The exact mechanisms underpinning these findings are not entirely known. However, the performance of exercise at moderate-to-high intensities requires a contribution from muscle glycogen (Chokkalingam *et al.* 2007, Jenni *et al.* 2008) and replenishment of this is a high metabolic priority after exercise (Jentjens and Jeukendrup 2003). Following exercise, glycogen synthesis is increased dramatically, thought to be driven primarily by depleted glycogen (Mikines *et al.* 1988, Nielsen *et al.* 2004), an up-regulation of insulin signalling pathways (Maarbjerg *et al.* 2011), and a prolonged permeability to the muscle cell membrane to glucose (Cartee *et al.* 1989). Taken together, an increased rate of glucose transport into the muscle, and an increased capacity to convert glucose to glycogen, results in a window where there is likely an increase in the potency of administered rapid-acting insulin (Zinman *et al.* 1977).

It would be naïve to suggest that the glycaemic responses of the patients under **Full** and **75%** in this study are solely due to depleted muscle glycogen stores however, as large carbohydrate boluses (1.0 g.carbohydrate.kg^{-1} BM) were ingested before and after exercise, which would have helped supplement glycogen reserves (Jentjens and Jeukendrup 2003). It is likely that the administration of larger doses of rapid-acting insulin (under the **Full** and **75%** conditions) created a milieu of relative hyperinsulinaemia, in turn supressing hepatic glucose production

and enhancing glucose uptake (Zinman *et al.* 1977), potentially into non-exercised tissue (Chokkalingam *et al.* 2007); if the destination of glucose was the liver, this may reduce the risk of hypoglycaemia later in the day (Chokkalingam *et al.* 2007). Indeed if this were true, the risk of developing hypoglycaemia during this initial period would be increased significantly if carbohydrate intake were not increased to match glucose disposal. Interestingly, blood glucose under **75%** and **Full** continued to decline beyond the action time of that typically expected for rapid-acting insulin analogues (Homko *et al.* 2003) with patients under these conditions requiring multiple boluses of carbohydrate supplementation. This is likely to explain a lack of statistical significance between the **50%** and **75%** conditions at 120 and 180 minutes.

Irrelevant of the exact mechanisms at play, these findings demonstrate an increased risk of early and late post-exercise hypoglycaemia when post-exercise rapid-acting dose is not adjusted. Furthermore, these data indicate that a **50%** reduction is necessary to prevent falls in blood glucose early after exercise and prevent hypoglycaemia, which is greater than the ~30% reduction current opinion would suggest (Lumb and Gallen 2009).

Unfortunately 75% of patients under the **50%** condition were exposed to hyperglycaemia (≥ 8.0 $mmol.l^{-1}$) following the post-exercise meal. Whereas the majority of these patients experienced only mild hyperglycaemia during the post-exercise post-prandial period, peak blood glucose ranged between 8.8 and 21.8 $mmol.l^{-1}$, with one patient averaging a blood glucose concentration of 19.3 $mmol.l^{-1}$ throughout this time. The finding that some patients may be exposed to periods of severe hyperglycaemia following reductions to post-exercise rapid-acting insulin highlights that patients differ in their sensitivity to insulin following exercise, and may therefore require a smaller reduction in post-exercise rapid-acting insulin dose. As such, clinicians and patients should be made aware that when heavily reducing pre- and post-exercise rapid-acting insulin dose there is potential for hyperglycaemia following the post-exercise meal. It is noteworthy that hyperglycaemia sustained in the present study occurred without employing currently recommended reductions to basal insulin (Lumb and

Gallen 2009, Maahs *et al.* 2009), which if applied, could exacerbate post-prandial hyperglycaemia.

Interstitial glucose concentrations revealed a preservation of glycaemia under **50%** for a further 4 hours after leaving the laboratory, meaning patients had been protected against hypoglycaemia for a total of 8 hours post-exercise. In comparison, glycaemia during the evening (between 4 and 8 hours post-exercise; Figure 3.3) was significantly lower under both **Full** and **75%** conditions, with patients under these conditions experiencing further hypoglycaemic episodes. However, at ~7 hours post-exercise, there was a decline in interstitial glucose concentrations under **50%**, such that at ~8 hours post-exercise glycaemia were similar between conditions. The decline in interstitial glucose was consistent across trials (Figure 3.3) and was coincidental with patients consuming their evening meal. Type 1 diabetes patients face particular difficulty in avoiding hypoglycaemia 7-11 hours post-exercise, as the requirement for glucose to maintain euglycaemia is increased (Mcmahon *et al.* 2007, Tamborlane 2007). Therefore, patients may need to make further adjustments to their rapid-acting insulin dose. However, this strategy is largely dependent upon time of exercise; if exercise is performed in the evening, falls in glycaemia will likely occur nocturnally. Therefore it may be prudent to implement a more specific feeding strategy following evening exercise. Considering that increased insulin sensitivity may persist for more than 48 hours after exercise, an additional strategy could be to reduce basal insulin dose.

So that it was possible to determine the impact of mealtime adjustments on glycaemia independent of basal insulin, basal insulin regimen was unaltered and standardised across trials. Reducing basal insulin dose requires planning exercise in advance, and may offer little flexibility if the timing of a reduction is not considered; if unforeseen circumstances prevent planned exercise from taking place, the patient may be exposed to prolonged and / or severe periods of hyperglycaemia (Lumb 2012), especially if patients display pre-existing post-prandial hyperglycaemia following the post-exercise rapid-acting insulin reduction. There are however no recommendations regarding dose or timing of basal dose adjustment for exercising

patients with type 1 diabetes. Moreover, it would be unwise to promote a reduction in basal dose in the setting of this present strategy without knowledge of the deeper metabolic, hormonal or inflammatory implications.

Of the total number of hypoglycaemic episodes experienced across all trials during the post-laboratory period, 82% occurred nocturnally. Therefore, sleep may be a contributing factor to the increased incidence of hypoglycaemia during the night (Macdonald 1987). As well as recent exercise (Sandoval *et al.* 2006, Briscoe *et al.* 2007), antecedent hypoglycaemia (Cryer 2008) may blunt symptomatic and autonomic responses to hypoglycaemia later in the day, particularly during sleep (Cryer and Childs 2002). This is important as those patients who experienced hypoglycaemia in the laboratory eliciting blood glucose concentrations less than 3 $mmol.l^{-1}$, also experienced hypoglycaemia again during sleep. These data suggest that if patients experience hypoglycaemia early after exercise, there is potential that they are at an increased risk of blood glucose falling again during the night. Although only 18% of patients experienced nocturnal hypoglycaemia under **50%**, the late fall in glycaemia means that the risk, albeit less, may still be present for patients despite large reductions in pre- and post-exercise rapid-acting insulin dose. Experiencing hypoglycaemia during sleep is a real fear for type 1 diabetes patients (Cryer and Childs 2002). This risk is likely to be exacerbated when exercise is performed during the afternoon or evening. Therefore, there is a need to further refine current exercise recommendations for type 1 diabetes patients, such that patients can engage in regular exercise with a reduced risk and fear of post-exercise nocturnal hypoglycaemia.

The aim of this study was to assess the acute and 24 hour glycaemic effects of reducing post-exercise rapid-acting insulin dose whilst employing current recommendations for reducing pre-exercise rapid-acting insulin dose. The results suggest that a 50% reduction in post-exercise rapid-acting insulin dose, under conditions of a large pre-exercise rapid-acting insulin reduction, preserves glycaemia and prevents hypoglycaemia for ~8 hours after exercise. However, patients may experience periods of post-prandial hyperglycaemia following the post-

exercise rapid-acting insulin reduction. It is important therefore to now assess the acute metabolic, inflammatory, and counter-regulatory hormonal effects of reducing post-exercise rapid-acting insulin dose under conditions of reduced pre-exercise rapid-acting insulin dose.

CHAPTER 3B

THE METABOLIC, INFLAMMATORY, AND COUNTER-REGULATORY-HORMONAL EFFECTS OF REDUCING RAPID-ACTING INSULIN DOSE AFTER EXERCISE

3.4 Introduction

As highlighted in chapter 3A, some patients may be exposed to periods of hyperglycaemia when combining pre and post-exercise rapid-acting insulin dose reductions (Campbell *et al.* 2013). With this is mind, it would be reasonable to speculate that low levels of circulating insulin combined with elevated concentrations of post-exercise counter-regulatory hormones may, in fact, precipitate a metabolic milieu promoting increased lipolysis (Khani and Tayek 2001) and ketogenesis (Laffel 2000). In addition, inflammatory cytokine responses are related to hyperglycaemia (Targher *et al.* 2001, Esposito *et al.* 2002, De Rekeneire *et al.* 2006, Rosa *et al.* 2008), lipid oxidation (Febbraio and Pedersen 2005), and/or hyperketonaemia (Stouthard *et al.* 1995, Karavanaki *et al.* 2011, Karavanaki *et al.* 2012). Although regular exercise has been demonstrated to reduce systemic inflammation, thus strengthening its therapeutic utility for patients with type 1 diabetes (Petersen and Pedersen 2005), somewhat paradoxically, these long-term adaptations occur despite opposing acute effects in which there is a pronounced increase in inflammatory markers early after exercise (Sprenger *et al.* 1992, Drenth *et al.* 1995, Nehlsen-Cannarella *et al.* 1997, Ostrowski *et al.* 1999, Pedersen and Hoffman-Goetz 2000, Nemet *et al.* 2002).

Potentially, performing exercise under conditions of concurrent or prior hyperglycaemia and/or hypoinsulinaemia might result in an inappropriately elevated level of inflammation after exercise. Additionally, metabolic disturbances could negate the over-all health benefits of exercise and accelerate the progression of diabetes related complications. Indeed, this may offer some explanation towards conflicting opinion regarding the efficacy of aerobic or endurance-based exercise for improvements in glycaemic control (for review see Tonoli et al (2012).

It is a well-established recommendation that patients reduce their pre-exercise rapid-acting insulin dose to prevent exercise-induced hypoglycaemia (West *et al.* 2010, West *et al.* 2011, West *et al.* 2011, Campbell *et al.* 2013) as the metabolic implications of this are well known

(Bracken *et al.* 2011). Having now demonstrated that a reduction in post-exercise rapid-acting insulin dose is also necessary to avoid early post-exercise hypoglycaemia, in the setting of post-prandial hyperglycaemia (Campbell *et al.* 2013; chapter 3A) it is important to investigate the deeper metabolic, hormonal and inflammatory consequences of this strategy to determine its efficacy. Therefore, a second arm of analysis was applied to chapter 3A to determine whether reducing pre- and also post-exercise rapid-acting insulin dose, as a strategy for preventing post-exercise hypoglycaemia in type 1 diabetes patients, causes metabolic or hormonal disturbances, influences ketonaemia, or alters inflammatory cytokine concentrations.

3.5 Methods

A second arm of analysis was performed on all patients from **Full** and **50%** trials from chapter 3A. **Full** and **50%** trials were chosen to illustrate both extremes of the intervention (normal dose versus large reduction). Blood lactate, serum cortisol, non-esterified-fatty-acids, β-hydroxybutyrate, and plasma glucagon, adrenaline, noradrenaline, IL-6 and TNF-α were measured for 180 minutes post-meal (Figure 3.4).

Figure 3.4. Schematic of experimental trial design. Note: Bold text indicates post-exercise intervention period.

3.6 Results

3.6.1 Counter-regulatory hormone and metabolite responses

There were no conditional differences in counter-regulatory hormones or metabolites (Table 3.2) up to 60 minutes post-exercise ($p > 0.05$). A significant interaction of condition and time ($F_{(11,77)} = 5.611$, $p = 0.006$, partial-eta$^2 = 0.445$), and a significant effect of time ($F_{(1,77)} = 71.424$, $p < 0.001$, partial-eta$^2 = 0.691$) and condition ($F_{(1,7)} = 13.070$, $p = 0.009$, partial-eta$^2 = 0.651$) was found when examining plasma glucagon concentrations during the trials (Table 3.2). Plasma glucagon concentrations following the post-exercise meal were significantly elevated from pre-meal values under both conditions, but were greater under **Full** (Table 3.2). There was no effect of insulin dose on plasma adrenaline, noradrenaline, serum cortisol, blood

lactate, serum NEFA (Table 3.2) or β-hydroxybutyrate following the post-exercise meal (Figure 3.5).

Mean post-exercise postprandial glucagon concentrations were positively related to corresponding mean noradrenaline concentrations under both conditions (**Full** $r = 0.929$, $p = 0.027$; **50%** $r = 0.788$, $p = 0.020$). During this time, there was a transient decline in β-hydroxybutyrate with concentrations similar to rest under **50%**, and lower than rest under **Full** (Figure 3.5). There was positive correlation observed between mean β-hydroxybutyrate concentrations and NEFA under both conditions (**Full** $r = 0.671$, $p = 0.024$; **50%** $r = 0.790$, $p = 0.001$), as well as mean blood glucose under **50%** ($r = 0.716$, $p = 0.013$), but not under **Full** ($r = 0.257$, $p = 0.446$). Moreover, mean NEFA concentrations were associated with greater blood glucose concentrations under **50%** (**50%** $r = 0.761$, $p = 0.007$; **Full** $r = 0.198$, $p = 0.559$) whereas NEFA were inversely related to mean circulating insulin concentrations under **Full** (**Full** $r = -0.746$, $p = 0.034$; **50%** $r = -0.291$, $p = 0.484$).

3.6.2 Inflammatory cytokine responses

The IL-6 and TNF-α responses are presented in Figure 3.5. Resting concentrations of IL-6 and TNF-α were positively related to length of diabetes (IL-6: $r = 0.701$, $p = 0.033$; TNF-α: $r = 0.632$, $p = 0.042$) and inversely related to HbA_{1c} (IL-6: $r = -0.699$, $p = 0.020$; TNF-α: $r = -0.698$, $p = 0.039$), but not $\dot{V}O_{2peak}$ (IL-6: $r = -0.463$, $p = 0.528$; TNF-α: $r = -0.327$, $p = 0.356$). Both plasma IL-6 and TNF-α concentrations were significantly raised from rest at 15 minutes post-exercise (IL-6: **Full** Δ+2.02 ± 1.01 (125 %) $pg.ml^{-1}$; $p = 0.032$, **50**% Δ+1.34 ± 0.98 (116 %) $pg.ml^{-1}$; $p = 0.030$; TNF-α: **Full** Δ+2.83 ± 0.86 (147 %) $pg.ml^{-1}$; $p = 0.025$, **50**% Δ+2.99 ± 0.74 (144 %) $pg.ml^{-1}$; $p = 0.021$). Following the post-exercise meal, IL-6 concentrations were significantly greater under **50%** (Figure 3.5), although TNF-α was similar between conditions. Despite this, both cytokines under **50%** remained similar to pre-meal and resting measures ($p > 0.05$; Figure 3.5). Under **50%** mean IL-6 concentrations over the post-exercise period were positively related to mean TNF-α concentrations ($r = 0.676$, $p < 0.001$) and serum insulin

concentrations were inversely related to IL-6 (r = -0.484, p = 0.017), but not TNF-α (r = -0.169, p = 0.430). No significant relationships existed between mean blood glucose and IL-6 (r = 0.299, p = 0.155) or TNF-α (r = 0.005, p = 0.980) over the post-meal period under **50**%. No relationships were found between any other measures under **Full**.

Table 3.2. Metabolic and counter-regulatory hormone responses to reductions in pre- and post-exercise rapid-acting insulin dose

															ANOVA *p*	
		Rest	**60**	**E**	**0**	**15**	**30**	**Pre-Meal**	**30**	**60**	**90**	**120**	**150**	**180**	**T**	**T*C**
Plasma Glucagon ($pg.ml^{-1}$)	**Full**	760±70	697±81		1152±99†	832±83†	698±61	710±69	1015±79†‡	1597±116†‡	1590±102†‡	1402±94†‡	1332±137†‡	1099±124†‡	<0.001	=0.006
	50%	789±85	628±85†*		1067±128†	854±127	815±89	609±98†	975±82†‡	1350±96†*‡	1251±97†*‡	1177±90†*‡	1085±86†*‡	889±82*‡		
Plasma Adrenaline ($nmol.l^{-1}$)	**Full**	0.29±0.05	0.12±0.03†		0.56±0.09†	0.40±0.10	0.31±0.07	0.21±0.05	0.20±0.05	0.18±0.07	0.12±0.04†	0.15±0.05	0.18±0.06	0.13±0.04†‡	=0.029	=0.577
	50%	0.27±0.05	0.19±0.04		0.59±0.07†	0.29±0.04	0.20±0.04	0.15±0.03	0.15±0.04	0.14±0.03	0.15±0.04	0.15±0.04	0.15±0.02	0.12±0.03		
Plasma Noradrenaline ($nmol.l^{-1}$)	**Full**	2.13±0.24	1.76±0.20		11.75±1.37†	4.30±0.56†	2.87±0.30†	2.49±0.27	2.51±0.25	2.86±0.33	2.53±0.29	2.61±0.40	2.37±0.31	2.36±0.32	<0.001	=0.537
	50%	2.51±0.28	2.42±0.29		12.68±1.38†	4.09±0.27†	3.35±0.21†	2.74±0.27	2.79±0.39	2.97±0.44	2.70±0.37	2.90±0.52	2.82±0.49	2.80±0.44		
Serum Cortisol ($\mu mol.l^{-1}$)	**Full**	0.22±0.03	0.21±0.03		0.19±0.02	0.23±0.03	0.20±0.03	0.17±0.01†	0.16±0.02†	0.15±0.03†	0.12±0.02†‡	0.11±0.02†‡	0.10±0.01†‡	0.10±0.01†‡	<0.001	=0.256
	50%	0.23±0.03	0.19±0.03		0.20±0.03	0.23±0.04	0.22±0.03	0.17±0.02†‡	0.14±0.02†‡	0.12±0.02†‡	0.11±0.02†‡	0.10±0.02†‡	0.08±0.01†‡	0.07±0.01†‡		
Blood Lactate ($mmol.l^{-1}$)	**Full**	0.49±0.12	0.82±0.13		3.63±0.61†	1.49±0.25†	1.07±0.16†	0.79±0.10	0.62±0.10	0.74±0.17	0.66±0.19	0.56±0.16	0.51±0.13‡	0.50±0.13‡	<0.001	=0.789
	50%	0.53±0.17	0.80±0.10		3.68±0.48†	1.64±0.29†	0.96±0.19†	0.74±0.15	0.68±0.19	0.69±0.11	0.59±0.13	0.54±0.12‡	0.55±0.1‡2	0.50±0.12‡		
Serum NEFA ($mmol.l^{-1}$)	**Full**	0.48±0.08	0.23±0.04†		0.24±0.04	0.42±0.11	0.34±0.06	0.35±0.07	0.30±0.06†	0.18±0.04†‡	0.17±0.04†‡	0.27±0.04†	0.25±0.03†	0.25±0.02†	=0.012	=0.448
	50%	0.40±0.06	0.24±0.07†		0.31±0.11	0.46±0.16	0.32±0.14	0.38±0.14	0.33±0.08	0.21±0.06†	0.24±0.05†	0.37±0.05	0.44±0.07	0.51±0.07		

Note**:** Data presented as mean ± SEM. **75%** trial was omitted from analysis. Test meal and insulin were administered immediately following rest and pre-meal sample points. ***** indicates significantly different from **Full** ($p \leq 0.05$). † indicates significantly different from rest. ‡ indicates significantly different from pre-meal. Exercise commenced 60 minutes after rest. **T** = Time, **C** = Condition, **E** = Exercise.

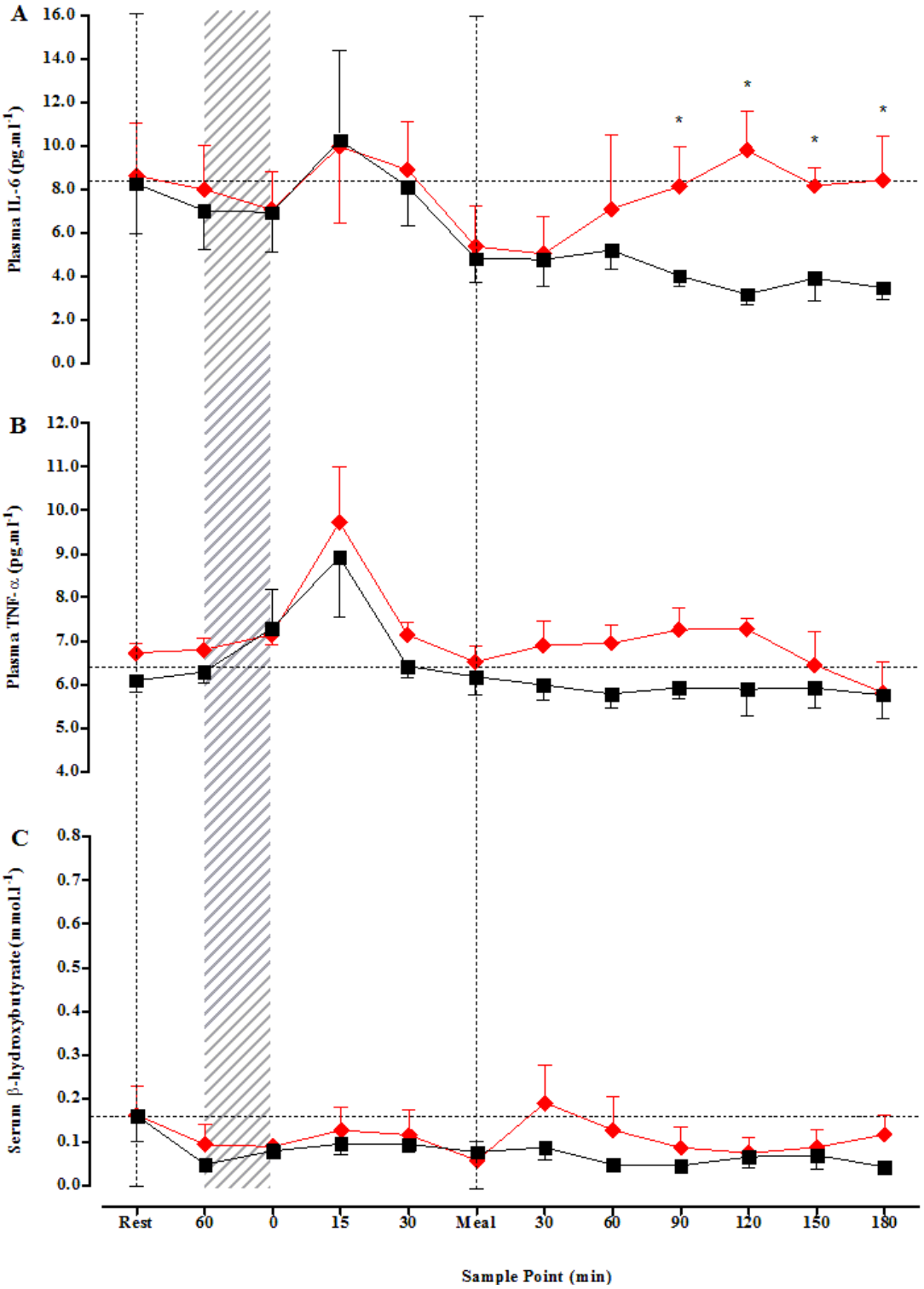

Figure 3.5 A-C. Time-course changes in (**A**) plasma IL-6, (**B**) plasma TNF-α, and (**C**) serum β-hydroxybutyrate from rest. Data presented as mean ± SEM. IL-6 and TNF-α (n = 8). Black squares = **Full**, red diamonds = **50%**. Transparent sample point within a condition indicates a significant difference from pre-meal concentrations ($p \leq 0.05$). * indicates significantly different from **Full** ($p \leq 0.05$). Thatched area indicates exercise. Vertical dashed line break indicates carbohydrate meal and insulin administration. Note: Test meal and insulin were administered immediately following 60 minutes post-exercise sample point.

3.7 Discussion

This study demonstrates that reducing pre- and also post-exercise rapid-acting insulin dose, as a strategy for preventing early-onset post-exercise hypoglycaemia, does not cause adverse metabolic, counter-regulatory-hormonal or inflammatory disturbances. Specifically, the data indicates that large reductions in rapid-acting insulin dose administered before and also after intensive running does not augment ketonaemia, nor cause significant elevations in inflammatory cytokines IL-6 and TNF-α above fasting concentrations, despite periods of postprandial hyperglycaemia following the post-exercise meal, in patients with type 1 diabetes.

Completing the exercise protocol caused a significant metabolic stress to patients, inducing large increases in blood lactate (~392 %) and catecholamines (adrenaline ~287 %, noradrenaline ~591 %), and large decreases in blood glucose (Δ~7.6 $mmol.l^{-1}$; chapter 3A). As described in chapter 3A, all patients under **50%** were protected from hypoglycaemia throughout their laboratory stay despite reductions in glycaemia following exercise and the administration of a second dose of rapid-acting insulin 60 minutes later. However, as a consequence of preventing hypoglycaemia, the majority of patients following the **50%** reduction (82 %) were exposed to periods of hyperglycaemia following the post-exercise meal.

Hyperglycaemia plays a central pathophysiological role in the development of long-term diabetes related complications (Nathan *et al.* 2005, Ceriello *et al.* 2013), but is also of immediate concern because hypoinsulinaemic hyperglycaemia is associated with an acute increase in lipolysis and ketogenesis (Laffel 2000, Wallace and Matthews 2004). Indeed, the present study revealed a positive association between increased blood glucose and NEFA, and increased blood glucose and β-hydroxybutyrate appearance. Although temporal changes in both NEFA and β-hydroxybutyrate concentrations were evident following the post-exercise meal under **50%**, concentrations remained similar between both conditions, and by 180 minutes both metabolites were similar to fasting rested concentrations. From a clinical

perspective, concentrations in these ranges are not deemed significant (< 1.0 mmol.l^{-1}) (Laffel 2000).

Although a large reduction in rapid-acting insulin dose was applied, serum insulin concentrations were elevated above resting and pre-meal measures under **50%** (chapter 3A). Despite unexplained differences in glucagon concentrations, the administration of even small amounts of rapid-acting insulin, under conditions of unchanged basal insulin dose, is likely to have raised circulating insulin concentrations whereby lipolysis is inhibited (through dephosphorlyation of hormone-sensitive lipase) and lipogenesis is increased (via activation of acetyl CoA carboxylase). Thus, reducing the capacity for β-oxidation of NEFA and ultimately limiting substrate availability for ketogenesis (Mcgarry 1996), and potentially promoting peripheral ketone body disposal (Balasse and Féry 1989). Moreover, temporal changes in catecholamine concentrations, the main lipolytic stimulus (Kalra and Tigas 2002), and cortisol (Fowler 2008) were not statistically significant between conditions. The consumption of a large carbohydrate based meal would have helped supplement muscle and liver glycogen, reducing the energy deficit created by exercise, and limiting the appearance of catecholamines and cortisol.

A logical extension of the results from chapter 3A was to question whether post-exercise hyperglycaemia would exacerbate the appearance of inflammatory cytokines in the patients in this investigation, as this would likely be further increased if patients experienced hyperketonaemia. Increased markers of inflammation are strongly related to glycaemic management and the pathogenesis of diabetes related complications (Targher *et al.* 2001, Fowler 2008). Patients with type 1 diabetes exhibit chronically elevated levels of inflammatory markers at rest (Targher *et al.* 2001, Esposito *et al.* 2002, De Rekeneire *et al.* 2006, Galassetti *et al.* 2006, Rosa *et al.* 2008). Indeed, a positive relationship was observed between resting inflammatory cytokine concentrations, and diabetes duration, which was inversely related to HbA_{1c}. It is worthy to note however, that baseline measures in this study were elevated above some of those previously reported (Galassetti *et al.* 2006, Galassetti *et al.* 2006, Rosa *et al.*

2008, 2010, Rosa *et al.* 2011). However it would be naïve to think that this was not influenced by the overnight fast or low circulating concentrations of insulin. Furthermore, most studies implement glucose and/or insulin clamp procedures and recruit children or adolescents who are usually recently diagnosed (Galassetti *et al.* 2006, Galassetti *et al.* 2006, Rosa *et al.* 2008, 2010, Rosa *et al.* 2011). This study population consisted of a relatively young (~24 years) group of individuals all in good glycaemic control (~7.7 % / 61 mmol/mol); exposure to inflammatory stimuli is likely to be much greater in the general diabetes population who are older, have a longer duration of diabetes, and in those with excess adiposity (De Rekeneire *et al.* 2006).

Only modest increases in IL-6 (~22%) and TNF-α (~45%) were observed following exercise, which is likely due to the consumption of the pre-exercise meal and concomitant insulin administration. The large carbohydrate bolus (1.0 g.carbohydrate.kg^{-1} BM) would have helped supplement glycogen reserves (Jentjens and Jeukendrup 2003), which may have attenuated the exercise-induced increases in IL-6 (Stouthard *et al.* 1995, Pedersen and Febbraio 2008) and even completely inhibited IL-6 release from contracting skeletal muscle (Pedersen and Febbraio 2008). In addition, insulin carries anti-inflammatory properties (Viardot *et al.* 2007), of which its administration, even in small doses, may have partially combatted the pro-inflammatory effects of TNF-α. Indeed, IL-6 concentrations were inversely related to circulating insulin concentrations. IL-6 has anti- as well as pro-inflammatory properties (Ostrowski *et al.* 1999, Petersen and Pedersen 2006), with some studies demonstrating IL-6 to exert inhibitory effects on TNF-α (Petersen and Pedersen 2006). In the present study there was a positive correlation between IL-6 and TNF-α; although a relationship does not necessarily indicate cause-effect, speculatively, increases in IL-6 may indeed have been in direct response to reductions in TNF-α (see section 1.5). Regardless of the underpinning mechanisms, data presented herein indicates that reductions in rapid-acting insulin after exercise do not significantly elevate the pro-inflammatory cytokine TNF-α, and that both TNF-α and IL-6 are not elevated above fasting concentrations.

IL-6 and TNF-α were selected, in part, because both display the greatest quantitative change in individuals with and without type 1 diabetes (Pedersen and Febbraio 2008), and therefore have a likelihood to yield distinct differences between study conditions (Ostrowski *et al.* 1999, Pedersen and Hoffman-Goetz 2000, Galassetti *et al.* 2006, Rosa *et al.* 2011). There is however, a known and marked inherent variability of many inflammatory markers (Rosa *et al.* 2008, Rosa *et al.* 2011), which reflects the remarkable metabolic complexity of the patient with type 1 diabetes, in which permutations in inflammation status are variable across patients and also within the same individuals over time (Rosa *et al.* 2008). Some of this variability can be attributed to antecedent hyperglycaemia (Sprenger *et al.* 1992, Drenth *et al.* 1995, Nehlsen-Cannarella *et al.* 1997, Ostrowski *et al.* 1999, Pedersen and Hoffman-Goetz 2000, Nemet *et al.* 2002, Galassetti *et al.* 2006, Rosa *et al.* 2008, 2010), however, it is important to note that patients in this study were kept under free-living conditions before experimentation and without correction using euglycaemic clamp procedures. Patients outside of this study are therefore likely to closely experience the responses in day-to-day life that were found in this study.

An interesting, if not surprising, finding was that plasma glucagon concentrations following the post-exercise meal were elevated under both **Full** and **50%**, but were significantly greater under **Full**. Although the majority of patients under this condition experienced hypoglycaemia (blood glucose ≤ 3.9 mmol.l^{-1}; **Full** n = 5, **50%** n = 0; chapter 3A), patients were treated with a corrective bolus of carbohydrate such that blood glucose levels (group mean blood glucose ~6.6 mmol.l^{-1}; chapter 3A) remained above the glycaemic threshold for plasma glucagon release (blood glucose ~ < 3.0 mmol.l^{-1}; Cryer 2008). Even if an appropriate glycaemic threshold was achieved to stimulate glucagon release, it would remain a surprise to find any increase in glucagon concentrations in these patients (length of diagnosis: range 4-31 years) as its secretion under hypoglycaemic conditions is largely attenuated in long-standing type 1 diabetes (Cryer 2008). One possible explanation for the increase in glucagon concentrations could be the consumption of a mixed-meal. Brown *et al* (2008) observed increased glucagon

concentrations in response to a mixed-meal in type 1 diabetes patients, as have other studies (Müller *et al.* 1970, Gerich *et al.* 1975, Ternand *et al.* 1982, Porksen *et al.* 2007), suggestive that the α-cell secretory reserve may be unaffected by the progression of the autoimmune process (Brown *et al.* 2008). If this were true, increases in glucagon may be attributed to neural stimulation, increased α-cell stimuli such as gastric inhibitory polypeptide (GIP) or a lack of glucagon-like peptide (GLP) which would otherwise promote postprandial endogenous glucagon secretion, although this is purely speculative. Whereas GLP usually inhibits glucagon secretion in non-diabetes patients, GLP is largely deficient in those with type 1 diabetes (Aronoff *et al*. 2004). Casual factors underpinning this are yet to be elucidated, although some authors suggest that this is consequential of lower intra-islet insulin levels, rather than systemic insulin concentrations *per se* (Greenbaum *et al.* 2002). Of note, the meal administered in the study by Brown et al (2008), was similar in nutritional content to the post-exercise meal given to patients in this study (Carbohydrate: 56 vs. 53 %, Protein: 21 vs. 25 %, Fat: 21 vs. 22 %), although smaller (~1.7 vs. ~2.8 MJ). In the present study, glucagon increased under both conditions, but was significantly greater following **Full**; as meals were identical in composition and weight, this may suggest a conditional effect following changes in rapid-acting insulin dose, however glucagon failed to correlate with changes in insulin concentrations, similarly to Brown et al (2008) and Potter and colleagues (1989). Interestingly, there was a positive association between glucagon and noradrenaline concentrations over the post-exercise post-prandial period. In individuals without diabetes, a sustained rise (≤ 120 minutes) in plasma noradrenaline has previously been demonstrated following a carbohydrate-rich mixed meal (Potter *et al.* 1989) with some initial mechanistic data indicating a complex synergy between glucagon and norepinephrine in appetite hormone regulation (Gagnon and Anini 2013). However, this has never been demonstrated in type 1 diabetes patients. Temporal rises in mean noradrenaline were evident at 60 minutes following the post-exercise meal under both conditions in this study, although changes from pre-meal concentrations were very small (~0.4 $nmol.l^{-1}$) and did not reach statistical significance.

The aim of this study was to assess the acute metabolic, inflammatory, and counter-regulatory hormonal effects of reducing post-exercise rapid-acting insulin dose under conditions of reduced pre-exercise rapid-acting insulin dose. The results from this study indicate that heavily reducing the dose of pre- and post-exercise rapid-acting insulin, as a measure to combat post-exercise hypoglycaemia, does not induce hyperketonaemia, increase the inflammatory cytokines IL-6 or TNF-α above fasting concentrations, or cause other metabolic or hormonal disturbances in type 1 diabetes patients. With this, diabetes care staff can have confidence that the only adverse effect of this strategy is hyperglycaemia. There is now a need to normalise post-exercise post-prandial glycaemia through modifications to dietary intake whilst under conditions of reduced rapid-acting insulin dose.

CHAPTER 4A

THE GLYCAEMIC RESPONSES TO MANIPULATING THE GLYCAEMIC INDEX OF CARBOHYDRATES CONSUMED FOLLOWING EVENING EXERCISE IN TYPE 1 DIABETES

4.0 Introduction

In chapter 3 it was demonstrated that meal time insulin adjustment, specifically reducing the dose of rapid-acting insulin before and after exercise is vital to minimise the risk of post-exercise hypoglycaemia (Campbell *et al.* 2013), although, this may cause post-prandial hyperglycaemia. However, there is currently little advice on optimal carbohydrate type for exercising patients with type 1 diabetes (Chu *et al.* 2011). Current recommendations place more focus on the *quantity* rather than the *composition* of the carbohydrate to be consumed following exercise (Bantle *et al.* 2008, Evert *et al.* 2014). Consumption of ~5.0g carbohydrate.kg^{-1} BM is typically recommended for moderate intensity exercise (Riddell and Perkins 2006, Perry and Gallen 2009), however, food *composition* is also an important consideration, as the type of carbohydrate can exert a major influence on post-prandial glycaemia in diabetes patients (Parillo and Riccardi 1995). Meals containing identical macronutrient compositions are digested and absorbed at varying rates producing a range of glycaemic responses (Jenkins *et al.* 1981), with carbohydrate foodstuffs with a low GI eliciting a more gradual rise and fall in blood glucose compared to their high GI equivalents. Resultantly, more favourable post-prandial glycaemic profiles have been shown following ingestion of low GI foods in patients with type 1 diabetes (Nansel *et al.* 2008, Parillo *et al.* 2011).

It may thus be possible to optimise post-exercise glycaemia by manipulating the composition of foods consumed during this time. The protracted absorption rates of low GI foods may be beneficial for reducing post-prandial hyperglycaemia. However, slower delivery of carbohydrate to post-exercise musculature, and potentially slower rates of muscle glycogen replenishment following exercise (Jentjens and Jeukendrup 2003, Jensen and Richter 2012), may increase the risk of post-exercise hypoglycaemia (Macdonald 1987, Riddell and Perkins 2006). Inversely, consuming high GI foods may promote accelerated muscle glycogen restoration (Jentjens and Jeukendrup 2003, Jensen and Richter 2012), reducing the incidence of post-exercise hypoglycaemia (Macdonald 1987, Riddell and Perkins 2006). However, the

need to reduce the insulin-to-carbohydrate ratio may be associated with post-prandial hyperglycaemia following ingestion of high GI carbohydrates (Nansel *et al.* 2008, Parillo *et al.* 2011).

In addition, studies have traditionally employed morning time exercise, whereas many individuals prefer to exercise in the evening. Considering that patients experience a delayed risk of hypoglycaemia 7-11 hours after exercise (Mcmahon *et al.* 2007), falls in glycaemia following evening time exercise are likely to occur nocturnally (Taplin *et al.* 2010). As such, patients are recommended to consume a bedtime snack (Hernandez *et al.* 2000) to ensure adequate carbohydrate availability during the night, avoiding nocturnal hypoglycaemia. Unfortunately however, there is currently no information regarding the optimum composition of this snack, nor whether a snack is required at all if adjustments in post-exercise rapid-acting insulin and meal composition are made. Therefore, the aim of this study was to examine the influence of the glycaemic index of the meal and subsequent bedtime snack consumed after evening-time exercise on post-prandial glycaemia and nocturnal glycaemic control in type 1 diabetes patients.

4.1 Methods

Patient demographic information is presented in Table 4.0. A schematic of the trial design is presented in Figure 4.0; this study was a randomised, counterbalanced, cross-over design. Patients arrived to the exercise laboratory on two separate evenings (~17:00 PM) having consumed a prescribed lunch meal (see 2.2.5.2, Table 2.3, MEAL 2) ~4 hours before arrival. Following a resting sample, patients self-administered a 75% reduced dose of rapid-acting insulin (2.0 ± 0.1 IU, see 2.2.6) into the abdomen (West *et al.* 2010, Campbell *et al.* 2013). Patients consumed a pre-exercise carbohydrate bolus equating to 1.0 g.carbohydrate.kg^{-1} BM (1.8 ± 0.2 MJ, see 2.2.5.6, Table 2.3, MEAL 3) within a 5 minute period. Patients remained at rest for 60 minutes following consumption of the pre-exercise carbohydrate bolus / rapid-acting insulin injection with blood samples at 60 minutes. Immediately after the 60 minute

blood draw, patients commenced 45 minutes of treadmill running at a speed calculated to elicit 70% of their $\dot{\mathbf{V}}O_{2peak}$. Immediately following exercise, a blood sample was taken, with further interval samples at 15, 30, and 60 minutes post-exercise. At 60 minutes, patients administered a 50% rapid-acting insulin dose (4.0 ± 0.2 IU; see 2.2.6) (determined from the results of chapter 3) and consumed one of two isoenergetic meals similar in macronutrient content equating to 1.0 g.carbohydrate.kg^{-1} BM but differing in glycaemic index (GI). The meals were of either a low (GI = 37; 1.7 ± 0.1 MJ, MEAL 5) or high GI (GI = 92; 1.7 ± 0.1, MEAL 6) (see 2.2.5.8, Table 2.3). Patients remained at rest for a further 180 minutes with periodic blood samples every 30 minutes. Fifteen minutes before the consumption of the post-exercise meal, and at 45, 105, and 165 minutes following the post-exercise meal, expired gases were collected for calculation of substrate oxidation rates (see 2.8). At 180 minutes post-exercise, patients consumed one of two isoenergetic bedtime snacks matched for macronutrient content and equating to 0.4 g.carbohydrate.kg^{-1} BM but differing in GI (low versus high) (Low: GI = 38, 0.9 ±0.3 MJ, MEAL 8; High: GI = 86, 0.9 ± 0.3 MJ, MEAL 9) (see 2.2.5.9, Table 2.3), corresponding to the GI of the post-exercise meal. As such, patients completed two trials in a randomised and counter-balanced fashion: a low GI (**LOW**) and a high GI trial (**HIGH**). Following the consumption of the bedtime snack, patients were discharged from the laboratory and returned home. Transport was provided to patients on their journey home to control travel across trials. Patients were instructed to replicate sleeping patterns as best possible over the course of the study. Continuous glucose monitoring captured interstitial glucose for a further 21 hours post-laboratory, and patients self-recorded β-hydroxybutyrate.

Table 4.0 Patients demographic information

		Patient ID											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Mean±SEM**
Insulin (IU)	**Basal**	38^{G}_{M}	20^{D}_{B}	22^{G}_{M}	26^{D}_{B}	34^{G}_{E}	18^{D}_{B}	20^{G}_{E}	31^{G}_{M}	24^{G}_{M}	30^{G}_{E}	-	26 ± 2
	Bolus	1^{L}	1^{A}	1^{A}	1^{A}	1^{A}	0.8^{A}	1^{A}	1^{A}	1^{A}	1^{A}	-	1.0 ± 0.0
HbA1c (%)		6.6	6.4	6.9	6.4	6.3	6.9	7.0	6.4	7.1	7.2	-	6.7 ± 0.7
BMI ($kg.m^2$)		26.7	24.5	25.4	23.1	25.4	24.5	26.9	26.1	25.4	27.2	-	25.5 ± 0.9
Diabetes duration (years)		15	16	16	14	15	4	26	14	16	15	-	15 ± 2
$\dot{V}O_{2peak}$ ($ml.kg.min^{-1}$)		50.1	54.3	53.1	56.3	53.2	54.3	49.1	54.3	45.3	48.1	-	51.9 ± 1.2
Age (years)		30	24	27	35	24	25	38	19	26	21	-	27 ± 5

Note: G = Glargine, D = Detemir, A = Aspart, L = Lispro, M = once daily (morning), E = once daily evening , B = bi-daily; bolus insulin calculated per 10g CHO.

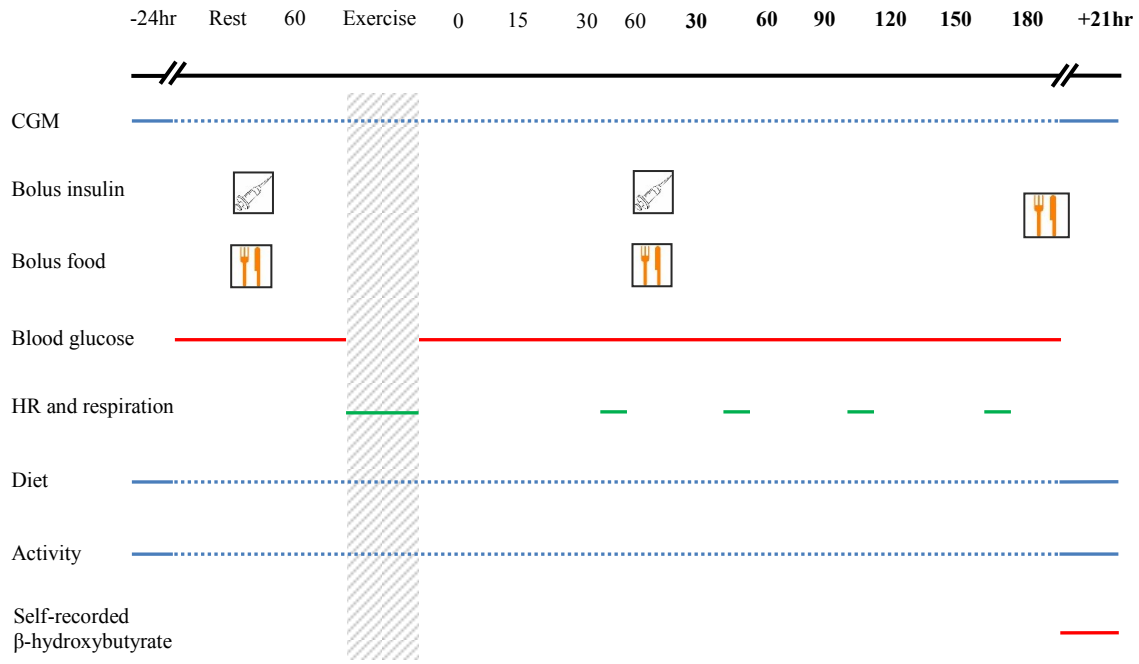

Figure 4.0. Schematic of experimental trial. Note: Bold text indicates post-exercise intervention period.

4.2 Results

4.2.1 Pre-laboratory phase

4.2.1.1 Pre-laboratory glycaemia

Glycaemic control was comparable over the 24 hours prior to patients' arrival at the laboratory for both experimental trials (CGM mean glucose: **HIGH** 7.9 ± 0.7, **LOW** 7.9 ± 0.7 mmol.l^{-1}; p = 0.465; and total interstitial glucose area under the curve: **HIGH** 11277 ± 1069, **LOW** 10971 ± 1126 mmol.l^{-1}.min^{-1}; p = 0.215).

4.2.1.2 Pre-laboratory dietary intake, insulin administration and activity

There were no differences in total energy consumed (**HIGH** 9.0 ± 0.8, **LOW** 9.9 ± 0.8 MJ; p = 0.508), with similar contribution from carbohydrate (**HIGH** 51 ± 3, **LOW** 46 ± 3 %; p = 0.869), fat (**HIGH** 30 ± 3, **LOW** 32 ± 4 %; p = 0.301) and protein (**HIGH** 20 ± 2, **LOW** 22 ± 3 %; p = 0.556). The total amount of rapid-acting insulin administered (**HIGH** 26 ± 4, **LOW** 26 ± 4 IU; p = 0.609) and levels of activity (**HIGH** 6949 ± 105, **LOW** 7041 ± 118 steps; p = 0.372) were comparable over the 24 hours before each trial.

4.2.2 Laboratory phase

The absolute blood glucose responses are presented in Figure 4.1. There was a significant condition*time interaction ($F_{(11,99)}$ = 15.972, p < 0.001, partial eta^2 = 0.666), and a significant time ($F_{(11,99)}$ = 39.827, p < 0.001, partial eta^2 = 0.833) and condition effect ($F_{(1,9)}$ = 15.049, p = 0.005, partial eta^2 = 0.653) for absolute blood glucose responses. Baseline blood glucose concentrations were similar (**HIGH** 6.5 ± 0.3, **LOW** 6.2 ± 0.7 mmol.l^{-1}; p = 0.715), as were those immediately before exercise (**HIGH** 12.1 ± 1.0, **LOW** 12.1 ± 0.5 mmol.l^{-1}; p = 0.762).

4.2.2.1 Exercise and recovery period

Patients ran at an average speed of 10.1 ± 0.3 km.hr^{-1}, completing 7.6 ± 0.2 km and expending 3.0 ± 0.2 MJ. Patients exercised at a similar intensity across trials (**HIGH** 77 ± 0, **LOW** 74 ± 0 %$\dot{V}O_{2peak}$; p = 0.352; **HIGH** 80 ± 2, **LOW** 79 ± 3 %HR_{peak}; p = 0.631) inducing comparable falls in blood glucose (**HIGH** -5.4 ± 0.7, **LOW** -6.8 ± 0.5 mmol.l^{-1}; p = 0.733; Figure 4.1) such that immediately following the cessation of exercise, blood glucose were comparable to baseline under both conditions (p > 0.05; Figure 4.1). There were no incidences of hypoglycaemia during exercise or throughout the 60 minutes recovery period, with blood glucose concentrations, on average, in euglycaemic ranges up to the administration of the post-exercise meal (**HIGH** 6.4 ± 0.9, **LOW** 5.5 ± 0.7 mmol.l^{-1}; p = 0.389; Figure 4.1).

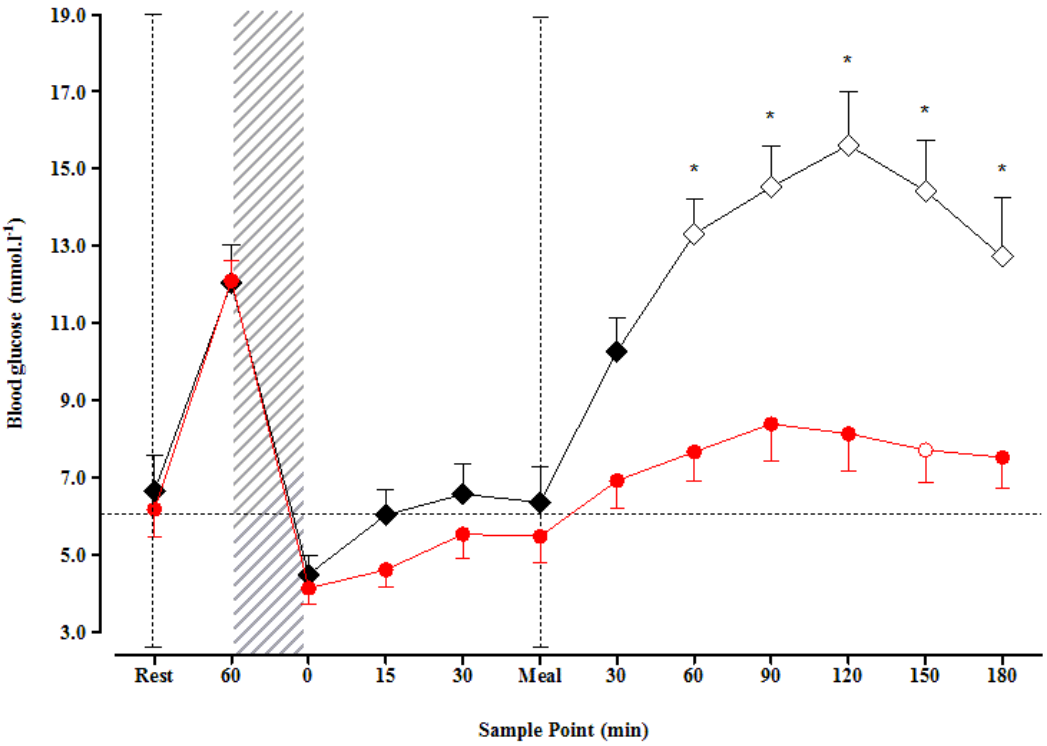

Figure 4.1. Time-course changes in blood glucose following the post-exercise meal intervention. Data presented as mean ± SEM. Black diamonds = **HIGH**, red circles = **LOW**. Transparent sample point within a condition indicates a significant difference from pre-meal concentrations ($p \leq 0.05$). * indicates a significant different between **HIGH** and **LOW** ($p \leq 0.05$). Vertical dashed line break indicates carbohydrate meal and insulin administration. Thatched area indicates exercise. Note: Test meal and insulin were administered immediately following 60 minutes post-exercise sample point.

4.2.2.2 Post-exercise intervention period

Both meal types induced an increase in blood glucose concentrations over the subsequent 180 minutes, although this rise was significantly attenuated following **LOW**, compared **HIGH** ($p < 0.05$; Figure 4.1). Despite clear differences in glycaemia, all patients were protected from hypoglycaemia during this time, regardless of meal GI. However, all patients were exposed to hyperglycaemia following **HIGH**, whereas fewer patients experienced hyperglycaemia following **LOW** (n = 4). Moreover, hyperglycaemia was less pronounced (mean peak blood glucose: **LOW** 8.8 ± 1.0 vs. **HIGH** 15.9 ± 1.2 mmol.l^{-1}; $p = 0.005$) and tended to be only transient (time spent hyperglycaemic: **LOW** 81 ± 26 vs. **HIGH** 165 ± 15 minutes; $p < 0.001$) following **LOW**. As such, immediately before the consumption of the bedtime snack, blood glucose concentrations were significantly greater following **HIGH** (**HIGH** 12.7 ± 1.5 vs. **LOW** 7.5 ± 2.6 mmol.l^{-1}; $p = 0.004$; Figure 4.1), resulting in more patients leaving the laboratory hyperglycaemic (**HIGH** n = 9 vs. **LOW** n = 4).

4.2.2.3 Substrate oxidation responses

There were no differences in substrate oxidation responses during the post-exercise post-prandial period, with average carbohydrate (**HIGH** 14.5 ± 2.6, **LOW** 14.7 ± 2.7 g.carbohydrate.hr^{-1}; $p = 0.927$) and lipid (**HIGH** 3.0 ± 0.9, **LOW** 3.1 ± 0.9 g.lipid.hr^{-1}; $p = 0.809$) oxidation rates similar.

4.2.3 Post-laboratory phase

4.2.3.1 Late evening glycaemic responses

Following discharge from the laboratory and having consumed the bedtime snack, glycaemia during the late evening was significantly greater under **HIGH** (mean interstitial glucose: **HIGH** 13.2 ± 1.6 vs. **LOW** 9.3 ± 0.9 mmol.l^{-1}; $p = 0.011$; Figure 4.2), with greater individualised mean peak interstitial glucose (**HIGH** 18.3 ± 1.1 vs. **LOW** 13.9 ± 0.8 mmol.l^{-1};

p = 0.009) and more patients in hyperglycaemic ranges before sleep (**HIGH** n = 9 vs. **LOW** n = 5). All patients under all conditions were protected from hypoglycaemia during this time.

4.2.3.2 Nocturnal glycaemia

During sleep, falling glucose levels were evident under both conditions, such that concentrations became comparable ~8 hours after exercise (p > 0.05; Figure 4.2). As such, mean interstitial glucose (**HIGH** 10.8 ± 1.4, **LOW** 8.4 ± 1.2 mmol.l^{-1}; p = 0.262; Figure 4.2) and total interstitial glucose area under the curve (**HIGH** 5876 ± 738, **LOW** 4562 ± 672 mmol.l^{-1}.min^{-1}; p = 0.236) during the night were similar between conditions. During the night, patients were exposed to hypoglycaemia under both conditions (**HIGH** n = 5, **LOW** n = 5), with similar time of onset (~8 hours post-exercise; Figure 4.2) and similar individualised interstitial glucose nadir (**HIGH** 3.6 ± 0.4, **LOW** 3.4 ± 0.3 mmol.l^{-1}; p = 0.650). Despite some patients experiencing multiple bouts of hypoglycaemia (total number of episodes: **HIGH** 10, **LOW** n = 8), total time spent in hypoglycaemic ranges (**HIGH** 237 ± 55, **LOW** 176 ± 32 minutes; p = 0.569) were similar between conditions, despite a tendency for fewer hypoglycaemic episodes and less times spent hypoglycaemic under **LOW**. There were no differences in time spent in euglycaemic (**HIGH** 202 ± 55, **LOW** 282 ± 55 minutes; p = 0.705) or hyperglycaemic ranges (**HIGH** 101 ± 44, **LOW** 81 ± 43 minutes; p = 0.765). As such, interstitial glucose concentrations immediately upon awakening were similar (**HIGH** 8.5 ± 0.9, **LOW** 8.3 ± 0.9 mmol.l^{-1}; p = 0.614). Glycaemia remained similar between conditions for the remainder of the 24 hours post-exercise window (p > 0.05; Figure 4.2).

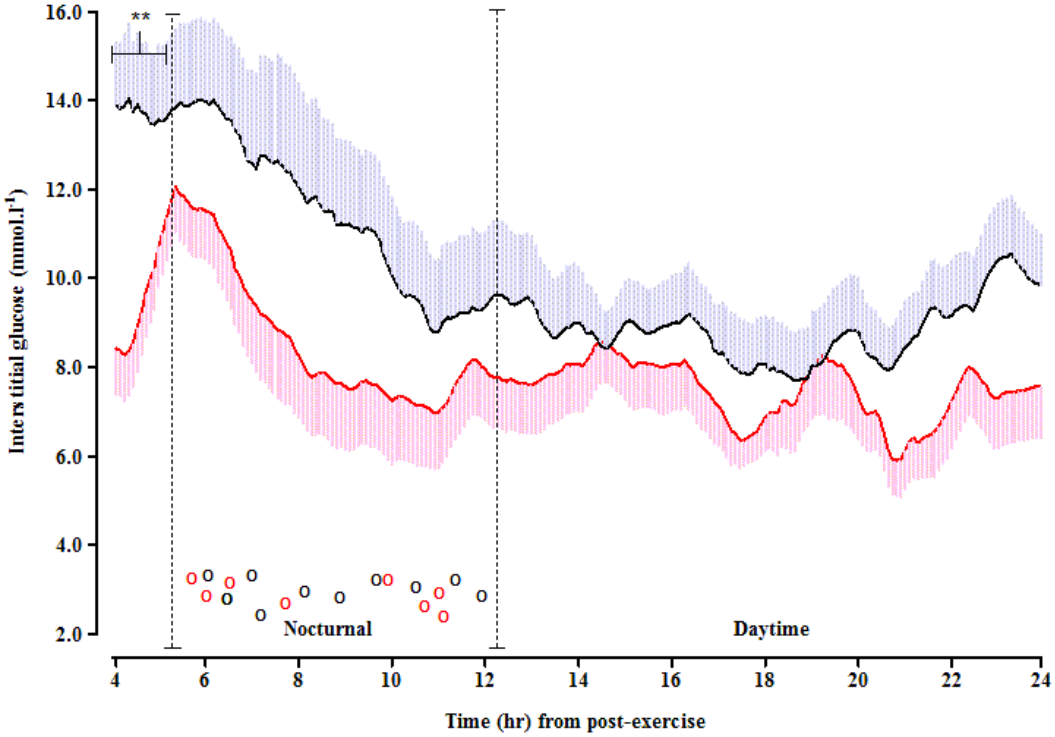

Figure 4.2. Time-course changes in interstitial glucose concentrations throughout the post-laboratory period. Data presented as mean ± SEM. Black solid trace = **HIGH**, red broken trace = **LOW**. ** indicates a significant difference in interstitial glucose area under the curve between **HIGH** and **LOW** ($p \leq 0.05$). Open circles represent hypoglycaemic episodes, as determined from CGM data Vertical dashed line break indicates nocturnal and daytime periods.

4.2.3.3 Post-laboratory dietary intake, insulin administration, activity, and self-recorded β-hydroxybutyrate

Self-recorded β-hydroxybutryate concentrations were undetectable in all patients throughout the post-laboratory period. During this time, total energy consumed (**HIGH** 3.0 ± 0.1, **LOW** 2.9 ± 0.2 MJ; $p = 0.774$) with contribution from carbohydrate (**HIGH** 72 ± 5, **LOW** 64 ± 8 %; $p = 0.767$), fat (**HIGH** 20 ± 5, **LOW** 22 ± 7 %; $p = 0.834$) and protein (**HIGH** 8 ± 3, **LOW** 14 ± 6 %; $p = 0.548$) was similar between all conditions, despite more carbohydrate consumed to correct blood glucose (**HIGH** 32 ± 7, **LOW** 6.0 ± 4 g; $p = 0.002$). The total amount of rapid-acting insulin administered (**HIGH** 17 ± 2, **LOW** 18 ± 2 IU; $p = 0.967$), and levels of activity (**HIGH** 6087 ± 94, **LOW** 6489 ± 118 steps; $p = 0.369$) were comparable during this time.

4.3 Discussion

The aim of this study was to determine whether manipulating the glycaemic index of carbohydrates consumed following evening-time exercise could modulate post-prandial glycaemia and metabolism, to provide protection from post-exercise, post-prandial hyperglycaemia and late-onset hypoglycaemia in patients with type 1 diabetes. This study demonstrates for the first time that consumption of low glycaemic index carbohydrates, under conditions of reduced rapid-acting insulin dose following evening exercise, improves post-prandial glycaemia, by reducing hyperglycaemia and offering protection from hypoglycaemia for approximately 8 hours after exercise. However, beyond this time risk of late-onset nocturnal hypoglycaemia persists regardless of the glycaemic index of the post-exercise meal and bedtime snack.

Chapter 3A demonstrated the importance of reducing rapid-acting insulin dose administered with the meal *after,* as well as before exercise to extend the period of protection from post-exercise hypoglycaemia (Campbell *et al.* 2013). This study now demonstrates that under these conditions, the composition of the post-exercise meal has an important role for modulating post-prandial glycaemia. Blood glucose concentrations following the high GI post-exercise meal were significantly greater than that with the low GI meal, consequently exposing all patients in the former condition to hyperglycaemia during the laboratory observation period. Conversely, the incidence of hyperglycaemia was reduced by 60% after the low GI meal (**LOW** = 40% vs. **HIGH** = 100%). Indeed, in those patients affected, hyperglycaemia was less pronounced and tended to be only transient and short lasting after the low GI meal. Despite clear post-prandial differences in glycaemia between the high and low GI meals, all patients remained protected from hypoglycaemia during their time in the laboratory. Presently, there are relatively few dietary guidelines to assist individuals with type 1 diabetes in managing post-exercise glycaemia. This study shows that by consuming a low glycaemic index post-exercise meal, post-prandial hyperglycaemia can be reduced, without exposure to hypoglycaemia. This is an important observation because the aim of diabetes management is to

normalise blood glucose concentrations (Thomas *et al.* 2007), especially when incorporating exercise into the lives of patients (Chu *et al.* 2011).

When exercise is performed in the evening, consumption of a carbohydrate-based snack before bed is recommended for type 1 diabetes patients (Hernandez *et al.* 2000). Blood glucose was typically within the euglycaemic ranges prior to the consumption of the bedtime snack following the low GI post-exercise meal, whereas following consumption of the high GI meal and snack, patients tended to stay in hyperglycaemic ranges over the entire duration of the post-exercise post-prandial period (**LOW** ~7.5 vs. **HIGH** ~12.2 $mmol.l^{-1}$). Outside of formal studies, patients within normal blood glucose ranges before bed often choose to raise glycaemia by consuming a carbohydrate-based snack (Hernandez *et al.* 2000) due to fear of nocturnal hypoglycaemia (Cryer and Childs 2002). However, patients who are severely hyperglycaemic before bed (such as those under **HIGH**) may be tempted to administer corrective insulin units, which, in an exercise-induced insulin sensitised state (Mikines *et al.* 1988, Maarbjerg *et al.* 2011) is likely to cause a rapid fall in glucose during the night. Avoidance of the bedtime snack, and hence missing a valuable source of carbohydrate before sleep, is likely to exacerbate the risk of nocturnal hypoglycaemia. Despite wide differences in blood glucose concentrations before sleep under all conditions, declines occurred irrelevant of post-exercise meal and snack composition, such that conditions became comparable 3 hours after consuming the bedtime snacks and with similar rates of nocturnal hypoglycaemia thereafter. This indicates that patients are at risk of late-onset nocturnal hypoglycaemia with predicted nadir more than 8 hours post-exercise (Mcmahon *et al.* 2007, Campbell *et al.* 2013) (chapter 3A) despite the consumption of a bedtime snack, regardless of GI, or blood glucose levels before bed. Thus, it would seem that elevating blood glucose before sleep through consumption of high GI carbohydrates in the post-exercise period confers no glycaemic benefit for avoiding nocturnal hypoglycaemia.

So that it was possible to investigate the impact of the GI of the evening carbohydrates, patients consumed enough carbohydrate (consuming 2.6 $g.carbohydrate.kg^{-1}$ BM during the

evening) to cover the cost of the exercise bout, with patients utilising ~1.7 g.carbohydrate.kg^{-1} BM in total during exercise, and with total daily carbohydrate intake matching current recommendations of ~5.0 g.carbohydrate.kg^{-1}BM (Riddell and Perkins 2006, Perry and Gallen 2009), thus establishing a positive carbohydrate balance. Despite consuming sufficient carbohydrate for the recovery of muscle glycogen post-exercise, and perhaps consuming more carbohydrate than is typical, hypoglycaemia was still encountered late after exercise in the early hours of the morning. Indeed this study shows that acute alterations in insulin dosage and carbohydrate feeding both before and after evening exercise are not enough to prevent late-onset hypoglycaemia in all patients. These findings direct attention towards the role of basal insulin administration in avoiding nocturnal hypoglycaemia after evening exercise. Considering once daily insulin Glargine administration is associated with a glucose nadir 4-14 hours after administration (Ashwell *et al.* 2006, Thomas *et al.* 2007), not only basal insulin dose but also the timing of administration may be of particular importance.

The aim of this study was to assess the acute and 24 hour glycaemic effects of manipulating the glycaemic index of carbohydrates consumed following evening exercise, under conditions of reduced pre- and post-exercise rapid-acting insulin dose. This study shows for the first time that consuming low glycaemic index carbohydrates in tandem with a reduced rapid-acting insulin dose following evening exercise can play an important role in normalising glycaemia, preventing post-prandial hyperglycaemia whilst protecting patients from hypoglycaemia for up to 8 hours after exercise. The clinical utility of these findings is clear, as carbohydrates which are part of a patients habitual diet can be easily exchanged with those that offer the same macronutrient content but are of a low GI (e.g. substituting particular types of breads, strains of rice, pastas and potatoes, or sports drinks with different carbohydrate compositions) facilitating more desirable post-prandial glycaemic responses. However, it does not seem that carbohydrate type, nor total carbohydrate intake alone, are factors in the development of late-onset hypoglycaemia, as patients may still be exposed to nocturnal hypoglycaemia following evening-time exercise. There is now a need to firstly: determine whether this strategy is

associated with adverse hormonal, metabolic, counter-regulatory disturbances, and whether there is an effect on appetite regulation, and secondly: to focus on basal insulin adjustment to determine whether late-onset nocturnal hypoglycaemia following evening-time exercise can be avoided, whilst harnessing improved post-prandial profiles following the consumption of low GI carbohydrates with a reduced rapid-acting insulin dose, during the post-exercise period.

CHAPTER 4B

THE METABOLIC, INFLAMMATORY, AND COUNTER-REGULATORY HORMONAL RESPONSES FOLLOWING MANIPULATION OF THE GLYCAEMIC INDEX OF CARBOHYDRATES CONSUMED AFTER EVENING EXERCISE IN TYPE 1 DIABETES

4.4 Introduction

Chapter 4A demonstrated that under conditions of reduced rapid-acting insulin dose, the consumption of low glycaemic index carbohydrates following evening exercise modulates post-prandial glycaemia such that hyperglycaemia is reduced without increased risk of early-onset post-exercise hypoglycaemia (Campbell *et al.* 2014). Whereas there is a clear glycaemic benefit for altering the composition of post-exercise meals following exercise whilst under conditions of reduced rapid-acting insulin dose, it remains unknown whether this strategy carries implications for hormonal, metabolic, or inflammatory parameters. Thus, the efficacy of manipulating post-exercise carbohydrates is yet to be determined. Considering the wide glycaemic variation attributed to differences in GI, it is likely that hormonal, metabolic and / or inflammatory measures could differ significantly between two meals with contrasting GI values.

It is known that hyperglycaemia is associated with an inflammatory cytokine response (Targher *et al.* 2001, Esposito *et al.* 2002, De Rekeneire *et al.* 2006, Rosa *et al.* 2008), and hypoinsulinaemic hyperglycaemia is associated with increased lipolysis and ketogenesis (Laffel 2000, Wallace and Matthews 2004). This was demonstrated in chapter 3B, in which a positive association between increased blood glucose and NEFA, and increased blood glucose and β-hydroxybutyrate appearance were evident. Although a clinically-meaningful difference in β-hydroxybutyrate concentrations was not established, nor were there clear differences observed in the pro-inflammatory cytokine TNF-α, patients were on average in only mildly hyperglycaemic ranges in chapter 3A (average blood glucose ~9.9 $mmol.l^{-1}$) compared to those observed in chapter 4A following the high GI post-exercise meals (average blood glucose ~13.2 $mmol.l^{-1}$). Furthermore, on average patients displayed a greater mean peak blood glucose (chapter 3A ~11.5, chapter 4A ~14.3 $mmol.l^{-1}$). With this in mind, there may be greater potential for wider differences in hormonal, metabolic and inflammatory parameters following the consumption of a high GI post-exercise meal. Whether normalisation of glycaemic profiles (Daneman 2006) through consumption of a low GI post-exercise meal can

avoid such disturbances is yet to be determined. Accordingly, the aim of this study was to investigate the influence of manipulating the GI of the meal consumed following evening exercise on hormonal, metabolic and inflammatory parameters in type 1 diabetes patients.

4.5 Methods

A second arm of analysis was performed on all patients from **HIGH** and **LOW** trials from chapter 4A. Blood lactate, serum cortisol, non-esterified-fatty-acids, β-hydroxybutyrate, and plasma glucagon, adrenaline, glycerol, IL-6 and TNF-α were measured for 180 minutes post-meal (Figure 4.3).

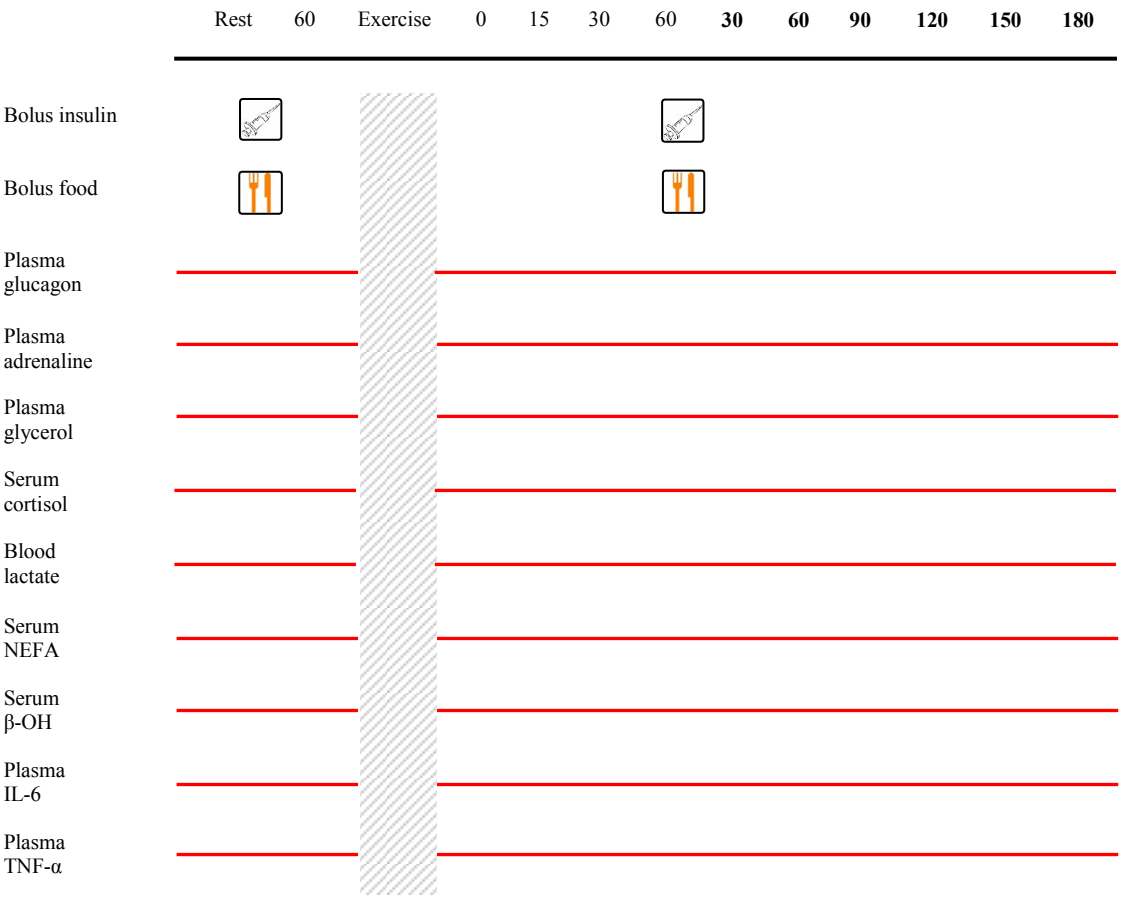

Figure 4.3. Schematic of experimental trial. Note: Bold text indicates post-exercise intervention period.

4.6 Results

4.6.1 Serum insulin, counter-regulatory hormone and metabolite responses

The serum insulin responses are presented in Figure 4.4. There was a significant time effect ($F_{(11,99)} = 13.232$, $p < 0.001$, partial-eta$^2 = 0.343$), but not a time*condition interaction ($F_{(1,9)} = 13.002$, $p < 0.001$, partial-eta$^2 = 0.652$) when examining insulin concentrations. Serum insulin concentrations peaked similarly at 60 minutes following the post-exercise meal / administration of rapid-acting insulin dose, before returning to pre-meal and resting concentrations under both conditions (Figure 4.4).

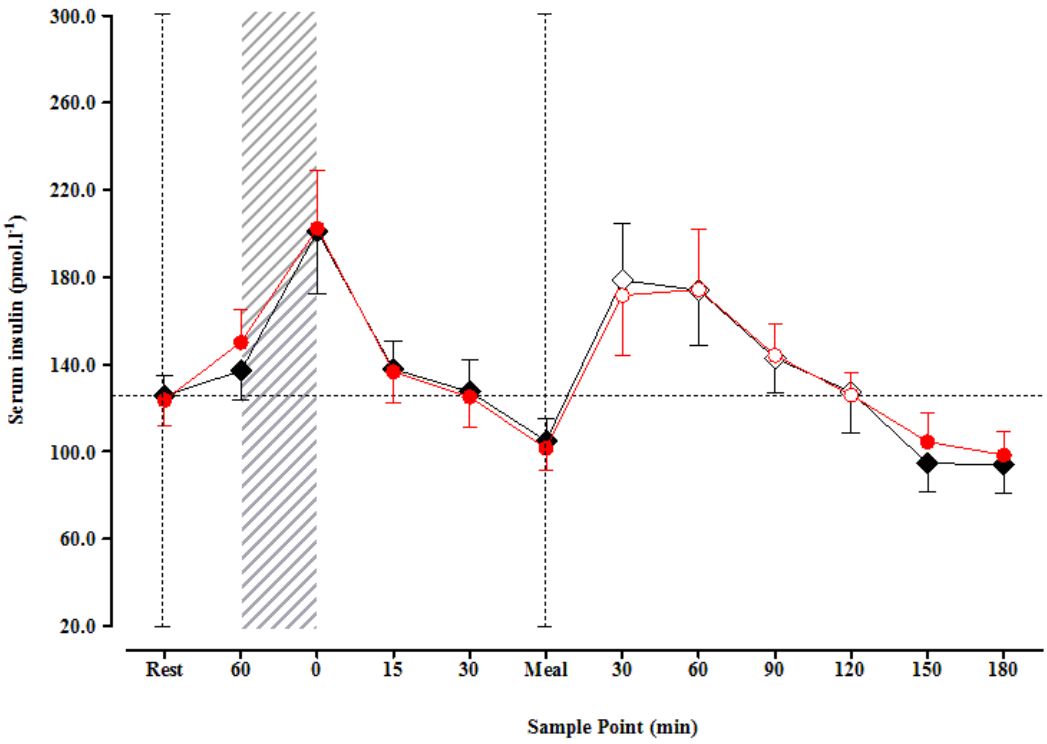

Figure 4.4. Time-course changes in serum insulin from rest. Data presented as mean ± SEM. Black diamonds = **HIGH**, red circles = **LOW**. Transparent sample point within a condition indicates a significant difference from pre-meal concentrations ($p \leq 0.05$). * indicates significantly different from **HIGH** ($p \leq 0.05$). Thatched area indicates exercise. Vertical dashed line break indicates carbohydrate meal and insulin administration. Note: Test meal and insulin were administered immediately following 60 minutes post-exercise sample point.

Hormonal and metabolite responses are presented in Table 4.1. There were no conditional differences in counter-regulatory hormones or metabolites up to 60 minutes post-exercise ($p > 0.05$; Table 4.1). There were no conditional differences in absolute concentrations of plasma glucagon, adrenaline, or serum cortisol (Table 4.1). Plasma glucagon concentrations peaked similarly 30 minutes following the post-exercise meals. Over the course of the remaining post-prandial period concentrations declined under **HIGH** such that at 150 and 180 minutes, concentrations were lower to those elicited at pre-meal, whereas under **LOW** this decline was largely attenuated (Table 4.1). Serum cortisol declined similarly such that concentrations under both conditions were lower than those observed pre-meal (Table 4.1).

Blood lactate was, on average, greater under **LOW** over the course of the post-exercise post-prandial period, although this failed to reach statistical significance between conditions and by 150 and 180 minutes concentrations were similar to resting and pre-meal concentrations (Table 4.1). Plasma glycerol concentrations were significantly lower following the **LOW** meal, with measures declining below pre-meal concentrations ($p < 0.05$; Table 4.1). No differences were observed in serum NEFA (Table 4.1) or β-hydroxybutyrate (Figure 4.5), with concentrations similar to pre-meal and rest at 180 minutes post-meal.

4.6.2 Inflammatory cytokine responses

The inflammatory cytokine responses are presented in Figure 4.5. There was a significant time*condition interaction ($F_{(11,99)} = 12.567$, $p < 0.001$, partial-eta$^2 = 0.583$), and a significant effect of time ($F_{(11,99)} = 11.792$, $p < 0.001$, partial-eta$^2 = 0.567$) and condition ($F_{(1,9)} = 9.664$, $p = 0.013$, partial-eta$^2 = 0.518$) when examining plasma IL-6 concentrations. There was also a significant time*condition interaction ($F_{(11,99)} = 17.027$, $p < 0.001$, partial-eta$^2 = 0.654$), and a significant effect of time ($F_{(11,99)} = 8.996$, $p < 0.001$, partial-eta$^2 = 0.500$) and condition ($F_{(1,9)} = 16.341$, $p = 0.03$, partial-eta$^2 = 0.645$) when examining plasma TNF-α concentrations.

Resting concentrations of IL-6 and TNF-α were positively related to length of diabetes (IL-6: $r = 0.762$, $p = 0.010$; TNF-α: $r = 0.786$, $p = 0.007$) and inversely related to HbA_{1c} (IL-6: $r = -$

0.708, p = 0.022; TNF-α: r = -0.600, p = 0.049), but not $\dot{\mathbf{V}}O_{2peak}$ (IL-6: r = -0.374, p = 0.288; TNF-α: r = -0.165, p = 0.650). Length of diagnosis and HbA_{1c} were negatively correlated (r = -0.942, p <0.001).

Both plasma IL-6 and TNF-α concentrations were significantly raised 15 minutes post-exercise (Figure 4.5) but returned to resting concentrations immediately before the administration of the post-exercise meals. Following **HIGH** post-prandial IL-6 and TNF-α significantly increased above pre-meal and resting concentrations (Figure 4.5), whereas IL-6 and TNF-α was attenuated following **LOW**. As such, at 180 minutes post-meal IL-6 and TNF-α decreased below resting measures under **LOW**, whereas concentrations remained elevated under **HIGH**. Average IL-6 and TNF-α concentrations over the post-exercise post-prandial period were positively correlated with average blood glucose concentrations (IL-6: r = 0.425, p = 0.049; TNF-α: r = 0.425, p = 0.049). No other relationships were found between any other measures.

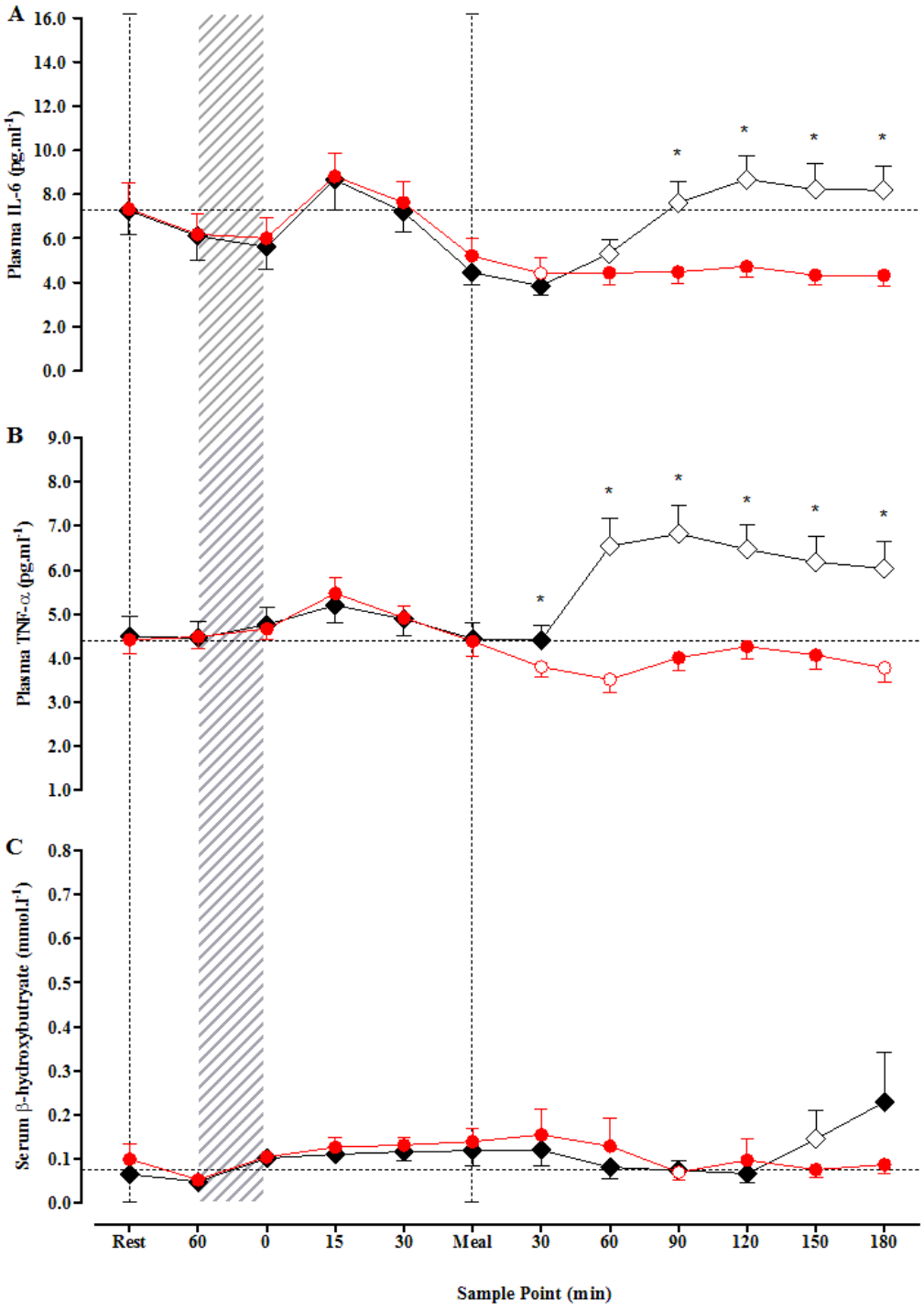

Figure 4.5 A-C. Time-course changes in (**A**) plasma IL-6, (**B**) plasma TNF-α and (**C**) serum β-hydroxybutyrate throughout the laboratory period. Data presented as mean ± SEM. Black diamonds = **HIGH**, red circles = **LOW**. Transparent sample point within a condition indicates a significant difference from pre-meal concentrations ($p \leq 0.05$). * indicates significantly different from **HIGH** ($p \leq 0.05$). Thatched area indicates exercise. Vertical dashed line break indicates carbohydrate meal and insulin administration. Thatched area indicates exercise. Note: Test meal and insulin were administered immediately following 60 minutes post-exercise sample point.

Table 4.1. Metabolic and counter-regulatory hormone responses to post-exercise meals of differing glycaemic index

															ANOVA *p*	
		Rest	**60**	**E**	**0**	**15**	**30**	**Pre-Meal**	**30**	**60**	**90**	**120**	**150**	**180**	**T**	**T*C**
Plasma Glucagon (pg.ml^{-1})	**HIGH**	730±99	591±72†		682±68	760±83	768±94	833±117	953±155†	922±154†	872±145†	798±141	690±100‡	669±101‡	<0.001	=0.306
	LOW	733±130	611±75†		658±68	792±82	818±100	816±125	947±170†‡	937±159†‡	907±149†	862±141	806±120	840±139		
Plasma Adrenaline (nmol.l^{-1})	**HIGH**	0.09±0.01	0.15±0.03		0.55±0.10†	0.35±0.11†	0.15±0.03	0.15±0.04	0.17±0.02	0.16±0.03	0.11±0.04	0.09±0.02	0.11±0.03	0.08±0.02	=0.013	=0.497
	LOW	0.08±0.02	0.15±0.02		0.54±0.12†	0.28±0.10†	0.14±0.04	0.14±0.04	0.15±0.02	0.13±0.03	0.11±0.02	0.11±0.02	0.10±0.02	0.07±0.02		
Serum Cortisol (µmol.l^{-1})	**HIGH**	0.17±0.03	0.18±0.02		0.28±0.02†	0.33±0.04†	0.24±0.03†	0.19±0.02	0.14±0.02	0.14±0.02	0.13±0.02‡	0.11±0.01†‡	0.08±0.01†‡	0.08±0.01†‡	<0.001	=0.099
	LOW	0.17±0.03	0.15±0.02		0.24±0.03†	0.32±0.05†	0.23±0.04†	0.18±0.03	0.13±0.02‡	0.12±0.02‡	0.10±0.01†‡	0.10±0.01‡	0.10±0.01‡	0.09±0.01‡		
Blood Lactate (mmol.l^{-1})	**HIGH**	1.0±0.2	1.1±0.3		4.1±0.8†	2.1±0.5†	1.3±0.3	1.0±0.3	0.7±0.2	0.9±0.2	0.8±0.1	0.7±0.2	0.5±0.1	0.4±0.1†	=0.001	=0.129
	LOW	0.9±0.2	1.0±0.2		4.2±0.5†	1.7±0.3	1.2±0.3	1.±0.2	0.8±0.2†	1.0±0.2	1.1±0.2	1.2±0.2‡	0.6±0.2	0.5±0.2		
Serum NEFA (mmol.l^{-1})	**HIGH**	0.18±0.05	0.12±0.03		0.25±0.06	0.35±0.06†	0.43±0.11†	0.53±0.15†	0.37±0.07	0.24±0.06‡	0.24±0.07‡	0.27±0.11‡	0.27±0.09‡	0.35±0.13	=0.011	=0.514
	LOW	0.27±0.07	0.18±0.03		0.27±0.07	0.34±0.07†	0.33±0.07	0.39±0.10†	0.39±0.10†	0.27±0.07	0.24±0.05	0.24±0.05	0.27±0.05	0.30±0.05		
Plasma Glycerol (mmol.l^{-1})	**HIGH**	4.23±0.58	4.35±1.23		6.83±1.51	5.02±1.13	5.69±1.31	7.21±1.83	7.52±1.78	9.50±2.23†	10.03±2.84†	8.99±2.99	8.36±2.21	6.15±1.80	=0.316	=0.032
	LOW	4.35±0.54	4.47±1.28		6.49±1.54	5.05±1.07	5.55±1.06	6.92±1.61	5.78±1.72*	5.42±1.61*	3.63±0.85*‡	2.96±0.69*‡	4.07±0.82*‡	2.89±0.85*‡		

Note**:** Data presented as mean ± SEM. Test meal and insulin were administered immediately following rest and pre-meal sample points. ***** indicates significantly different from **HIGH** ($p \leq 0.05$). † indicates significantly different from rest. ‡ indicates significantly different from pre-meal. Exercise commenced 60 minutes after rest. **T** = Time, **C** = Condition, **E** = Exercise.

4.7 Discussion

The aim of this study was to determine whether manipulating the glycaemic index of foods consumed following evening-time exercise influence hormonal, metabolic or inflammatory parameters and appetite responses in patients with type 1 diabetes. This study demonstrates that consumption of a low glycaemic index post-exercise meal administered in tandem with reduced rapid-acting insulin dose reduces circulating inflammatory markers, whereas these inflammatory markers are significantly elevated following substitution for a high GI meal. Other hormonal and metabolic measures remain unaffected.

The clinical utility of consuming meals with a low GI around the time of exercise is clear; specifically, low GI meals after exercise offer more favourable postprandial glycaemic profiles, without an increased risk of post-exercise hypoglycaemia in type 1 diabetes patients (West *et al.* 2011, West *et al.* 2011, Campbell *et al.* 2014) (chapter 4A). This is important because the inclusion of exercise into the lives of patients is severely hampered by difficulties in managing post-exercise glycaemia. Now, this study demonstrates that manipulating the glycaemic index of meals under conditions of a reduced rapid-acting insulin dose also carries important implications on inflammatory markers. This is important, as regular exposure to metabolic, hormonal or inflammatory disturbances could significantly influence long-term diabetes-related complications in regularly exercising patients (Rabasa-Lhoret *et al.* 2001). This study shows that meal GI has significant implications for post-prandial circulating inflammatory markers; specifically, for the first time, under non-clamp techniques and replicating free-living conditions, inflammatory cytokines TNF-α and IL-6 were dramatically increased following a high GI meal. An otherwise comparable low GI meal completely prevented rises in these inflammatory cytokines. The clinical relevance of these findings should not be underestimated, as offsetting hyperglycaemia and inflammation is important for preventing early pathogenetic diabetes-related complications (Rosa *et al.* 2011). Indeed, resting inflammatory cytokine concentrations were positively related to length of diabetes, supporting the notion that diabetes is a long-standing inflammatory disease (Devaraj *et al.*

2007). Moreover, resting inflammatory cytokine concentrations were positively correlated with average blood glucose concentrations and inversely related to HbA_{1c}, strengthening the hypotheses of hyperglycaemia as a mediator of inflammation (Devaraj *et al.* 2005, De Rekeneire *et al.* 2006, Galassetti *et al.* 2006) and that the normalisation of glycaemic profiles through tight diabetes management reduces inflammatory disturbances. Moreover, anti-IL-6 therapy has demonstrable effects for reducing HbA_{1c} (Ogata *et al.* 2011) and neutralisation of TNF-α improves glucose metabolism (Hotamisligil *et al.* 1993).

However, the pathological versus the beneficial nature of IL-6 remains to be fully understood. Indeed, during contraction, muscles can produce IL-6 strictly independently of TNF-α (Keller *et al.* 2006), strengthening the argument that muscular IL-6, of which is quantitatively more important than that released from any other tissue (Hiscock *et al.* 2004), plays a role in metabolism and not just inflammation *per se*. IL-6 expression is increased when intramuscular glycogen is low, consuming carbohydrate during exercise diminishes the exercise induced increases in IL-6 (Nehlsen-Cannarella *et al.* 1997, Nieman *et al.* 1998), and studies in humans show increased hepatic glucose output in response to injections of recombinant human IL-6 (Stouthard *et al.* 1995). Taken together it would seem that IL-6 is related to hepatic glycogen content, plays a role in endogenous glucose production, and is involved in muscle-to-liver communication (Febbraio *et al.* 2004). This seems plausible as muscle-derived IL-6 has been shown to contribute to improved glycaemia following exercise (Pedersen and Febbraio 2008) by increasing glucose uptake (Ellingsgaard *et al.* 2011).

In addition, IL-6 has been shown to mediate post-exercise increases in GLP-1 secretion (Allen *et al.* 2012) indicating a potential role for insulin-mediated glucose uptake. The incretin response in type 1 diabetes is known to be diminished however (Greenbaum *et al.* 2002, Aronoff *et al.* 2004). Data taken from mice models in which β-cells were destroyed with streptozotocin have failed to show any improvement in insulin secretion with exogenous administration of IL-6 (Allen *et al.* 2012), which, when considering the pathology of type 1 diabetes, may make the role of IL-6 an unlikely antagonist for hyperglycaemia.

Additionally, β-hydroxybutyrate concentrations did not significantly rise under either of the two conditions (Figure 4.5), remaining similar to pre-meal and resting concentrations. Basal insulin dose remained unchanged, and despite a reduction in rapid-acting insulin dose, circulating insulin concentrations likely remained sufficient for suppression of β-hydroxybutyrate production (Mcgarry 1996), potentially driving ketone body disposal (Balasse and Féry 1989). Concentrations during both trial conditions were well below those levels deemed clinically significant (> 1.0 $mmol.L^{-1}$) (Laffel 2000). All other hormonal and metabolite measures remained similar between conditions during the post-exercise post-prandial period.

The aim of this study was to assess the metabolic, inflammatory, and counter-regulatory hormonal effects of manipulating the glycaemic index of post-exercise carbohydrates consumed following evening exercise, under conditions of reduced pre- and post-exercise rapid-acting insulin dose. This study demonstrates that consumption of a low GI post-exercise meal under conditions of a reduced rapid-acting insulin dose reduces markers of inflammation, whereas substitution for a high GI meal significantly elevates inflammatory parameters. Other hormonal and metabolic measures remain unaffected. There is now a need to assess the impact of this strategy on appetite responses.

CHAPTER 4C

APPETITE RESPONSES FOLLOWING MANIPULATION OF THE GLYCAEMIC INDEX OF CARBOHYDRATES CONSUMED AFTER EVENING EXERCISE IN TYPE 1 DIABETES

4.8. Introduction

Current recommendations stipulate that exercising patients consume adequate amounts of carbohydrate to avoid hypoglycaemia (Gallen *et al*. 2011). As evidenced in chapter 4A however, it would seem that the risk of developing hypoglycaemia late-after exercise remains in spite of consuming enough carbohydrate to cover the cost of exercise. Following exercise, over-consumption of carbohydrate (Dubé et al. 2014), and ultimately excessive energy intake (Robertson et al. 2009), as a preventative measure against further falls in glycaemia, may negate the benefits exercise offers and could potentially contribute to a deterioration in wider diabetes management (Kennedy et al. 2013).

Research has shown that insufficient exercise and excessive energy intake can confer detrimental long-term implications for glycaemic control and cardiovascular risk in patients (Wadén *et al*. 2008; Salem *et al*. 2010). Conversely, elevating energy expenditure through regularly exercising, and thus inducing a negative energy balance could be advantageous to glycaemic control; reduced energy and carbohydrate intake may assist in the prevention of adiposity accumulation and the associated insulin resistance which occurs following diagnosis of type 1 diabetes (de Vries *et al*. 2013). However, even in people without diabetes there is a risk of over-compensation of energy intake in response to energy expenditure (King *et al*. 2012), potentially due to increased appetite (King *et al*. 2012). Nonetheless, a negative energy balance can be induced further when combined with a dietary strategy (Shaw *et al*. 2006). Indeed, modulating post-exercise appetite through nutritional strategies could be advantageous for type 1 diabetes patients, thus, appetite regulation following exercise is emerging as an important component of diabetes care (Specht *et al*. 2013, Dubé *et al*. 2014).

The composition of the foods consumed following exercise is of importance to type 1 diabetes patients. Chapter 4A illustrated improved glycaemia in the acute post-exercise period when LGI carbohydrates are consumed after exercise, compared HGI. This is important, as patients with type 1 diabetes are faced with particular difficulty in normalising glycaemia around the

time of exercise and more so following exercise (chapter 3A and 4A), repeated exposure to severe glycaemic variability on a regular basis may indeed negate the benefits that exercise offers (Chimen *et al*. 2012, Kennedy *et al*. 2013). However, the impact of food composition on appetite in type 1 diabetes is less well understood.

In people without type 1 diabetes, diets that contain LGI carbohydrates are associated with reductions in appetite (Stevenson *et al*. 2009), however this may not be the case when fibre content is matched (Gonzalez and Stevenson 2012). The acute impact of glycaemic index on appetite in a healthy population may be largely driven by insulinaemia rather than glycaemia, as postprandial insulin concentrations are inversely related to hunger, whereas postprandial glycaemia does not (Flint *et al*. 2006). Another potential factor in the appetite response to HGI vs. LGI meals is the gastrointestinal peptide glucagon-like peptide-1 (GLP-1), which suppresses appetite and energy intake (Verdich et al. 2001). GLP-1 is secreted by enteroendocrine cells in response to nutrient exposure and displays a differential response following ingestion of carbohydrates that differ in their rate of appearance into the circulation (Wachters-Hagedoorn *et al*. 2006). Whether these assumptions can be applied to patients with type 1 diabetes remains to be established, as differences in post-prandial glucose and GLP-1 excursions are vastly different to those witnessed in people without diabetes (Kamoi *et al*. 2011). In addition, studying appetite responses following HGI and LGI meals in type 1 diabetes patients offers a unique insight into the impact of meal glycaemic index, whereby insulin-induced satiety is not confounded by dissimilar insulinaemia (Air *et al*. 2002), as administration of insulin dose is typically based on carbohydrate amount and not type.

Accordingly, this study had two main aims: 1) to investigate the appetite and GLP-1 response to HGI and LGI post-exercise meals in type 1 diabetes patients, thereby reflecting a typical daily situation in which exercise recommendations for minimising the risk of hypoglycemia are adhered; 2) to examine the influence of the glycaemic index on appetite independent of insulinaemia and fibre content.

4.9. Methods

HIGH and **LOW** trials from chapter 4A were repeated in a randomised and counterbalanced fashion. Such that, the same cohort of patients performed **HIGH** and **LOW** trials twice, to avoid repetition of data. Blood glucose, serum insulin, plasma glucagon, and total GLP-1, and subjective appetite scores (via visual analogue scales) were measured for 180 minutes post-meal (Figure 4.6).

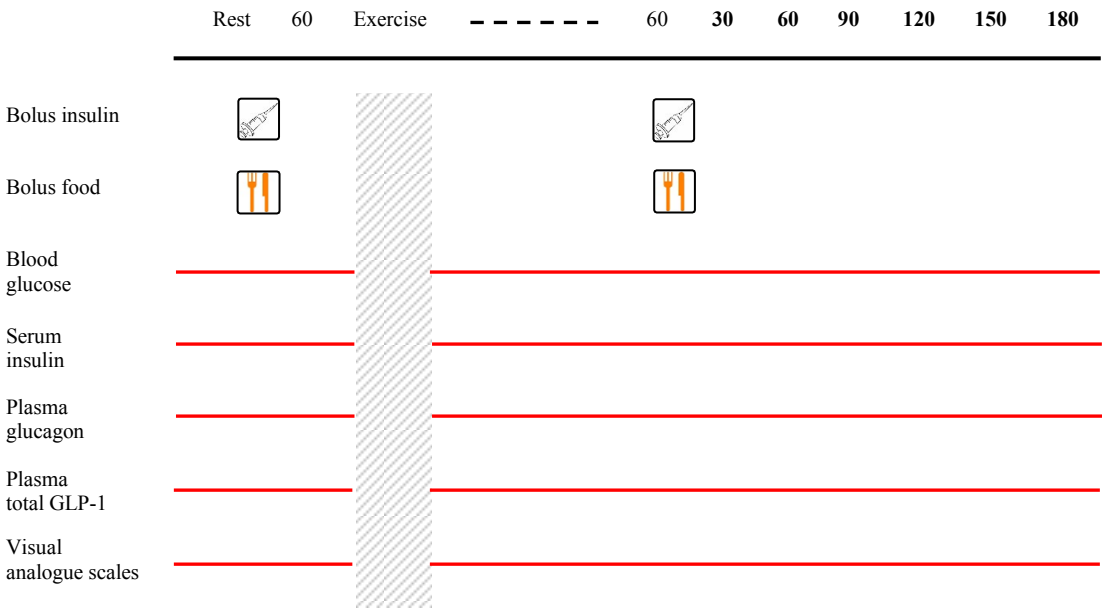

Figure 4.6. Schematic of experimental trial. Note: Bold text indicates post-exercise intervention period.

4.10. Results

4.10.1 Pre-intervention phase

There were no differences in glycaemia, serum insulin, plasma glucagon concentrations or appetite scores prior to the consumption of the post-exercise test meals ($p > 0.05$), such that immediately before administration, patients displayed similar blood glucose (**HIGH** 6.2 ± 0.7 vs. **LOW** 5.8 ± 0.5 mmol.l^{-1}, $p = 0.169$; Figure 4.7), serum insulin (**HIGH** 106 ± 15 vs. **LOW**

102 ± 14 pmol.l^{-1}, p = 0.986; Figure 4.7), plasma glucagon (**HIGH** 732 ± 99 vs. **LOW** 735 ± 103 pg.ml^{-1}, p = 0.884; Figure 4.8), and total GLP-1 (**HIGH** 1.95 ± 0.21 vs. **LOW** 2.47 ± 0.87 pmol.l^{-1}, p = 0.620; Figure 4.8). At this time, sensations of hunger (**HIGH** 68 ± 3 vs. **LOW** 67 ± 2, p = 0.925) and fullness (**HIGH** 60 ± 2 vs. **LOW** 61 ± 2, p =0.791) were similar between conditions.

4.10.2 Post-intervention phase

Following administration of rapid-acting insulin and test meals, serum insulin peaked similarly at 60 minutes under both conditions (**HIGH** 181 ± 26 vs. **LOW** 175 ± 30 pmol.l^{-1}, p = 0.773; Figure 4.7). Blood glucose increased from periprandial concentrations over the postprandial period under both conditions, but elevations were significantly more pronounced under **HIGH**, with greater mean peaks (**HIGH** +10.2 ± 0.5 vs. **LOW** +3.2 ± 0.6 mmol.l^{-1}, $p < 0.001$; Figure 4.7). Temporal changes in serum insulin remained similar beyond this time ($p > 0.05$; Figure 4.7), with concentrations returning to periprandial measures at 180 minutes ($p > 0.05$; Figure 4.7). Moreover, total insulin AUC were similar between conditions over the postprandial period (**HIGH** 49576 ± 6786 vs. **LOW** 43924 ± 6196 pmol.l^{-1}.min^{-1}, p = 0.332). Total blood glucose AUC was significantly greater under **HIGH** (**HIGH** 2205 ± 90 vs. **LOW** 1437 ± 107 mmol.l^{-1}.min^{-1}, p = 0.002), displaying a significantly greater average change in absolute blood glucose concentrations over the post-meal period (**HIGH** +6.6 ± 0.9 vs. **LOW** +1.7 ± 0.4 mmol.l^{-1}, $p < 0.001$). As such, patients under **HIGH** were, on average, hyperglycaemic (**HIGH** 12.8 ± 0.5 mmol.l^{-1}; Figure 4.7), whereas patients under **LOW** typically remained within euglycaemic ranges (**LOW** 7.6 ± 0.6 mmol.l^{-1}, p = 0.002; Figure 4.7).

Glucagon concentrations were significantly increased following the administration of both meals peaking similarly 30 minutes after consumption (Figure 4.8). Following this, concentrations declined under **HIGH** such that at 150 and 180 minutes concentrations were lower than pre-meal, whereas the decline under **LOW** was largely attenuated (Figure 4.8). However, total glucagon AUC was not statistically different between conditions (**LOW**

264150 ± 98209 vs. **HIGH** 247054 ± 79042 pg.ml^{-1}.min^{-1}; p = 0.141). Temporal increases in total GLP-1 at 60 minutes following the meal were not statistically significant (p = 0.223), with concentrations similar between conditions and baseline over the course of the post-prandial period (Figure 4.8).

Sensations of hunger peaked at 60 minutes under both conditions, which was matched with suppression in feelings of fullness (Figure 4.9). Over the remaining 120 minutes hunger sensations decreased under **HIGH**, whereas there was an increase in fullness. Inversely, under **LOW** increases in hunger and decreases in fullness were largely attenuated. Total AUC for feelings of hunger and fullness were significantly greater (**LGI** 7619 ± 1130 vs. **HIGH** 6961 ± 1050 mm.min^{-1}, p < 0.001), and lower (**LOW** 2669 ± 421 vs. **HIGH** 3345 ± 561 mm.min^{-1}, p < 0.001) under **LOW**, respectively.

Under **LOW**, a negative relationship was observed between total post-meal blood glucose AUC and hunger AUC (r = 0.840, p = 0.039), but not fullness AUC (r = 0.006, p = 0.910) or serum insulin AUC (r < 0.001, p = 0.977), plasma total GLP-1 (r = 0.018, p = 0.543). Neither hunger (r = 0.004, p = 0.900) nor fullness (r = 0.040, p = 0.699) were associated with changes in serum insulin AUC. Neither glucagon AUC nor total GLP-1 were associated with any other variable under **LOW**. No other correlations were observed between measures under **HIGH** (p > 0.05).

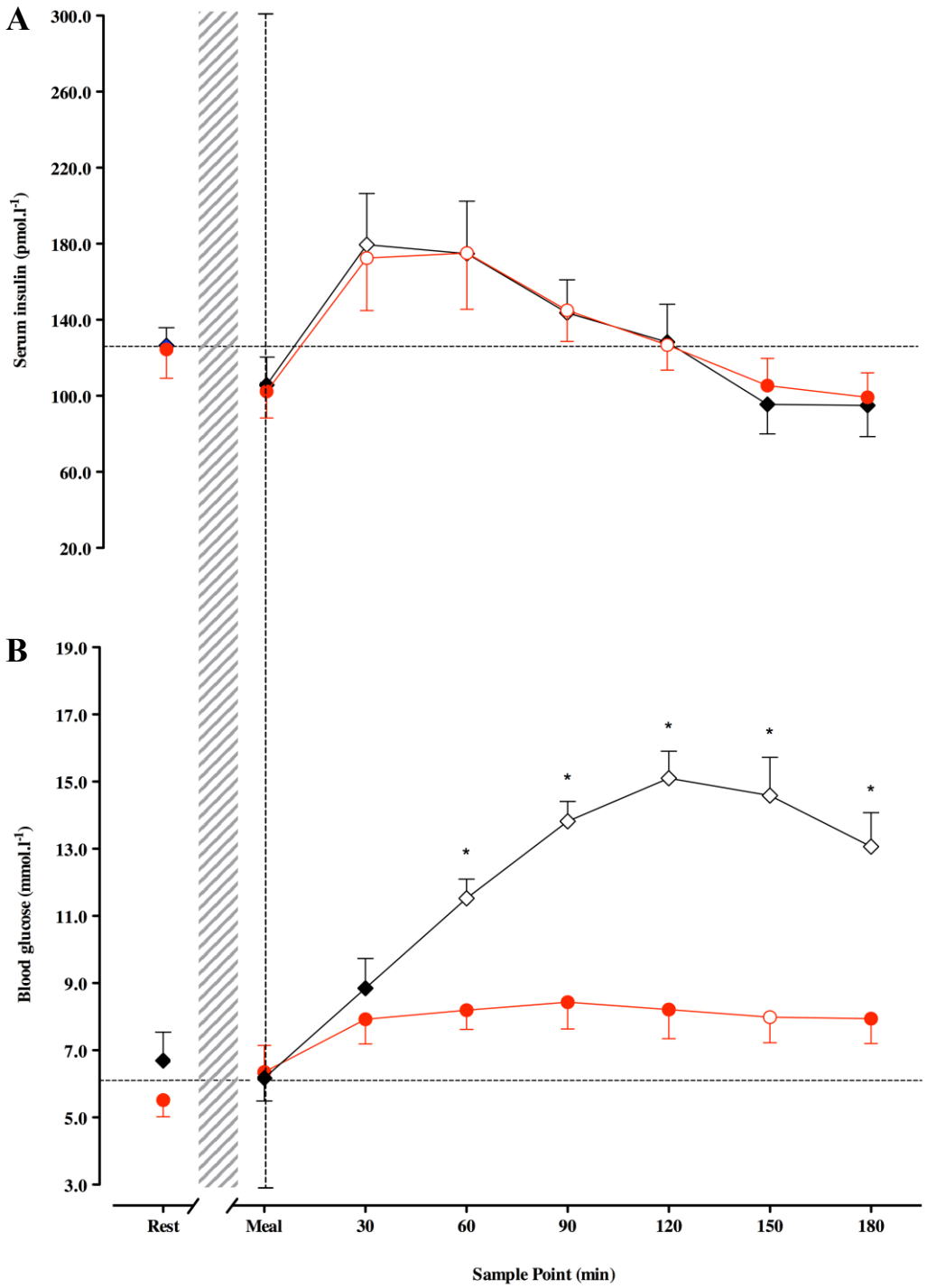

Figure 4.7 A-B. Time-course changes in (**A**) serum insulin and (**B**) blood glucose. Data presented as mean ± SEM. Black diamonds = **HIGH**, red circles = **LOW**. Transparent sample point within a condition indicates a significant difference from pre-meal concentrations ($p \leq 0.05$). * indicates significantly different from **HIGH** ($p \leq 0.05$). Thatched area indicates exercise. Vertical dashed line break indicates post-exercise intervention. Note: Test meal and insulin were administered immediately following 60 minutes post-exercise sample point.

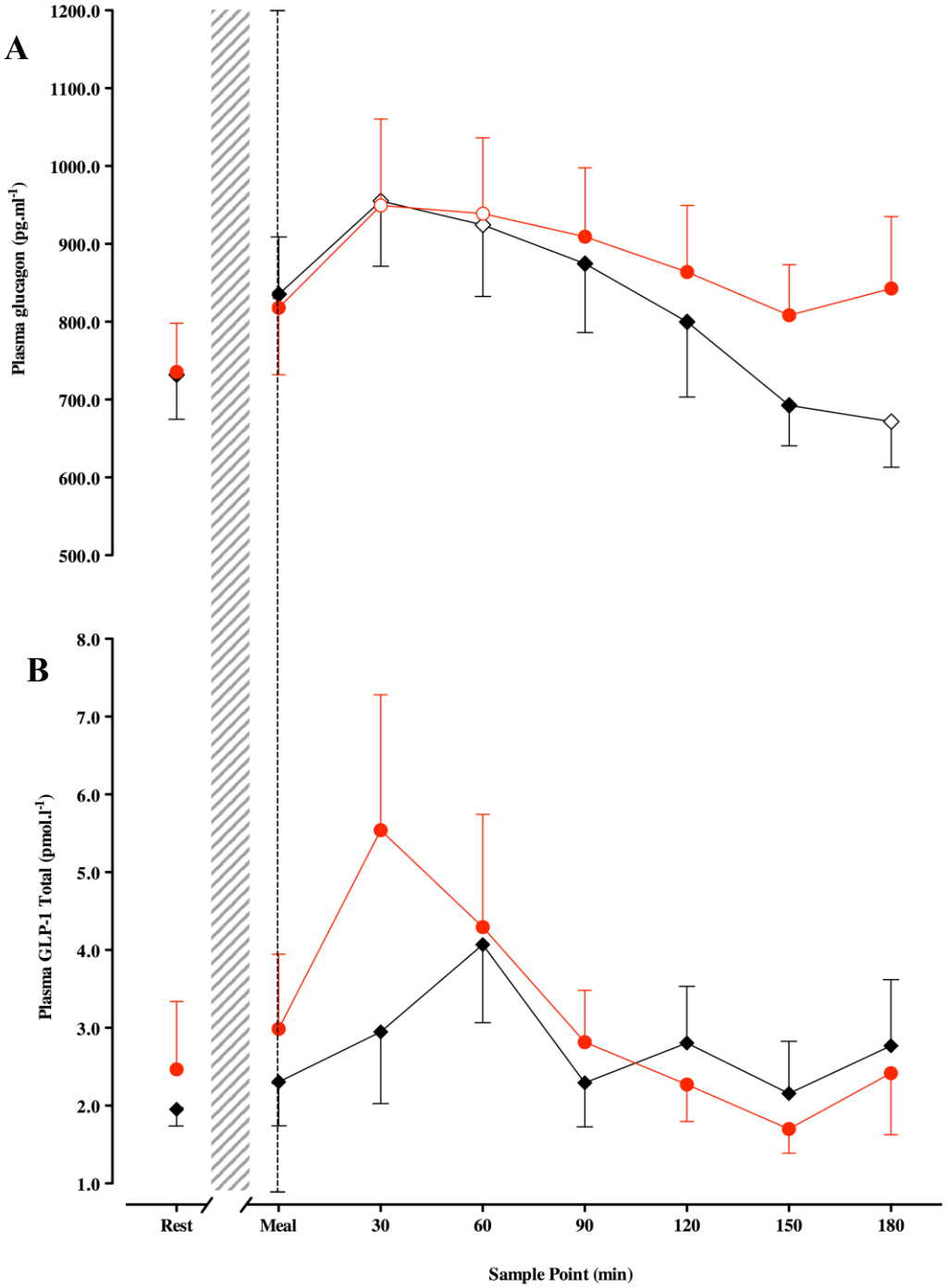

Figure 4.8 A-B. Time-course changes in (**A**) plasma glucagon and (**B**) plasma GLP-1 total. Data presented as mean ± SEM. Black diamonds = **HIGH**, red circles = **LOW**. Transparent sample point within a condition indicates a significant difference from pre-meal concentrations ($p \leq 0.05$). Thatched area indicates exercise. Vertical dashed line break indicates post-exercise intervention. Note: Test meal and insulin were administered immediately following 60 minutes post-exercise sample point.

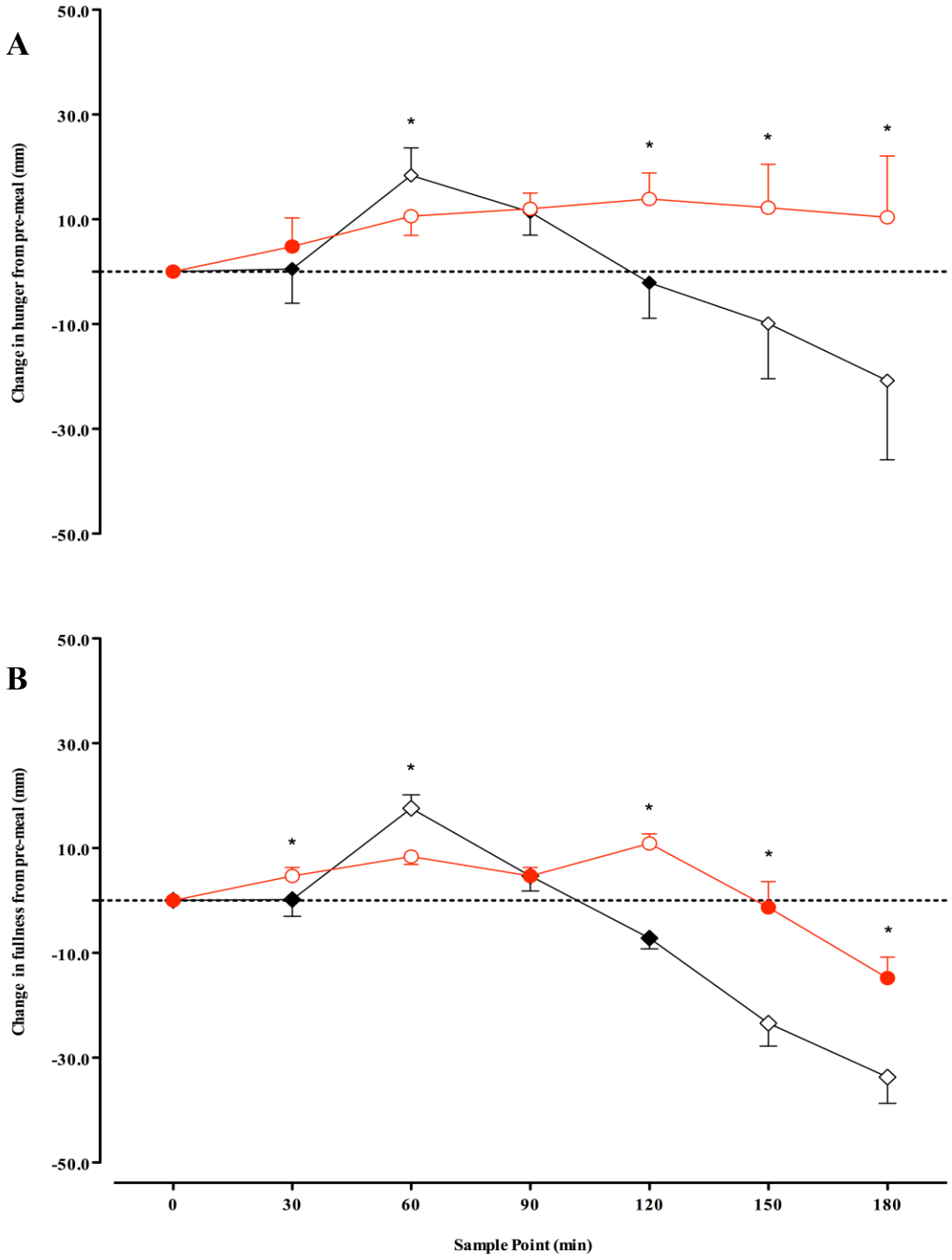

Figure 4.9 A-B. Time-course changes from pre-meal in (**A**) hunger, and (**B**) fullness. Data presented as mean ± SEM. Black diamonds = **HIGH**, red circles = **LOW**. Transparent sample point within a condition indicates a significant difference from pre-meal concentrations ($p \leq 0.05$). * indicates significantly different from **HIGH** ($p \leq 0.05$). Post-exercise meal and insulin administration at 0 minutes sample point. Positive change on Y axis denotes an increase in hunger (**A**), and fullness (**B**), negative change denotes a decrease.

4.11. Discussion

The aim of this study was to investigate the influence of manipulating the GI of the meal consumed following exercise on appetite responses in type 1 diabetes patients. This study shows for the first time that high GI carbohydrates consumed following exercise, elevates subjective feelings of fullness and supresses sensations of hunger in patients with type 1 diabetes. It is important to note that these responses were observed under comparable insulinaemia and when meals were matched for macronutrient composition and fibre content.

Consuming meals with a low GI before and after exercise offer more favourable postprandial glycaemic profiles without increasing the risk of post-exercise hypoglycaemia in type 1 diabetes patients (West *et al.* 2011, West *et al.* 2011, Campbell *et al.* 2014)(chapter 4A). This is important because the inclusion of exercise into the lives of patients is severely hampered by difficulties in managing post-exercise glycaemia. The present study now reveals that meals with a low GI may be less satiating in the post-exercise recovery period in patients with type 1 diabetes, as determined via visual analogues scales. Although it would be naïve to infer these findings to longer-term observations, our data may indicate likelihood for increased calorie intake following exercise due to increased appetite, rather than avoidance of hypoglycaemia *per se,* as commonly reported (Tonoli *et al.* 2012, Kennedy *et al.* 2013). This may have important implications for long-term weight management in this population, and may contrast data in non-diabetes individuals which demonstrate an improvement in weight management following low GI consumption (Larsen *et al.* 2010).

It has previously been demonstrated that with fibre-matched meals, a higher glycaemic response is associated with greater postprandial feelings of fullness (Gonzalez and Stevenson 2012). Based on strong positive correlations of fullness and postprandial insulinemia in humans (Flint *et al.* 2006), taken in concert with the acute induction of satiety in animal models (Air *et al.* 2002), it would be reasonable to speculate that insulin was a major confounding factor in the appetite response observed. However, insulin concentrations were

controlled manually in this study, under non-clamp procedures and from matched insulin administration. Accordingly, insulin concentrations were similar at every time point in the postprandial period (Figure 4.7), whereas marked increases in postprandial glucose concentrations were evident with **HIGH** vs. **LOW** (Figure 4.8) as expected. Therefore these results indicate that high GI meals induce greater satiety independent of the insulin response that is typical of these meals (Stevenson *et al.* 2006).

These findings are consistent with previous infusion studies in people with and without type 1 diabetes, whereby hyperglycaemic (~14 and ~10 $mmol.l^{-1}$) intravenous infusion reduced hunger sensations compared to euglycaemia (~6 $mmol.l^{-1}$) (Chapman *et al.* 1998, Russell *et al.* 2001). Interestingly, these effects are more apparent in the postprandial state (Russell *et al.* 2001), suggesting an interaction with the gastrointestinal tract. Another potential mechanism to explain the reduced hunger sensations with **HIGH** versus **LOW** could be through portal vein signalling (Mithieux *et al.* 2005). With **HIGH**, high concentrations of glucose would likely be present in the portal vein. Animal models have shown decreased food consumption following portal glucose infusions (Mithieux *et al.* 2005), suggesting that portal glucose is associated with appetite suppression. Furthermore, this response is attenuated by portal vein denervation (Mithieux *et al.* 2005), demonstrating the importance of this pathway for glucose sensing and appetite. However, it is worthy to note that circulating insulin concentrations in the present study resulted from subcutaneous administration opposed to insulin release into the portal vein from the pancreas. Even under conditions of peripheral circulating hyperinsulinaemia, normal postprandial portal venous insulin patterns are not fully reproduced (Rizza *et al.* 1980), leading to an impaired assimilation of splanchnic glucose (Felig *et al.* 1978), and an inability to supress glucagon through a paracrine effect thus favouring the release of hepatic glucose (Baron *et al.* 1987). Whilst glucagon displays anorectic properties (Chan *et al.* 1984), it is improbable that this influences the appetite response observed in this study, since glucagon concentrations did not significantly differ between trials and in fact, were on average, greater with **LOW**.

In addition, appetite responses may be mediated by the release of incretins such as GLP-1 (Wachters-Hagedoorn *et al.* 2006, Krog-Mikkelsen *et al.* 2011). Although the evidence for a differential GLP-1 response to **HIGH** vs. **LOW** mixed-meals is equivocal (Stevenson *et al.* 2009). The present study demonstrates no difference in the GLP-1 response to **HIGH** vs. **LOW** meals consumed following exercise in type 1 diabetes patients. This indicates that the appetite responses observed were independent of both insulinaemia and plasma GLP-1 concentrations.

The difference in fibre content between the **HIGH** and **LOW** meals was 0.5 g. Meta-analyses indicate that fibre does reduce subjective appetite sensations and subsequent energy intake (Wanders *et al.* 2011). The difference between meals in this study however is not likely to have played a role in the response observed, as a 1 g increase in fibre intake suppresses appetite by ~0.18% (Wanders *et al.* 2011). In the current investigation a ~9 % and ~25 % difference was observed in the postprandial AUC for hunger and fullness, respectively. Given the ~0.5 g difference in fibre would influence these responses by at least 2 orders of magnitude less (~0.09 %) it can be considered a negligible difference.

The aim of this study was to assess the appetite responses following the manipulation of the glycaemic index of carbohydrates consumed following evening exercise, under conditions of reduced pre- and post-exercise rapid-acting insulin dose. The findings of this study should be considered in the context of more global diabetes care, as low GI post-exercise meals produce more suitable glycaemic responses than otherwise comparable high GI meals (chapter 4A) (Campbell *et al.* 2014). However, this study demonstrates that a post-exercise high GI meal produces greater fullness and less hunger, independent of insulin, in patients with type 1 diabetes. It is worthy to note that this is the first study to assess post-prandial appetite responses in exercising type 1 diabetes patients. With this in mind, the clinical application of these findings should not be underestimated; interventions were carried out in the evening, in a non-fasted state, thereby facilitating greater translation to daily life (Gonzalez and Stevenson 2013). Further work is needed to clarify the mechanisms of this effect in patients and to

establish the long-term implications of this response in patients regularly participating in exercise as VAS measures do not necessarily translate to changes in behaviour. In conclusion, this is the first study to demonstrate that high GI post-exercise meals induce greater postprandial feelings of fullness and lower postprandial hunger sensations in type 1 diabetes patients, independent of insulinaemia. There is now a need to assess the acute and 24 hour glycaemic effects of a combined basal-bolus insulin reduction and carbohydrate feeding strategy for preventing hypoglycaemia following evening exercise.

CHAPTER 5A

THE EFFECTS OF REDUCING BASAL INSULIN DOSE ON GLYCAEMIA AFTER EVENING EXERCISE IN TYPE 1 DIABETES

5.0 Introduction

Making meal-time adjustments, in both rapid-acting insulin dose (chapter 3) and food composition (chapter 4), is a pragmatic and effective strategy to achieve euglycaemia in the early hours following exercise for patients with type 1 diabetes (chapters 3 and 4). More specifically, consuming post-exercise carbohydrates with a low GI, whilst under conditions of reduced pre- and post-exercise rapid-acting insulin dose, offers protection from early-onset post-exercise hypoglycaemia (~8 hours) whilst reducing exposure to post-prandial hyperglycaemia and inflammation (chapters 3 and 4).

Unfortunately, these acute prandial adjustments do not offer complete protection; falls in glycaemia are likely to occur beyond ~8 hours post-exercise, particularly during the hours of sleep if exercise is performed in the evening (chapter 4) (Campbell *et al.* 2014). Exposure to late-onset nocturnal hypoglycaemia would suggest that acute prandial modifications to rapid-acting insulin dosage and carbohydrate intake are inadequate in preventing *late* falls in glycaemia following evening exercise, indicating a more long-lasting intervention may be required to extend this window of protection considering the prolonged period of heightened insulin sensitivity after exercise.

Under non-exercise conditions, there is typically a glucose nadir 4-14 hours after administration of basal insulin (Ashwell *et al.* 2006, Thomas *et al.* 2007). Considering late falls in glycaemia following exercise typically coincide at this time, application of a *basal* dose reduction could be a potential strategy to combat the risk of late-onset hypoglycaemia. Moreover, adjustments to both meal composition and rapid-acting insulin dose leaves basal dose open to modification. Unfortunately, there is currently a lack of evidence to support such advice with accompanying acute strategies, in spite of widespread recommendation in current clinical practice (Gallen 2012). Within the literature, alterations to the basal component of a patients' regimen have been predominantly trialled in individuals treated with continuous subcutaneous insulin infusion therapy (CSII) with demonstrable success (Edelmann *et al.*

1986, Sonnenberg *et al.* 1990, Admon *et al.* 2005, Tsalikian *et al.* 2006). For example, Tsalikian and colleagues (2006) found hypoglycaemia was reduced by a factor of two thirds when basal insulin was suspended. This form of treatment involves continuous infusion of rapid-acting insulin delivered subcutaneously at a variable rate controlled via an electronically controlled pump. However, UK based patients are predominantly treated using an injectable basal-bolus regimen, whereby the basal component consists of a slowly-absorbed long-acting insulin analogue (insulin Glargine [Lantus], sanofi-aventis, USA; Determir [Levemir], Novo Nordisk, Denmark) that is self-administered once or twice per day. This is a far less flexible method of insulin delivery than CSII meaning it would be inappropriate to infer findings from these studies across different treatment regimens. Furthermore, there is currently no literature examining the effects of reducing basal insulin when employing acute prandial adjustments to diet and rapid-acting insulin. Potentially, reducing basal dose could spare glucose in the hours following exercise protecting patients from late-onset hypoglycaemia. Conversely, reducing basal insulin when large reductions to pre- and post-exercise rapid-acting insulin dose are also applied may induce periods of sustained hyperglycaemia which could potentially be detrimental to glycaemic control (Gallen 2012).

Therefore, the aim of this study was to examine the effects of reducing basal insulin dose when employing acute prandial recommendations (chapters 3 and 4) on glycaemic control for 24 hours after exercise in patients with type 1 diabetes.

5.1 Methods

Details of patients and patents' insulin regimen are presented in Table 5.0. A schematic of the experimental design in presented in Figure 5.0; this study was a randomised counterbalanced cross-over design. Patients completed two experimental arms, in which basal insulin dose was maintained (**100%**) or reduced by 20% (**80%**) over the course of one day where exercise was performed in the evening. Timing of basal dose (changed / unchanged) was maintained between trials and performed as per each patient's individual regimen. On the morning of each

trial (~08:00 AM) patients arrived to the laboratory, having fasted overnight, for a resting venous blood sample before consuming a standardised breakfast meal equating to 1.3 g.carbohydrate.kg^{-1}BM (534 ± 27 kcal; 2.2 ± 0.11 MJ, MEAL 1, see 2.2.5.3, Table 2.3). Patients returned in the evening at (~17:00 PM) having consumed a prescribed lunch meal equating to 1.3 g.carbohydrate.kg^{-1} BM (4.0 ± 0.11 MJ, MEAL 2, see 2.2.5.4, Table 2.3), ~4 hours before arrival. Following a resting sample, patients self-administered a 75% reduced dose of rapid-acting insulin (2 ± 0.5 IU, see 2.2.6.1) into the abdomen (West *et al.* 2010, Campbell *et al.* 2013, Campbell *et al.* 2014). Patients consumed a pre-exercise carbohydrate bolus equating to 1.0 g.carbohydrate.kg^{-1} BM (1.7 ± 0.03, MEAL 3, see 2.2.5.6, Table 2.3) within a 5 minute period. Patients remained at rest for 60 minutes following consumption of the pre-exercise carbohydrate bolus / rapid-acting insulin injection with blood samples at 60 minutes (Figure 5.0). Immediately after the 60 minute blood draw, patients commenced 45 minutes of treadmill running at a speed calculated to elicit 70% of their $\dot{\mathbf{V}}O_{2peak}$. Immediately following exercise, a blood sample was taken, with further interval samples at 15, 30, and 60 minutes post-exercise. At 60 minutes, patients administered a 50% rapid-acting insulin dose (4 ± 0.3 IU, see 2.2.6.1), as determined from chapter 3, and consumed a low glycaemic index (GI) meal equating to 1.0 g.carbohydrate.kg^{-1} BM (1.7 ± 0.1 MJ, MEAL 7, see 2.2.5.8, Table 2.3), as determined from chapter 4, before being discharged. Patients were provided with a standardised low GI bed-time snack equating to 0.4 g.carbohydrate.kg^{-1} BM (0.9 ± 0.04 MJ, MEAL 10, see 2.2.5.10) which was consumed 180 minutes after the post-exercise meal (before sleep at 22:45 PM); patients were contacted at 180 minutes post-exercise meal to ensure compliance. Patients were instructed to replicate sleeping patterns as best possible over the course of the study. Over the course of this day, patients either maintained their regular basal insulin dose (**100%**; 32 ± 4 IU), or performed a global basal insulin reduction, so that total daily amount of basal insulin administered was reduced by 20% (**80%**; 26 ± 3 IU) (see 2.2.6.1). The following morning, patients returned to the laboratory, having fasted overnight, for a further resting blood sample and a breakfast meal (MEAL 11, see 2.2.5.11, Table 2.3). Blood glucose and serum insulin were measured on the morning before and after each

laboratory visit, and throughout each laboratory visit. CGM was used to capture interstitial glucose concentrations for 24 hours post-exercise (see 2.2.3.2).

Table 5.0 Patients demographic information

	Patient ID											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Mean±SEM**
Insulin (IU) Basal	38^{G}_{M}	20^{D}_{B}	22^{G}_{M}	26^{D}_{B}	34^{G}_{E}	18^{D}_{B}	20^{G}_{E}	31^{G}_{M}	24^{G}_{M}	30^{G}_{E}	-	26 ± 2
Bolus	1^{L}	1^{A}	1^{A}	1^{A}	1^{A}	0.8^{A}	1^{A}	1^{A}	1^{A}	1^{A}	-	1.0 ± 0.0
HbA1c (%)	6.9	6.7	6.8	6.8	6.6	7.1	6.1	6.2	6.2	7.7	-	6.9 ± 0.2
BMI ($kg.m^2$)	22.9	24.2	26.3	25.1	25.4	25.8	22.9	26.1	27.1	27.2	-	25.0 ± 0.8
Diabetes duration (years)	11	14	11	14	7	6	22	12	13	8	-	12 ± 2
$\dot{V}O_{2peak}$ ($ml.kg.min^{-1}$)	56.3	53.2	54.3	49.1	53.2	54.3	45.3	48.8	46.3	51.2	-	51.3 ± 2.1
Age (years)	30	24	26	26	26	34	38	24	26	26	-	27 ± 2

Note: G = Glargine, D = Detemir, A = Aspart, L = Lispro, M = once daily (morning), E = once daily evening , B = bi-daily; bolus insulin calculated per 10g CHO.

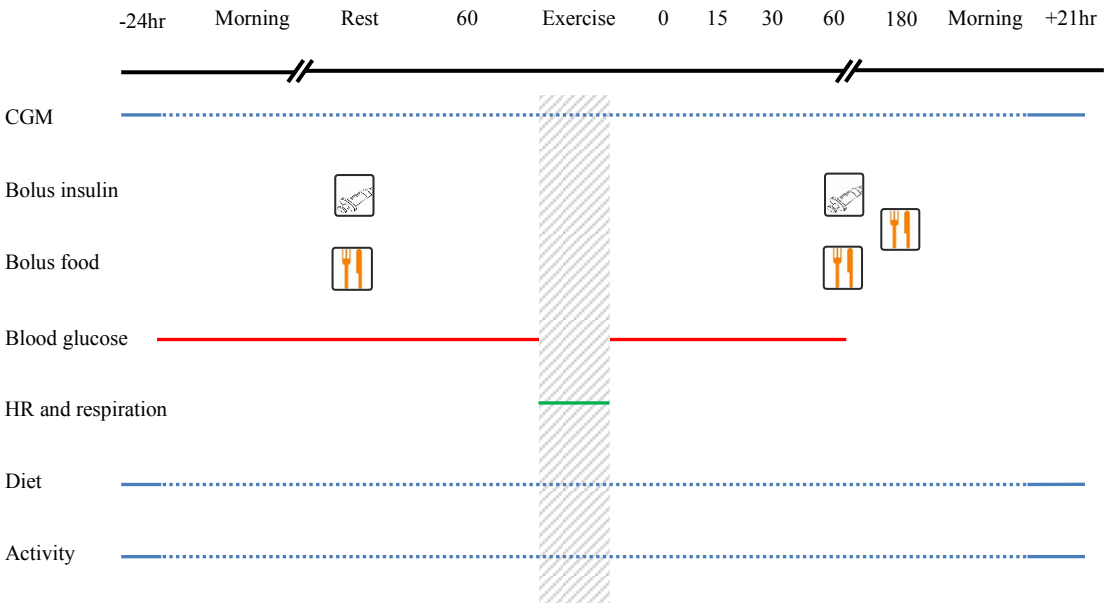

Figure 5.0. Schematic of experimental trial.

5.2 Results

5.2.1 Pre-laboratory phase

5.2.1.1 Pre-laboratory glycaemia

Over the course of the 24 hours prior to patients' arrival to the laboratory, glycaemic control was comparable between both experimental trials (CGM mean interstitial glucose: **100%** 8.1 ± 0.3, **80%** 8.2 ± 0.4 $mmol.l^{-1}$; $p = 0.962$; and total interstitial glucose area under the curve: **100%** 11593 ± 547, **80%** 12024 ± 949 $mmol.l^{-1}.min^{-1}$; $p = 0.876$). Moreover, resting, fasted pre-trial morning blood glucose concentrations were similar between trials (**100%** 5.9 ± 0.5, **80%** 6.1 ± 0.7 $mmol.l^{-1}$; $p = 0.459$; Figure 5.1). In addition, fasting serum insulin was also similar between conditions (**100%** 111 ± 23, **80%** 112 ± 23 $mmol.l^{-1}$; $p = 0.933$)

5.2.1.2 Pre-laboratory dietary intake, insulin administration and activity

No differences were observed in total energy consumed (**100%** 9.8 ± 0.7, **80%** 9.9 ± 0.8 MJ; $p = 0.982$), with contribution from carbohydrate (**100%** 53 ± 3, **80%** 53 ± 3 %; $p = 0.998$), fat (**100%** 28 ± 3, **80%** 28 ± 3 %; $p = 0.998$) and protein (**100%** 19 ± 2, **80%** 19 ± 1 %; $p = 0.991$) similar. In addition, total rapid-acting insulin units administered (**100%** 30 ± 3, **80%** 30 ± 3 IU; $p = 0.922$) as well as levels of activity (**100%** 5721 ± 96, **80%** 5703 ± 101 steps; $p = 0.901$) were comparable over the 24 hours before each trial.

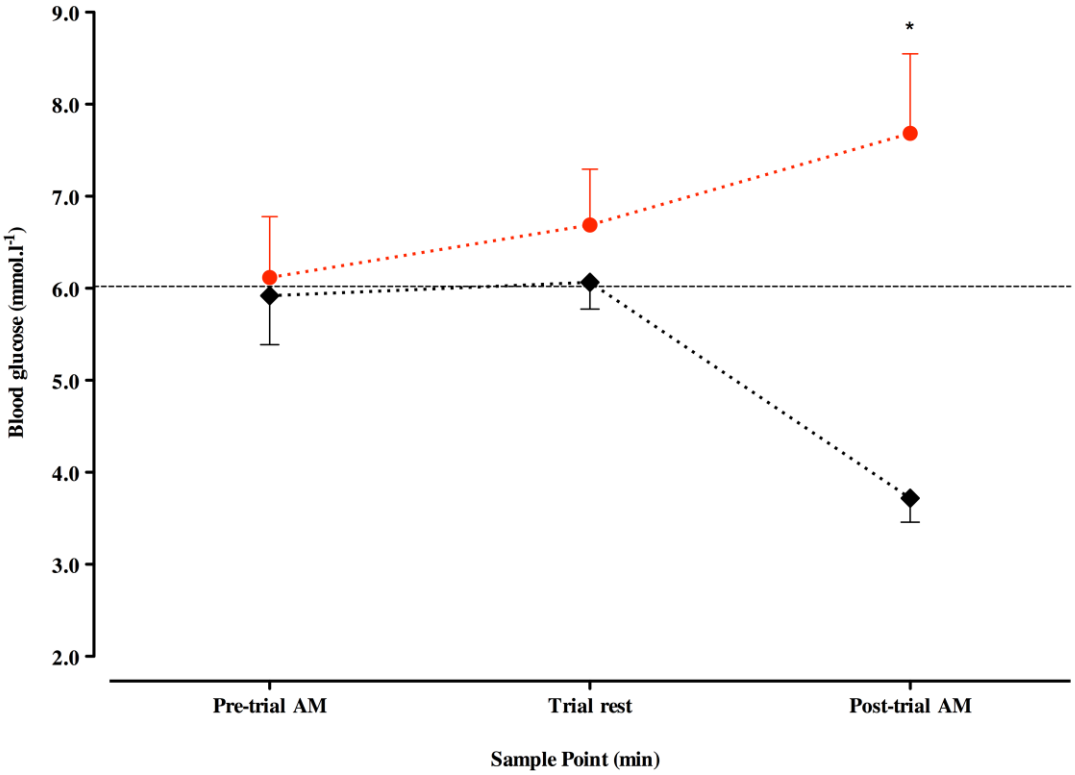

Figure 5.1. Time-course changes in morning time fasted, and daytime rested blood glucose concentrations. Data presented as mean ± SEM. Black diamonds = **100%**, red circles = **80%**. * indicates a significant difference in blood glucose between **100%** and **80%** ($p \leq 0.05$).

5.2.2 Laboratory phase

Patients displayed similar serum insulin (**100%** 132 ± 19, **80%** 129 ± 18 mmol.l^{-1}; $p = 0.936$) and blood glucose concentrations upon arrival to the laboratory (**100%** 6.1 ± 0.3, **80%** 6.7 ± 0.6 mmol.l^{-1}; $p = 0.290$; Figure 5.1). Blood glucose increased similarly following the pre-exercise bolus and reduced rapid-acting insulin dose, such that concentrations immediately before exercise were similar (**100%** 10.9 ± 0.6, **80%** 11.1 ± 0.7 mmol.l^{-1}; $p = 0.772$). Serum insulin concentrations during this time remained similar between conditions ($p > 0.05$).

5.2.2.1 Exercise and recovery period

Patients ran at an average speed of 9.7 ± 0.4 km.hr^{-1}, completing 7.3 ± 0.3 km and expending 3.1 ± 0.2 MJ. Patients exercised at a similar intensity across trials (**100%** 74 ± 0.1, **80%** 73 ± 0.1 %$\dot{V}O_{2peak}$; p = 0.993; **100%** 78 ± 1, **80%** 78 ± 2 %HR_{peak}; p = 0.991) inducing comparable falls in blood glucose (**100%** Δ -6.4 ± 0.4, **80%** Δ-5.9 ± 0.6 mmol.l^{-1}; p = 0.688), such that immediately following the cessation of exercise, blood glucose were lower than baseline under both conditions ($p < 0.05$). However, blood glucose remained within euglycaemic ranges up to the administration of the post-exercise meal (**100%** 6.3 ± 0.2, **80%** 6.9 ± 0.1 mmol.l^{-1}; p = 0.180), with all patients remaining protected from hypoglycaemia.

5.2.3 Post-laboratory phase

5.2.3.1 Late evening glycaemic responses

There was a significant condition*time interaction ($F_{(44,440)}$ = 4.021, p = 0.002, partial-eta^2 = 0.287), and a significant time effect ($F_{(22,220)}$ = 18.054, $p < 0.001$, partial-eta^2 = 0.659) for interstitial glucose concentrations over the course of the entire post-laboratory period. Following discharge from the laboratory, glycaemia remained similar throughout the late evening (1-5 hours post-exercise; 19:30-00:30 PM; Figure 5.2) with interstitial glucose, under free-living conditions, typically in euglycaemic ranges prior to the consumption of the bedtime snack (mean interstitial glucose: **100%** 5.7 ± 0.5, **80%** 5.8 ± 0.6 mmol.l^{-1}; p = 0.817; Figure 5.2). Furthermore, interstitial glucose concentrations were comparable immediately before sleep (**100%** 7.2 ± 1.0, **80%** 8.0 ± 0.7 mmol.l^{-1}; p = 0.217; Figure 5.2), and all patients under both conditions were protected from hypoglycaemia during this time.

5.2.3.2 Nocturnal glycaemia

Under the **100%** condition glucose levels fell at ~6 hours post-exercise with the first hypoglycaemic episode occurring at 8 hours post-exercise, and mean interstitial glucose nadir occurring at ~8-12 hours post-exercise and during hours of sleep (mean interstitial nadir:

100% 2.6 ± 0.2 mmol.l^{-1}; Figure 5.2). Conversely, glycaemia was preserved throughout the night under **80%** (mean interstitial glucose: **80%** 8.7 ± 0.6 vs. **100%** 7.0 ± 0.9, mmol.l^{-1}; p = 0.032; total interstitial glucose area under the curve: **100%** 2574 ± 246, **80%** 3657 ± 190 mmol.l^{-1}.min^{-1}; p = 0.021; Figure 5.1). As such, all patients under **80%** were protected from nocturnal hypoglycaemia, whereas, 9 patients (90 %) experienced nocturnal hypoglycaemia under **100%** with 3 of those patients encountering 2 or more nocturnal hypoglycaemic episodes (total of 14 individual hypoglycaemic episodes). Moreover, total time spent in hypoglycaemic ranges was significantly less under **80%** (**80%** 0 ± 0, **100%** 286 ± 35 minutes; p < 0.001), with a significantly greater amount of time spent euglycaemic (**80%** 397 ± 56, **100%** 122 ± 28 minutes; p < 0.001), but not hyperglycaemic (**80%** 143 ± 56, **100%** 132 ± 52 minutes; p = 0.188).

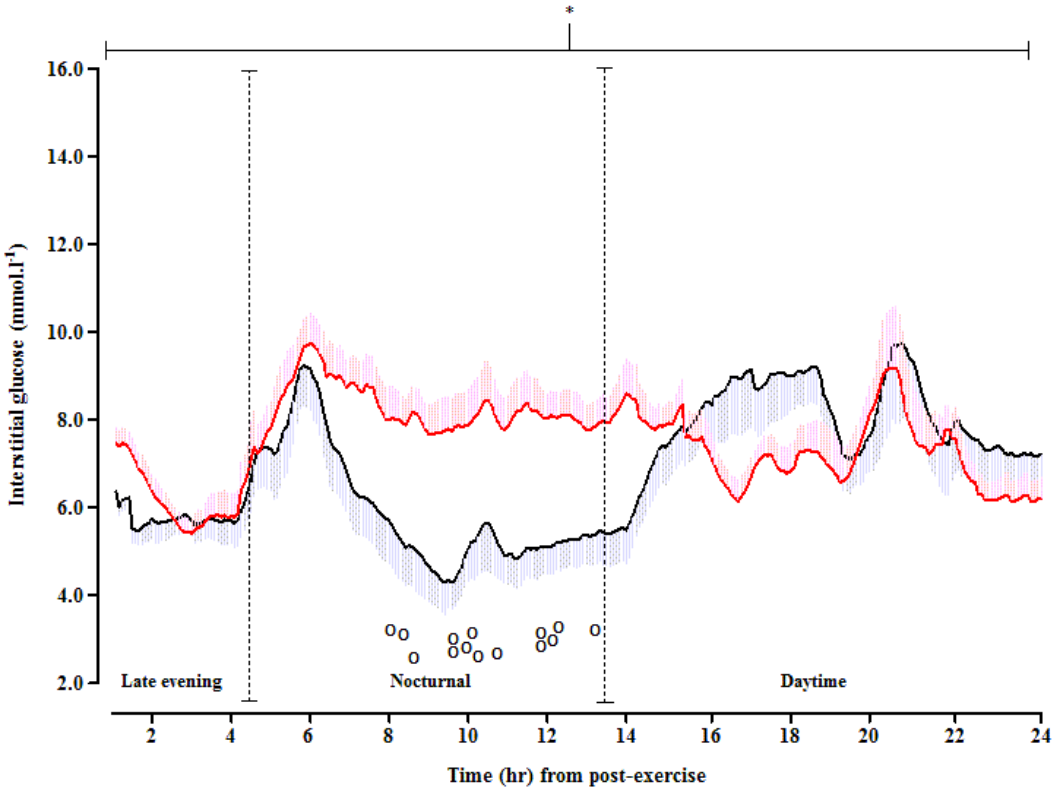

Figure 5.2. Time-course changes in interstitial glucose concentrations throughout the post-laboratory period. Data presented as mean ± SEM. Black solid trace = **100%**, red broken trace = **80%**. ** indicates a significant difference in interstitial glucose area under the curve between **100%** and **80%** ($p \leq 0.05$). Open circles represent hypoglycaemic episodes, as determined from CGM data. Vertical dashed line break indicates nocturnal and daytime periods.

5.2.3.3 Next-day glycaemia

Immediately upon awakening (~13 hours post-exercise; 07:30 AM; Figure 5.2), interstitial glucose was significantly less under **100%** with patients typically in hypoglycaemic ranges (**100%** 5.3 ± 0.6, **80%** 8.1 ± 0.6 mmol.l^{-1}; $p = 0.008$; Figure 5.1). Fasted, resting blood glucose was significantly lower under **100%** (**100%** 3.7 ± 0.3, **80%** 7.7 ± 0.9 mmol.l^{-1}; $p < 0.001$; Figure 5.1), and significantly less than those concentrations measured on the morning before exercise ($p < 0.001$). In comparison, patients under **80%** were typically in euglycaemic ranges upon awakening, and displayed similar blood glucose concentrations to those measures on the morning before exercise ($p > 0.05$).

After this time, patients under **100%** spent more time in hypoglycaemic (**100%** 264 ± 22 vs. **80%** 5 ± 6 minutes; $p < 0.001$) and hyperglycaemic (**100%** 123 ± 21 vs. **80%** 67 ± 16 minutes; $p = 0.004$) ranges, and less time in euglycaemia (**100%** 643 ± 54 vs. **80%** 1048 ± 71 minutes; $p = 0.007$) than those under the **80%** condition. In addition, patients under **80%** also tended to elicit less glycaemic variability during this time, although a significant difference between measures of glycaemic variability was not observed (Table 5.1).

Table 5.1. Estimates of next day glycaemic variability

	Median	**Mean**	**SD**	**CV%**	**MAGE**	**CONGA**	**MODD**	**Mr**	**J-Index**
100%	7.5	7.7	2.4	30.4	35.1	2.12	5.0	6.4	0.1
80%	7.1	7.4	1.7	23.4	32.2	1.8	5.2	6.4	0.01
***p* value**	=0.654	=0.631	=0.320	=0.284	=0.634	=0.367	=0.472	=0.994	=0.351

Note: MAGE = mean amplitude of glycaemic excursions; Mr = weighted average of glucose values; CONGA = continuous overall net glycaemic action; MODD = mean of daily differences.

5.2.3.4 Post-laboratory dietary intake, insulin administration and activity

Over the course of the next day, total energy consumed was significantly greater under **100%** (**100%** 4.8 ± 0.2, **80%** 3.0 ± 0.2 MJ; $p < 0.001$), with increased contribution from carbohydrate (**100%** 76 ± 3, **80%** 69 ± 3 %; $p = 0.004$). All patients under **100%** required carbohydrate bolus' to correct blood glucose, whereas this was avoided under **80%** (**100%** 26.8 ± 19.9, **80%** 0 ± 0 g; $p < 0.001$). Total meal-time carbohydrate (excluding carbohydrate consumed to correct blood glucose), was greater (**100%** 117.6 ± 12.3, **80%** 46.6 ± 3.3 g; $p < 0.001$) as was fat (**100%** 17.2 ± 2.7, **80%** 13.7 ± 2.8 g; $p = 0.014$) and protein (**100%** 16.08 ± 4, **80%** 11.6 ± 3.3 g; $p = 0.023$) consumption under **100%**. As such, patients administered significantly less meal-time rapid-acting insulin under **80%** (**80%** 12.5 ± 1.2, **100%** 6.7 ± 0.8 IU; $p = 0.001$), although insulin administered to correct blood glucose was greater (**80%** 2.1 ± 2.5, **100%** 1.0 ± 2.0 IU; $p = 0.040$). Levels of activity were similar between conditions with total amount of steps comparable (**100%** 4231 ± 102, **80%** 4301 ± 132 steps; $p = 0.602$).

5.3 Discussion

The aim of this study was to examine the effects of reducing basal dose under conditions acute prandial adjustments to rapid-acting insulin and meal composition on glycaemia for 24 hours after performing evening exercise in patients with type 1 diabetes. This study demonstrates for the first time that a combined basal-bolus insulin reduction and carbohydrate feeding strategy provides full protection from exercise-induced hypoglycaemia for 24 hours after exercise. Notably, when basal dose was reduced by 20% a clear preservation of glycaemia during the night was observed, completely abating the risk of nocturnal hypoglycaemia. In addition, it would seem patients tend to experience fewer glycaemic fluctuations, with more time spent in euglycaemic ranges across the following day.

An inability to manage blood glucose following exercise has, until now, proved great tribulation to patients wishing to engage in exercise (Brazeau *et al.* 2008). Indeed, the connection between poor rates of exercise participation and adherence in this population has a

tenable link with the risk and fear of developing exercise-induced hypoglycaemia (Bernardini *et al.* 2004, Brazeau *et al.* 2008). As such, hypoglycaemia remains the primary obstacle to exercise (Brazeau *et al.* 2008). Heretofore, protection from exercise induced-hypoglycaemia was limited to a narrow window after exercise (~8 hours; chapter 4A). Indeed, 90 % of patients under the non-reduction trial encountered hypoglycaemia beyond 8 hours post-exercise. It is therefore clear that prandial adjustments to rapid-acting insulin, and carbohydrate feeding carry only acute protective effects and do not influence late-onset nocturnal hypoglycaemia risk following evening exercise (Campbell *et al.* 2014) (chapter 4A).

Late falls in glycaemia appear coincidental with reported glucose nadirs on non-exercise days occurring 4-14 hours after basal insulin administration (Ashwell *et al.* 2006, Mcmahon *et al.* 2007, Thomas *et al.* 2007). It is noteworthy that these findings were observed in insulin Glargine users (Porcellati *et al.* 2007, Heller *et al.* 2009). Insulin Glargine reaches a metabolic plateau after 3-6 hours after injection (Heinemann *et al.* 2000, Lepore *et al.* 2000, Rave *et al.* 2003, Klein *et al.* 2007), and remains in steady state activity close to 100% for 24 hours after administration (Porcellati *et al.* 2007). The majority of patients in this study were treated with basal insulin Glargine (Glargine n = 8 vs. Detemir n = 2), all of whom encountered nocturnal hypoglycaemia on the non-reduction trial. Insulin Detemir shares similar action-time profiles to that of insulin Glargine over the first 12 hours, but beyond this time, Detemir exhibits a progressive decrease in activity to ~55% by 24 hours post-administration (Porcellati *et al.* 2007). The aim of this study was not to determine the optimal basal insulin regimen (insulin type or timing) *per se*, but to establish the effectiveness of a global reduction in basal dose. Considering the relatively small sample size in this study it would be in appropriate to draw comparisons against different regimens. Future work should be directed towards this aim however, considering the differences in pharmacokinetic and pharmacodynamic effects of insulin Glargine and Detemir and the future release of ultra-long-acting insulin Degludec. Irrelevant of basal insulin (Glargine versus Detemir), time of administration (morning versus evening) or frequency of injection (once versus twice daily), the results of this study show, for

the first time, that when total daily basal insulin dose is reduced by 20% on the day of evening exercise, in concert with acute prandial adjustments, patients are protected from hypoglycaemia for a total of 24 hours after exercise.

To maximise applicability to everyday diabetes management, the experimental design was conceived trying to reproduce real life conditions, capturing events of the intervention on the proceeding day after exercise, and under free living conditions. It is important to understand whether strategies which adapt patients' treatment regimens influence their ability to manage glycaemia on subsequent non-exercise days as glycaemic dysregulation could offset the beneficial effects exercise carries. The results of this study indicate that patients adopting the basal reduction tend to experience tighter glucose control compared to those administering their full basal dose. Patients under **80%** spent significantly more time in euglycaemic ranges, and less time in hypoglycaemic and hyperglycaemic ranges. Measures of glycaemic variability revealed that there was a propensity for fewer and less severe fluctuations in glucose under **80%**, although this was not statistically significant. Lower basal insulin concentrations may have a direct effect on next-day glycaemia considering the duration of basal insulin action may be longer than 24 hours (Lepore *et al.* 2000). However, reductions in basal dose may carry indirect effects also; patients under **80%** consumed significantly less food *ad libitum*, in particular, consuming less carbohydrates and administering significantly fewer units of insulin, which in a state of continued insulin sensitivity (Mikines *et al.* 1988) may lend some explanation to differences in glycaemia during this time. Patients were instructed to continue habitual food consumption, with patients not required to replicate diet, over the course of day following the exercise trials. Potentially, reductions in basal dose may carry prospective effects on appetite regulation and energy balance, although a more longitudinal study would be required to confirm this hypothesis. In light of the above, it would seem that reducing basal dose carries important implications for next day glycaemic control. In addition, glycaemia was similar between conditions over the course of exercise day, with differences establishing after 6 hours post-exercise and during sleep. This would indicate that reducing basal dose on the day

of exercise does not significantly disrupt glycaemic control in the time preceding exercise. This is an important observation because the aim of diabetes management is to normalise blood glucose concentrations (Thomas *et al.* 2007), especially when incorporating exercise into the lives of patients (Chu *et al.* 2011).

The aim of this study was to assess the acute and 24 hour glycaemic effects of a combined basal-bolus insulin reduction and carbohydrate feeding strategy for preventing hypoglycaemia following evening exercise. This is the first study to demonstrate that a combined basal-bolus insulin reduction and carbohydrate feeding strategy provides full protection from exercise-induced hypoglycaemia for 24 hours after exercise. The clinical importance of these findings is self-evident, as patients adopting this strategy can now participate in exercise without fear of exercise-induced hypoglycaemia. However, these findings may not be directly transferable to patients treated on different regimens, and the optimum regimen (insulin type and timing) are yet to be determined. There is now a need to establish whether this strategy is associated with wider metabolic, hormonal, and inflammatory implications which could, if repeated on a regular basis affect longer-term diabetes management.

CHAPTER 5B

THE METABOLIC, INFLAMMATORY, AND COUNTER-REGULATORY HORMONAL RESPONSES FOLLOWING EVENING EXERCISE IN TYPE 1 DIABETES PATIENTS UNDER CONDITIONS OF REDUCED BASAL INSULIN DOSE

5.4 Introduction

Reducing basal dose whilst employing a reduced pre- and post-exercise rapid-acting insulin and low GI carbohydrate feeding strategy protects type 1 diabetes patients from hypoglycaemia for 24 hours following exercise (chapter 5A). However, there is now a need to determine the hormonal, metabolic and inflammatory implications of this strategy.

Whereas hypoinsulinaemia augments lipolysis and ketogenesis (Laffel 2000, Wallace and Matthews 2004) and concomitant exposure to hyperglycaemia increases the appearance of circulating inflammatory cytokines (Targher *et al.* 2001, Esposito *et al.* 2002, De Rekeneire *et al.* 2006, Rosa *et al.* 2008), heavily reducing pre- and post-exercise rapid-acting insulin dose does not cause clinically meaningful increases in β-hydroxybutyrate (> 1.0 $mmol.L^{-1}$) (chapter 3B). Moreover, when these reductions are applied in concert with the consumption of a low GI meal, the appearance of IL-6 and TNF- α are also reduced (chapter 4B). Thus, it would appear that: 1) despite a large reduction in insulin dose, circulating concentrations remain great enough to supress ketone body production (Balasse and Féry 1989, Mcgarry 1996), and 2) that normalisation of glycaemia via modification of post-exercise food composition can reduce inflammatory disturbances. Whether the application of a basal dose reduction *in combination* with rapid-acting insulin adjustments would affect counter-regulatory hormonal, metabolic and inflammatory parameters is yet to be established.

Accordingly, the aim of the study was to examine the counter-regulatory hormonal, metabolic, and inflammatory responses of reducing basal insulin dose when evening exercise is performed in patients with type 1 diabetes adopting acute prandial recommendations.

5.5 Methods

A second arm of analysis was performed on both trials from chapter 5A. Blood lactate, serum cortisol, non-esterified-fatty-acids, β-hydroxybutyrate, and plasma glucagon, adrenaline, IL-6 and TNF-α were measured for 60 minutes post-meal (Figure 5.3).

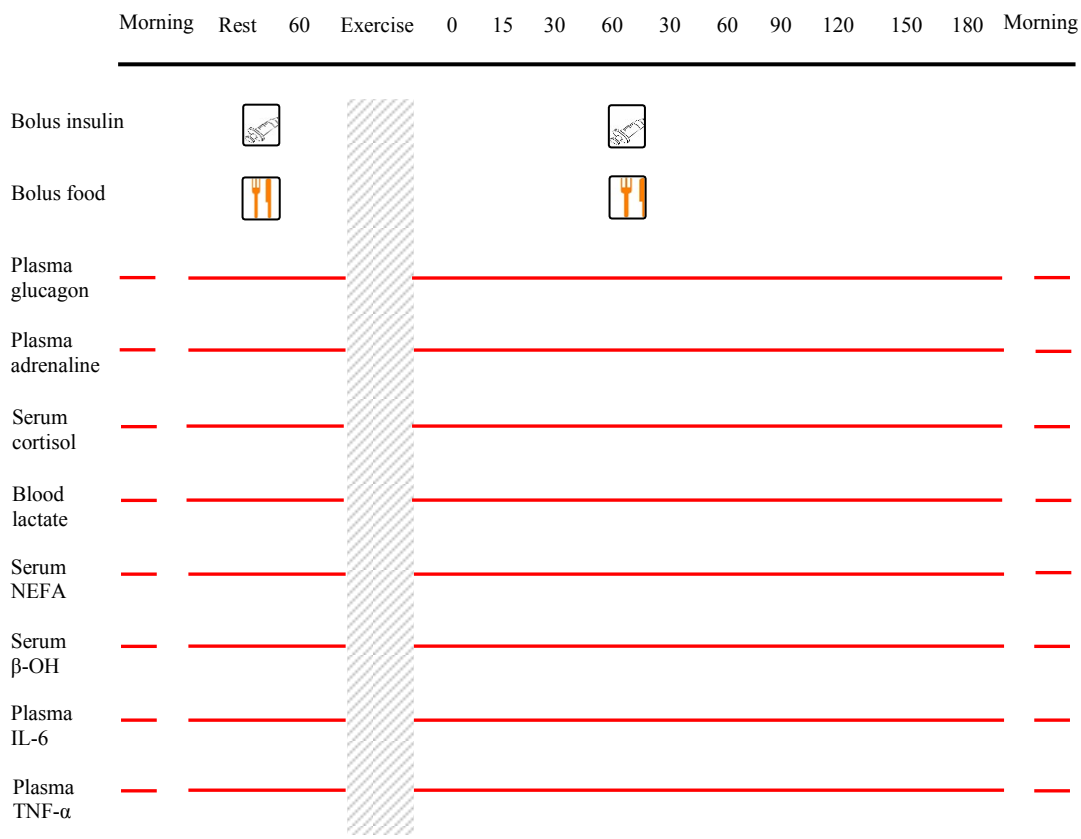

Figure 5.3. Schematic of study trial design.

5.6 Results

5.6.1 Pre-laboratory phase

5.6.1.1 Counter-regulatory hormone and metabolite responses

The counter-regulatory hormone and metabolite responses are presented in Table 5.2. There were no conditional differences in fasted, resting morning-time concentrations in any measures.

5.6.1.2 Inflammatory cytokine responses

The inflammatory cytokine responses are presented in Figure 5.4. There were no conditional differences in IL-6 or TNF-α concentrations in fasted, resting pre-trial morning concentrations in either measure. Concentrations of IL-6 and TNF-α were positively related to length of

diabetes (IL-6: r = 0.811, p = 0.008; TNF-α: r = 0.799, p = 0.003) and inversely related to HbA_{1c} (IL-6: r = -0.722, p = 0.032; TNF-α: r = -0.771, p = 0.034), but not $\dot{V}O_{2peak}$ (IL-6: r = -0.322, p = 0.257; TNF-α: r = -0.102, p = 0.893). Length of diagnosis and HbA_{1c} were negatively correlated (r = -0.799, $p < 0.029$).

5.6.2 Laboratory phase

5.6.2.1 Counter-regulatory hormone and metabolite responses

There were no conditional differences in counter-regulatory hormone or metabolite responses during the laboratory phase. Serum cortisol was lower than pre-trial morning concentrations under both conditions at rest, and throughout the trial period. All other resting measures were similar to those observed on the pre-trial morning, with temporal changes remaining similar between conditions (Table 5.2).

5.6.2.2 Inflammatory cytokine responses

Trial resting concentrations in both IL-6 and TNF-α were similar to those elicited on the pre-trial morning (Figure 5.4) and remained similar in the 60 minutes before exercise. Following exercise, TNF-α was significantly raised from both rest and pre-trial morning concentrations under both conditions before returning to trial resting and morning concentrations immediately before the post-exercise meal at 60 minutes post-exercise (Figure 5.4). There were no conditional differences in TNF-α during this time ($p > 0.05$). Temporal changes in IL-6 were evident during the post-exercise period whereby concentrations peaked similarly at 15 minutes post-exercise (Figure 5.4). IL-6 decreased under both conditions following this point, however the decline was significantly attenuated under **80%**. Conversely, IL-6 under **100%** decreased such that concentrations were significantly lower than both resting and pre-trial morning concentrations (Figure 5.4).

5.6.3 Post-laboratory phase

5.6.3.1 Counter-regulatory hormone and metabolite responses

Plasma adrenaline was significantly lower under both conditions than the pre-trial morning and trial resting sample (Table 5.2). Blood lactate, and serum NEFA and β-hydroxybutyrate (Table 5.2) were significantly increased under both conditions. Serum cortisol, was increased from rested concentrations, but remained similar to those measured on the pre-trial morning under both conditions (Table 5.2). All other hormones and metabolites remained unchanged (Table 5.2).

5.6.3.2 Inflammatory cytokine responses

Both conditions displayed similarly raised TNF-α concentrations compared to the pre-trial morning sample, although these were similar to resting concentrations elicited during the main trials. Conversely, IL-6 under **100%** was significantly lower than both pre-trial morning and trial resting measures (Figure 5.4). Concentrations under **80%** remained similar to both pre-trial morning and trial resting samples (Figure 5.4).

Table 5.2 Metabolic and counter-regulatory hormone responses during manipulation to basal-bolus insulin

											ANOVA *p*	
		Morning 1	**Rest**	**E**	**60**	**0**	**15**	**30**	**Pre-Meal**	**Morning 2**	**T**	**T*C**
Plasma Glucagon ($pg.ml^{-1}$)	**100%**	637±148	549±115		396±80#†	526±94	573±125	555±118	576±136	634±147	=0.049	=0.663
	80%	595±118	532±111		397±77#†	501±84	593±138	540±128	536±121	611±106		
Plasma Adrenaline ($nmol.l^{-1}$)	**100%**	0.22±0.04	0.14±0.03		0.10±0.04#	0.47±0.10#†	0.26±0.08	0.12±0.03	0.12±0.04	0.13±0.03#	=0.017	=0.322
	80%	0.29±0.06	0.14±0.04		0.11±0.03#	0.45±0.12†	0.29±0.11	0.13±0.05	0.13±0.03	0.08±0.02#		
Serum Cortisol ($\mu mol.l^{-1}$)	**100%**	0.65±0.06	0.32±0.06#		0.20±0.37#	0.37±0.10#	0.41±0.12	0.42±0.12	0.30±0.08#	0.61±0.08†	= 0.042	= 0.654
	80%	0.71±0.06	0.28±0.04#		0.24±0.04#	0.29±0.09#	0.42±0.14	0.36±0.13	0.27±0.09#	0.68±0.07†		
Blood Lactate ($mmol.l^{-1}$)	**100%**	0.01±0.01	0.44±0.21		0.95±0.15#†	3.27±0.58#†	1.83±0.29#†	0.77±0.22#	1.41±0.93#†	0.27±0.15#	<0.019	=0.798
	80%	0.11±0.11	0.53±0.19		1.18±0.23#†	3.40±0.66#†	1.77±0.34#†	0.86±0.30#	0.64±0.17#	0.40±0.18#		
Serum NEFA ($mmol.l^{-1}$)	**100%**	0.39±0.06	0.26±0.05		0.19±0.03#	0.16±0.02#	0.24±0.04	0.20±0.02#	0.29±0.06	0.65±0.15#†	=0.049	=0.663
	80%	0.36±0.05	0.30±0.10		0.35±0.13#	0.25±0.04#	0.38±0.09	0.31±0.08	0.33±0.08	0.52±0.07#†		

Note: Data presented as mean ± SEM. Test meal and insulin were administered immediately following rest and pre-meal sample points. * indicates significantly different from **100%** ($p \leq 0.05$). # indicates significantly different to morning 1. † indicates significantly different from rest. Morning 1 = pre-trial morning sample, Morning 2 = post-trial morning visit sample, T = Time, C = Condition, **E** = Exercise.

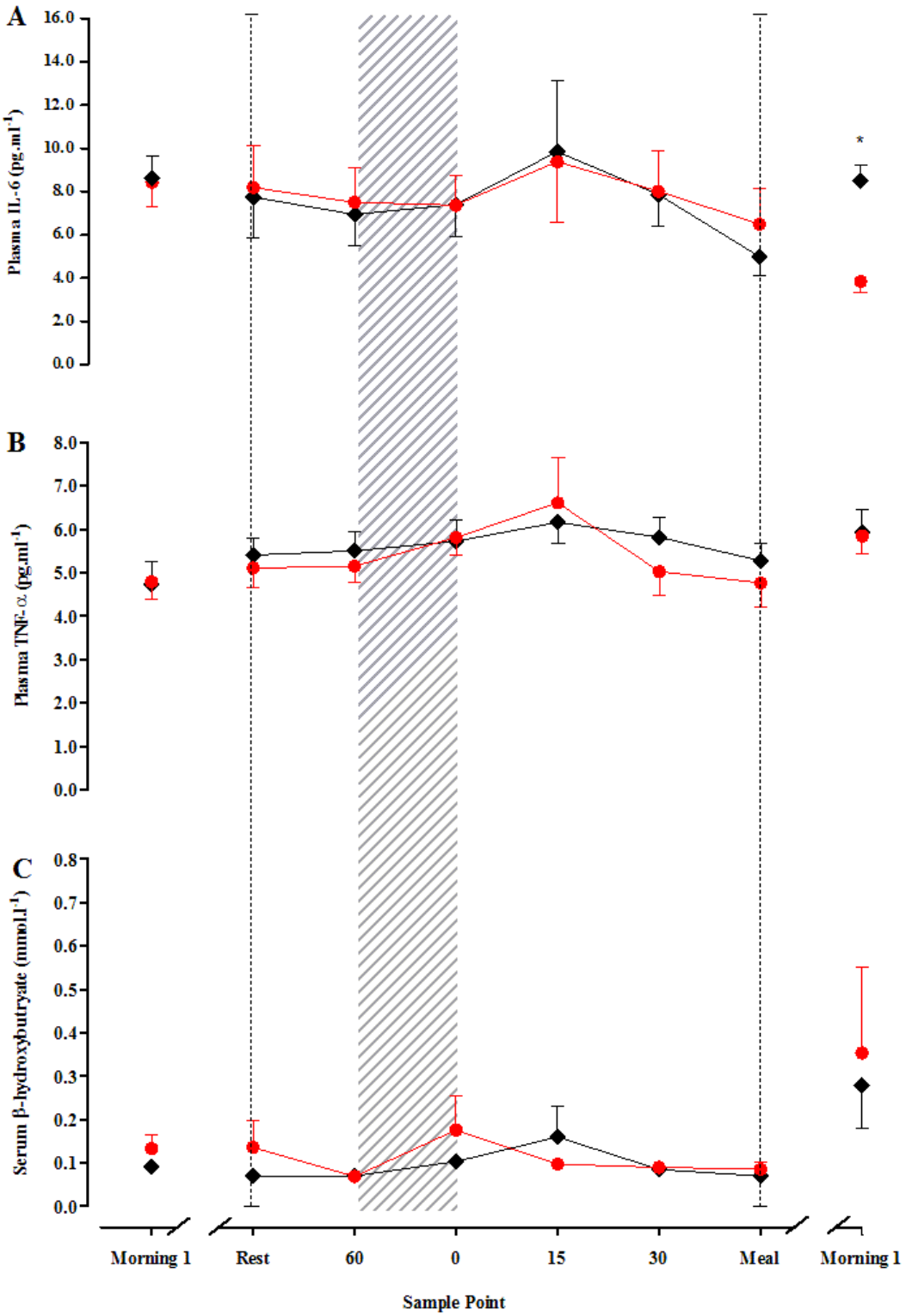

Figure 5.4 A-C. Time-course changes in (**A**) plasma IL-6, (**B**) plasma TNF-α and (**C**) serum β-hydroxybutyrate. Data presented as mean ± SEM. Black diamonds = **100%**, red circles = **80%**. Transparent sample point within a condition indicates a significant difference from pre-meal concentrations ($p \leq 0.05$). * indicates significantly different from **100%** ($p \leq 0.05$). Thatched area indicates exercise. Vertical dashed line break indicates carbohydrate meal and insulin administration. Thatched area indicates exercise. Note: Basal insulin dose was administered as per individual patient regimen.

5.7 Discussion

The main findings in this study were that a reduction in basal insulin dose, in concert with acute prandial adjustments, does not significantly augment ketonaemia to clinically meaningful concentrations. Alike, elevations in the inflammatory marker TNF-α were similar between conditions, with concentrations on the morning after exercise greater than those on the morning before, but similar to resting pre-exercise measures. IL-6 concentrations were not significantly raised from morning or pre-exercise measures under the reduction trial, and there were no other hormonal or metabolic disturbances associated with this strategy.

Exercise-induced hypoglycaemia is avoidable for type 1 diabetes patients by applying acute prandial adjustments with a reduction in basal insulin dose (chapter 5A). The importance of a strategy which achieves this cannot be underestimated as hypoglycaemia remains the primary obstacle to patients wishing to engage in exercise (Brazeau *et al.* 2008). Now, this study demonstrates that this strategy does not significantly raise ketonaemia. Decrements in insulin and elevations in counter-regulatory hormones combine to stimulate lipolysis in adipose tissue, and ketogenesis in the liver (Nosadini *et al.* 1994, Delaney *et al.* 2000). Under such conditions, lipases are activated increasing circulating NEFA and impairing their re-esterification, catalysing their transport into the mitochondria (Mcgarry and Foster 1980, Kruszynska 1997), and subsequently converting them into ketone bodies (Keller *et al.* 2009).

Rapid-acting insulin was omitted with the bedtime snack, and there were no reported occasions of rapid-acting insulin being administered to correct blood glucose through the late evening and night, meaning rapid-acting insulin administration was matched over the course of the laboratory period and throughout the night (1-13 hours post-exercise) between the two trials (chapter 5A). As the rapid-acting analogue insulin Aspart is 100% cross-reactive with insulin Glargine (Pennartz *et a*l. 2011), any changes in serum concentrations can be considered a result of manipulating basal

dose. However, serum insulin concentrations remained similar between conditions across the exercise trial and post-laboratory period, meaning differences in circulating basal insulin were undetectable at the specified time points. Similarly, adrenaline and cortisol, the main lipolytic stimulators (Keller *et al.* 2009), returned to pre-exercise concentrations under both conditions by 60 minutes post-exercise, and by the following morning were lower than those obtained on the preceding morning. Although increases in NEFA concentrations were apparent under the **80%** condition on the morning after exercise, the rise was comparable to **100%**, indicating that the reduction in basal dose was not great enough to significantly elevate lipolysis. Furthermore, it would appear that this metabolic milieu was insufficient to augment ketonaemia, as β-hydroxybutyrate was alike between conditions. Moreover, concentrations were less than those considered clinically meaningful (> 1.0 $mmol.l^{-1}$; Laffel (2000)).

Blood lactate concentrations represent a balance between lactate production and utalisation. Small elevations in blood lactate would support a notion for increased glycolysis, probably in exercised musculature as this tissue is the major component of production and use (Gladden 2008), and maybe the liver too although to a lesser extent (Connor and Woods 1982); subsequent production of pyruvate, the precursor for oxaloacetate, and its condensation with acetyl CoA prevents its diversion from the citric acid cycle to ketogenesis (Jain *et al.* 1998, Jain *et al.* 1999, Jain *et al.* 2006), hence low levels of β-hydroxybutyrate. Indeed, lactate forms from the reduction of pyruvate by lactate dehydrogenase, which if oxidised in the citric acid cycle would increase lactate production. Post-trial morning elevations in blood lactate were comparable between conditions suggesting no conditional effect of insulinaemia on this metabolite. Indeed, acute regulation of endogenous glucose production by insulin is demonstrated to occur mainly via changes in glycogenolysis rather than gluconeogenesis in type 1 diabetes patients (Boden *et al.* 2003). However, depleted glycogen stores following exercise, and their subsequent restoration in the

post-exercise period, may have promoted an increased contribution from gluconeogenesis to endogenous glucose production.

An important observation was the response in inflammatory cytokines following the intervention as the avoidance of inflammation is important for preventing the early-onset of diabetes related complications (Rosa *et al.* 2011). This study shows that the inflammatory cytokine TNF-α is not affected by reductions in basal insulin dose when acute prandial adjustments are also made for evening exercise. In addition, IL-6 is not significantly increased from fasted, rested morning-time concentrations, although concentrations are significantly lower when a basal dose reduction is not applied. At 1 hour post-exercise, IL-6 concentrations under **100%** were significantly lower than those on the pre-morning trial and trial resting sample point, and would have likely remained stable, at least in the early hours following the post-exercise meal (chapter 4B). Conversely, concentrations under **80%** were significantly greater than **100%** immediately prior to the post-exercise meal and on the post-exercise morning. Insulin carries anti-inflammatory properties (Viardot *et al.* 2007). Indeed an inverse relationship between circulating insulin and IL-6 found in chapter 3B would support findings herein. However, insulin concentrations at the post-trial morning sample point were comparable (chapter 5A). It is possible that differences in insulin concentrations occurred during the night and were simply not captured due to sampling frequency. If this were the case, it may lend some explanation as to why post-trial morning IL-6 concentrations differed between conditions; as concentrations were lower under the **100%** condition, potentially, higher insulin concentrations through the night may have supressed IL-6 or increased IL-6 clearance. However, with no visible differences in other metabolic parameters at this time point, and considering the multiple roles IL-6 plays (e.g. anti- versus pro-inflammatory effects, molecular signalling and metabolic cross-tissue communicator; see Ellingsgaard *et al.* (2011)), it would be inappropriate to speculate on the exact mechanisms underpinning this finding.

In support of the findings in chapter 4B, resting inflammatory cytokine concentrations were positively related to length of diabetes and inversely related to HbA_{1c} in this study, strengthening the premise of diabetes as a long-standing inflammatory disease (Devaraj *et al.* 2007), and the association between intensive diabetes management and reduced inflammatory disturbances (Devaraj *et al.* 2005, De Rekeneire *et al.* 2006, Galassetti *et al.* 2006). All other hormonal and metabolite measures remained similar between conditions during the post-exercise post-prandial period.

The aim of this study was to assess the metabolic, counter-regulatory hormonal, and inflammatory responses following a combined basal-bolus insulin reduction and carbohydrate feeding strategy for evening exercise. In summary, this study demonstrates that a reduction in basal insulin dose, in concert with acute prandial adjustments to rapid-acting insulin and diet, following evening exercise in type 1 diabetes does not significantly alter ketone body formation or increase IL-6 above fasted, rested morning-time concentrations, and that TNF-α is not increased above day-time pre-exercise levels. Future work would benefit from a more detailed profiling of inflammatory and ketogenic markers to better capture potential changes in these parameters in the post-exercise period. In addition, it is suggested that future thought be given to the impact of different insulin species and treatment regimens.

CHAPTER 6

GENERAL DISCUSSION

6.0 Introduction

The research presented in this thesis has examined the impact of alterations in insulin administration and carbohydrate feeding on acute and 24 hour post-exercise glycaemic control. In addition, acute metabolic, counter-regulatory hormonal and inflammatory parameters were investigated to understand the deeper underlying physiological consequences of the interventions employed. This chapter will collate and consider the findings of chapters 3 to 5. A schematic is provided in Figure 6.0 which summarises and incorporates these findings into a workable strategy which can be applied in clinical practice.

6.1 Acute glycaemic control and avoidance of early-onset hypoglycaemia

Patients are recommended to reduce the amount of rapid-acting insulin administered before exercise, to prevent hypoglycaemia during and immediately after exercise (Rabasa-Lhoret *et al.* 2001, Mauvais-Jarvis *et al.* 2003, West *et al.* 2010, West *et al.* 2011, West *et al.* 2011). Across all chapters, patients heavily reduced the amount of rapid-acting insulin dose administered 60 minutes before exercise. Despite the performance of an intensive bout of aerobic exercise during a time of peak insulin absorption (Plank *et al.* 2002), there were no incidences of hypoglycaemia during exercise, and all patients remained protected for up to one hour post-exercise without further feeding. In addition, patients were, on average, within euglycaemic ranges over the duration of the 60 minute post-exercise period (chapters 3, 4, and 5). This emphasises the importance of large reductions in pre-exercise rapid-acting insulin dose for protecting patients from hypoglycaemia during, and immediately after exercise, and highlights that this strategy exposes patients to only transient hyperglycaemia in the period before exercise. It is worthy to note however, that such large reductions in pre-exercise rapid-acting insulin may not necessary if the exercise modality is altered. For example, performing resistance exercise or including intermittent high-intensity periods throughout aerobic exercise, is likely to increase acute counter-regulatory hormones which

may assist in the preservation of glycaemia acutely after exercise (<1 hour post-exercise) (Campbell *et al*. 2014).

Chapter 3 demonstrated that it is also important to reduce the dose of rapid-acting insulin administered with the meal after exercise to extend this window of protection. A 50 % reduction in post-exercise rapid-acting insulin was necessary to prevent falls in glycaemia, and protect all patients from hypoglycaemia for a further 7 hours (8 hours post-exercise). This contrasts to current opinion which advocates a reduction of only ~30 % (Lumb and Gallen 2009). In addition, it can be advised that post-exercise rapid-acting insulin reductions should be applied irrelevant of exercise modality, although alterations in dose should be tailored to individual exercise preferences.

GI heavily influences post-prandial glycaemia in type 1 diabetes (Nansel *et al.* 2008, Parillo *et al.* 2011). Now, it is clear that the GI of meals consumed following exercise carry important implications for post-exercise glycaemia. Evidenced by chapter 3, hyperglycaemia is a likely consequence for the majority of patients reducing post-exercise rapid-acting insulin dose; the incidence of hyperglycaemia was greater than double when a 50 % dose was implemented, with individual peak blood glucose as great as 21.8 $mmol.l^{-1}$ and one patient averaging blood glucose concentrations of 19.3 $mmol.l^{-1}$ across the post-meal period (chapter 3). Notably, the post-exercise meal in this study elicited a moderate GI (GI = 57), and in chapter 4, a high GI (GI = 92) also induced severe hyperglycaemia in the majority of patients. However, when an otherwise similar meal identical in macronutrient composition but of a low GI (GI = 37), the incidence of post-prandial hyperglycaemia was reduced by 60 % and in those patients affected, hyperglycaemia tended to be shorter-lasting and less pronounced. Currently, there are relatively few dietary guidelines to assist patients in managing their blood glucose after exercise (Chu *et al.* 2011). Now however, it is clear that the composition of post-exercise carbohydrate is an important consideration for patients with type 1 diabetes. Specifically, low GI carbohydrate consumption facilitates more desirable post-prandial glycaemia responses by normalising blood glucose. This is

an important contribution to the literature as current recommendations place more focus on the quantity rather than the composition of the carbohydrate to be consumed following exercise (Bantle *et al.* 2008, Evert *et al.* 2014).

6.2 Avoidance of late-onset hypoglycaemia

Chapters 3 and 4 illustrate that acute prandial adjustments to rapid-acting insulin and carbohydrate feeding carry only short-lasting protective effects from exercise-induced hypoglycaemia. Patients in both chapters were exposed to hypoglycaemia 8 hours after exercise, irrelevant of the dose administered with the post-exercise meal (chapter 3), or post-exercise meal composition (chapter 4). It would not seem that carbohydrate intake alone is sufficient for preventing late falls in glycaemia, as patients across chapters consumed adequate carbohydrate to cover the energy expended during exercise and establish a positive energy balance across the course of the day. Notably, when exercise is performed in the evening, late falls in glycaemia occur nocturnally (chapter 4 and 5), meaning it would be impractical for patients to consume carbohydrate at this time. Moreover, hypoglycaemia occurred despite the consumption of a bedtime snack, irrespective of its composition, and notwithstanding ambient glucose levels before sleep. Typically, patients within normal blood glucose ranges before sleep often choose to raise glycaemia before bed. However, the findings from chapter 4 and 5 would suggest that simply elevating glycaemia before sleep does not confer any glycaemic benefit for avoiding late-onset nocturnal hypoglycaemia.

It was important to assess whether late-onset hypoglycaemia could be acutely managed, as adjusting basal dose may be daunting for patients. In addition, there is no data regarding the deeper, metabolic, hormonal and inflammatory implications of adjusting basal insulin dose. However, the findings of chapter 4 direct attention towards the need for a longer-lasting intervention. Late falls in glycaemia appear coincidental with reported glucose nadirs on non-exercise days occurring 4-14 hours after basal insulin administration (Ashwell *et al.* 2006,

Mcmahon *et al.* 2007, Thomas *et al.* 2007). Chapter 5 has somewhat confirmed this; reducing the total amount of basal insulin administered over the course of the exercise day by 20 % prevented falls in glycaemia late after exercise, completely abating the risk of hypoglycaemia for a total of 24 hours after exercise. This is the first strategy to date that has provided complete 24 hour protection from exercise-induced hypoglycaemia in patients with type 1 diabetes. This is particularly pertinent considering the intensive nature of exercise employed compared to that in other studies.

In addition, it would seem that a reduction in basal dose of this magnitude does not significantly alter glycaemic control in the time preceding exercise, as differences in glycaemia were not established until 6 hours after exercise and during sleep (chapter 5). Moreover, it would seem that glycaemic control over the course of the next day is improved with this strategy, with fewer and less severe fluctuations in glucose following a reduction in basal insulin. It is important to understand whether strategies that adapt patients' treatments regimens influence their ability to manage glycaemia on subsequent non-exercise days as glycaemic dysregulation in the time following exercise could offset the beneficial effects that exercise carries. Moreover, it would seem that when comparing post-prandial glycaemia, there are only minimal, if any differences between chapters 4 and 5, suggesting the influence of basal dose adjustment on acute fluctuations is negligible.

6.3 Implications for ketonaemia and counter-regulatory hormones

As hypoinsulinaemic hyperglycaemia is associated with acute increases in lipolysis and ketogenesis (Laffel 2000, Keller *et al.* 2009) manipulating insulin administration and carbohydrate feeding may promote ketonaemia. In support of this, chapter 3 revealed a positive association between increased blood glucose and NEFA, and increased blood glucose and β-hydroxybutyrate following reductions to rapid-acting insulin after exercise. Despite large reductions in pre-exercise

rapid-acting insulin dose and exposure to transient hyperglycaemia however, ketonaemia was not significantly elevated immediately before exercise. During exercise, patients demonstrated an average respiratory exchange ratio of ~0.97 which reflects the utilisation of carbohydrate as the predominant fuel source; low levels of lipolysis would have limited substrate availability for ketogenesis. As with previous literature, β-hydroxybutyrate was not significantly increased over the course of the post-exercise period (Bracken *et al.* 2011). Following the post-exercise meal, β-hydroxybutyrate did not rise significantly under any of the interventions indicating that the administration of even small amounts of rapid-acting insulin was enough to maintain circulating concentrations to a level whereby lipolysis was inhibited, and lipogenesis increased, thus reducing the capacity for β-oxidation of NEFA and ultimately limiting substrate availability for ketogenesis (Keller *et al.* 2009). In chapter 5, a rested morning sample was taken on the day after exercise. Comparable NEFA and β-hydroxybutyrate concentrations would imply that the application of a 20 % basal dose reduction was insufficient to significantly increase lipolysis or ketonaemia. This is important considering that the role of basal dose is to restrict excessive hepatic glucose output, and prevent ketoacidosis. Indeed, altering basal dose may be daunting for patients, especially as there is no experimental data pertaining to the implications of basal dose adjustment for exercise. However, it would seem that ketonaemia is not significantly affected despite acute alterations in rapid-acting insulin, diet, and now also basal dose.

In addition, catecholamines and cortisol, the main lipolytic stimulus, remained unchanged between conditions across chapters. Importantly, β-hydroxybutyrate concentrations across chapters remained well below those levels deemed clinically significant (> 1.0 $mmol.l^{-1}$) (Laffel 2000). Consumption of carbohydrate would have reduced the energy deficit created by exercise thereby limiting the appearance of catecholamines and cortisol.

6.4 Inflammatory cytokine responses

Given the tight interplay between insulin, glycaemia, and inflammation, a logical extension of the interventions employed in this thesis was to investigate their influence on the appearance of inflammatory markers. Increased markers of inflammation are strongly related to glycaemic management and the pathogenesis of diabetes related complications (Targher *et al.* 2001, Fowler 2008). All patients recruited in this thesis exhibited chronically elevated levels of inflammatory markers at baseline, although concentrations of IL-6 and TNF-α were typically greater than those previously observed (Galassetti *et al.* 2006, Rosa *et al.* 2008, 2010, Rosa *et al.* 2011). The majority of previous studies have recruited children or adolescents recently diagnosed, which differs to the patients in this series of studies who were older and had a longer diabetes duration. Across chapters, a positive relationship was observed between inflammatory cytokine concentrations and diabetes duration, which was inversely related to HbA_{1c}. Therefore, concentrations reported herein are more likely to be reflective of an exercising adult population with type 1 diabetes. Exercise has the capacity to increase inflammation (Ostrowski *et al.* 1999, Nemet *et al.* 2002, Petersen and Pedersen 2005), which could be exacerbated under conditions of hypoinsulinaemia (Fishel *et al.* 2005, Viardot *et al.* 2007) and hyperglycaemia (De Rekeneire *et al.* 2006, Devaraj *et al.* 2007). However, only modest increases in IL-6 and TNF-α were observed following exercise.

Whereas it would seem that reductions in rapid-acting insulin alone are not sufficient to induce a significant increase in the inflammatory markers IL-6 and TNF-α above those at rest (chapter 3), these inflammatory cytokines are dramatically increased following a high GI meal (chapter 4). Conversely, consumption of a low GI meal completed prevented these rises. This strengthens current opinion that hyperglycaemia is a mediator of inflammation, but more importantly, normalisation of glycaemia via acute prandial adjustments in rapid-acting insulin and carbohydrate feeding can abate this inflammatory response. Moreover, IL-6 was supressed following a full dose of post-exercise rapid-acting insulin in chapter 3, which further highlights the capacity of insulin

to act as a potent anti-inflammatory (Viardot *et al.* 2007). In addition to these findings, chapter 5 illustrates that TNF-α is not affected by a reduction in basal insulin dose when these acute adjustments are applied, and that IL-6 is not significantly increased from fasted, resting morning-time concentrations. Collectively, this data would indicate that a combined basal-bolus reduction and post-exercise carbohydrate feeding strategy does not significantly increase the inflammatory markers IL-6 and TNF-α.

6.5 Appetite responses

The appetite responses to specific post-exercise dietary interventions in type 1 diabetes patients is an important consideration as excessive energy intake relative to energy expenditure can confer detrimental long-term implications for glycaemic control and cardiovascular risk in patients (Wadén *et al*. 2008; Salem *et al*. 2010). Over-consumption of carbohydrates as a compensatory strategy to prevent hypoglycaemia is fairly typical in type 1 diabetes patients (Dubé *et al*. 2014). However, even in those without type 1 diabetes, excessive energy intake may occur due to increased appetite (Kamoi *et al*. 2011). Thus, it was important to establish whether normalising glycaemia would improve or adversely affect satiety in patients. Chapter 4 revealed that consumption of carbohydrate based foods, match for macronutrient composition and fibre, but differing in GI significantly influence subjective sensations of appetite. Specifically, a high GI meal acutely induces greater fullness and less hunger than an otherwise equivalent low GI meal. Notably, these findings occurred under matched insulin administration, and similar plasma glucagon and GLP-1 concentrations, suggesting that ambient blood glucose concentrations play a large role in regulating appetite responses. Readers must consider however, that these findings are based on acute observations only; whereas it is possible that patients may increase calorie intake due to increased appetite rather than the avoidance of hypoglycaemia *per se*, further studies should be conducted to determine whether the strategies implemented within this thesis carry important implications for long-term weight management in this population.

6.6 Limitation and future directions

Specific limitations are addressed in respective experimental chapters. The findings in this thesis are based upon a series of acute, relatively short-term studies, which require careful interpretation if translated directly into clinical practice. It is important to consider that any short-term effect of an intervention needs to be maintained over a longer period of time, and thus this body of work would benefit from longer observational studies assessing the impact of the strategies employed under true free-living conditions and without experimental control parameters. It is suggested that the recommendations generated from this thesis be advertised in-clinic and promoted to type 1 diabetes patients with further follow-up assessment to determine whether such recommendations are effective within a real-world setting, and to establish whether these recommendations impact upon exercise participation, adherence, and improvements in glycaemic control.

Although typical of the vast majority of applied, laboratory-based exercise studies in type 1 diabetes (Bussau *et al.* 2006, Bussau *et al.* 2007, West *et al.* 2010, West *et al.* 2011, West *et al.* 2011, Yardley *et al.* 2012, Davey *et al.* 2013, Turner *et al.* 2013, Yardley *et al.* 2013, Turner *et al.* 2014, Turner *et al.* 2014), the sample size employed across chapters was relatively small. However, a power assessment revealed that the number of patients recruited in chapter 3 was sufficient to achieve a statistical power of 80 %, with chapters 4 and 5 achieving a statistical power of 73 %. In addition, this study recruited relatively young, male patients, all in excellent glycaemic control and who were already actively engaged in regular exercise. Caution should be taken when inferring results obtained from this series of studies to the wider diabetes community who may be older, less well controlled, less accustomed to exercise, and treated on different insulin regimens than those employed herein. Indeed, reduced compliance in some patients may make these recommendations difficult to translate and implement. A further limitation is that the strategies employed within this thesis are based upon one exercise model. The glycaemic, metabolic, hormonal and inflammatory responses in type 1 diabetes patients contrast greatly across exercise

modalities and intensities, and it would therefore be inappropriate to apply these findings to a range of different exercises. Nevertheless, these limitations should not detract from the clinical importance of these findings as patients and clinicians can tailor these strategies based on individual glycaemic responses, treatment regimens, and exercise preferences.

It would be advantageous to follow on from this body of work by investigating wider markers of glycaemic control and diabetes management. Although a fairly broad ranging and comprehensive assessment of the deeper implications arising from these interventions was conducted, due to financial resources, it was only possible to measure two inflammatory cytokines, with a limited number of hormones and metabolites. It would be of great interest, and of potential importance, to determine the deeper physiological mechanisms behind these findings, with an investigation into liver and muscle to determine specific effects on both glucose output and storage, as well as the interplay between these tissues in their role for late-onset hypoglycaemia. To strengthen the clinical application of these strategies it would be beneficial to investigate more global pathological markers for cardiovascular health, such as lipoproteins, hypertension, and endothelial progenitor cells.

6.7 Conclusions

The findings in this thesis have demonstrated:

1. Reducing the dose of rapid-acting insulin administered after exercise, whilst under conditions of heavily reduced pre-exercise rapid-acting insulin dose protects patients for a total of 8 hours after exercise. During this time, patients may experience periods of post-prandial hyperglycaemia, but are not exposed to other metabolic, counter-regulatory hormonal or inflammatory disturbances. Beyond this time, risk of late-onset hypoglycaemia remains. This fully addresses aims 1 and 2 (section 1.10).

2. The composition of foods consumed after evening exercise carry important implications for type 1 diabetes patients. Specifically, consuming foods that elicit a low GI in the post-exercise period, whilst employing reductions in pre- and post-exercise rapid-acting insulin, reduces post-prandial hyperglycaemia whilst maintaining protection from early-onset hypoglycaemia for a total of 8 hours after exercise. During this time, a high GI meal was shown to increase inflammatory markers, whereas a low GI meal completely prevented this rise and was not associated with any other metabolic or counter-regulatory disturbance. Beyond this time, risk of late-onset nocturnal hypoglycaemia remains. In addition, acute prandial adjustments to rapid-acting insulin and food composition carry implications for appetite, whereby a low GI post-exercise meal induces greater sensations of hunger and lower feelings of satiety early into the post-prandial period. This fully addresses aims 3, 4, and 5 (section 1.10).

3. Combining acute prandial adjustments in rapid-acting insulin and food composition with a reduction in the amount of basal dose administered over the course of an exercise day offers complete protection from hypoglycaemia for a total of 24 hours after exercise. In addition, this strategy does not augment ketonaemia, does not raise inflammatory markers IL-6 and TNF-α above fasted rested concentrations, and is not associated with any other hormonal or metabolic disturbances. In addition, this strategy carries important implications for next day glycaemic control and appetite regulation. This fully addresses aims 6 and 7 (section 1.10).

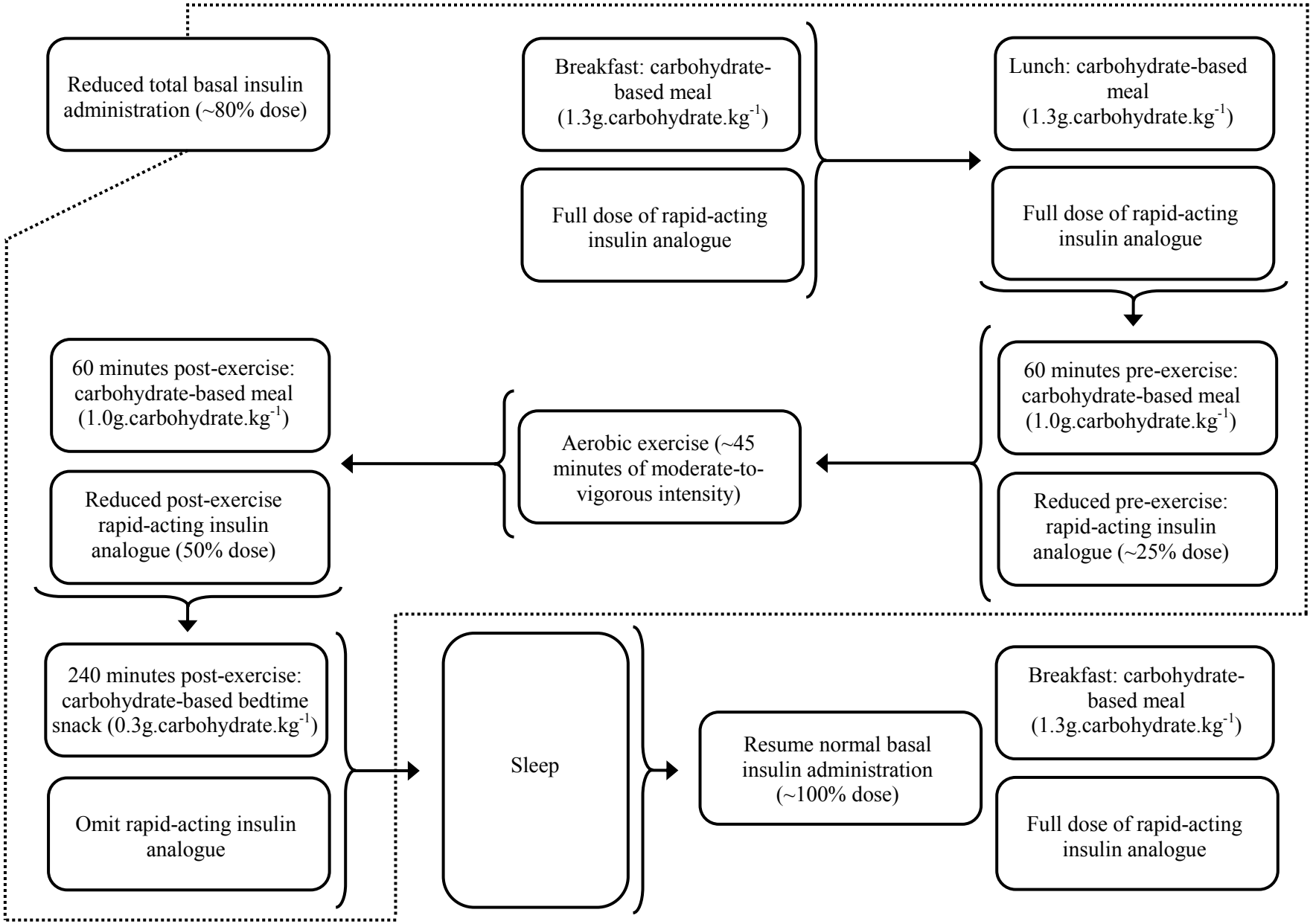

Figure 6.0. Schematic of recommended course of action for preventing exercise-induced hypoglycaemia.

CHAPTER 7

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CHAPTER 8

APPENDICES

Appendix A. Local National Health Service Ethics Committee favourable opinion

Appendix A1. Chapter 3 A-B

NHS
Health Research Authority

NRES Committee North East - Sunderland
Room 002
TEDCO Business Centre
Viking Business Park
Jarrow
Tyne & Wear
NE32 3DT

Telephone: 0191 4283563
Facsimile: 0191 4283432

30 December 2011

Dr Daniel J West
Northumbria University
Department of Sport and Exercise
School of Life Sciences
Northumbria Building
Newcastle upon Tyne
NE1 8ST

Dear Dr West

Study title: **The metabolic and glycaemic responses to reductions in rapid-acting insulin dose after running exercise in people with Type 1 Diabetes Mellitus**

REC reference: **11/NE/0343**

Thank you for your letter received 12 December, responding to the Committee's request for further information on the above research [and submitting revised documentation].

The further information was considered [in correspondence] by a sub-committee of the REC. A list of the sub-committee members is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a **favourable ethical opinion (with conditions)** for the above research on the basis described in the application form, protocol and supporting documentation [as revised], subject to the conditions specified below.

The Committee require confirmation when the research passport has been received.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Appendix A2. Chapter 4 A-C

NHS
Health Research Authority

NRES Committee North East - Sunderland
Room 002
TEDCO Business Centre
Viking Business Park
Jarrow
Tyne & Wear
NE32 3DT

Telephone: 0191 4283563
Facsimile: 0191 4283432

01 February 2013

Dr Daniel J West
Lecturer in Exercise and Health Nutrition
Northumbria University
Department of Sport Exercise Science
Faculty of Health and Life Sciences
Newcastle upon Tyne
NE1 8ST

Dear Dr West

Study title:	**The metabolic and glycaemic responses to changes in the glycaemic index of the meal consumed after performing evening exercise in Type 1 Diabetes Mellitus**
REC reference:	**13/NE/0016**
IRAS project ID:	**118634**

The Research Ethics Committee reviewed the above application at the meeting held on 28 January 2013. Thank you and Mr Campbell for attending to discuss the application.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Mrs Helen M Wilson, nrescommittee.northeast-sunderland@nhs.net.

Ethical opinion

The members of the Committee present gave a **favourable ethical opinion (with conditions)** of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

You and Mr Campbell joined the meeting.

Appendix A3. Chapter 5 A-B

NHS
Health Research Authority
NRES Committee North East - Sunderland
Room 002
TEDCO Business Centre
Viking Business Park
Jarrow
Tyne & Wear
NE32 3DT

Tel: 0191 428 3384

04 February 2014

Mr Matthew Campbell
Clinical Exercise Specialist
Northumberland Building Room 431
Northumbria University
Newcastle
NE1 8ST

Dear Matthew,

Study title:	**The metabolic and glycaemic responses to changes in the glycaemic index of the meal consumed after performing evening exercise in Type 1 Diabetes Mellitus**
REC reference:	**13/NE/0016**
Amendment number:	**Substantial Amendment 1**
Amendment date:	**16 January 2014**
IRAS project ID:	**118634**

The above amendment was reviewed at the meeting of the Sub-Committee held on 03 February 2014 by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a **favourable** ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Covering Letter	Email from Matthew Campbell	14 January 2014
Protocol	Version 2	06 January 2014
Participant Information Sheet	Version 3	06 January 2014
Notice of Substantial Amendment (non-CTIMPs)	Substantial Amendment 1	16 January 2014

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Appendix A4. Written informed consent form

Consent Form

Study Number:____ Patient Identification Number:___

CONSENT FORM

Title of Project:

Name of Researcher/s: Dr Daniel J West and Matthew D Campbell

Please Initial Box:

1	I confirm that I have read and understand the information sheets 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 prejudice and 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 this study may be looked at by responsible individuals from Northumbria University, from regulatory authorities or from the NHS Trust where it is relevant to my taking part.	☐
4	I give permission for the individuals described above to have access to my records, and I understand that accessible data will not be stored or copied.	☐
5	I agree to take part in this study.	☐

Name of Patient	**Date**	**Signature**
Name of Person Taking Consent	**Date**	**Signature**
Researcher/s	**Date**	**Signature**

Appendix B. Assessment for impaired awareness of hypoglycaemia (Clarke et al. 1995)

Table 1—*Survey items used to categorize aware or having reduced awareness of hypoglycemia in subjects*

1) Check the category that best describes you: (check one only)
_____ I always have symptoms when my blood sugar is low (A)
_____ I sometimes have symptoms when my blood sugar is low (R)
_____ I no longer have symptoms when my blood sugar is low (R)

2) Have you lost some of the symptoms that used to occur when your blood sugar was low?
_____ yes (R) _____ no (A)

3) In the past six months how often have you had moderate hypoglycemia episodes? (Episodes where you might feel confused, disoriented, or lethargic and were unable to treat yourself)
_____ Never (A) _____ Once or twice (R) _____ Every other month (R)
_____ Once a month (R) _____ More than once a month (R)

4) In the past year how often have you had severe hypoglycemic episodes? (Episodes where you were unconscious or had a seizure and needed glucagon or intravenous glucose)
_____ Never (A) _____ 1 time (R) _____ 2 times (R) _____ 3 times (R)
_____ 5 times (R) _____ 6 times (R) _____ 7 times (R) _____ 8 times (R)
_____ 9 times (R) _____ 10 times (R) _____ 11 times (R)
_____ 12 or more times (U)

5) How often in the last month have you had readings <70 mg/dl with symptoms?
___ Never ___ 1 to 3 times ___ 1 time/week ___ 2 to 3 times/week ___ 4 to 5 times/week
___ Almost daily

6) How often in the last month have you had readings <70 mg/dl without any symptoms?
_____ Never _____ 1 to 3 times _____ 1 time/week _____ 2 to 3 times/week
_____ 4 to 5 times/week _____ Almost daily

(R = answer to 5 < answer to 6, A = answer to 6 > answer to 5)

7) How low does your blood sugar need to go before you feel symptoms?
_____ 60–69 mg/dl (A) _____ 50–59 mg/dl (A) _____ 40–49 mg/dl (R)
_____ <40 mg/dl (R)

8) To what extent can you tell by your symptoms that your blood sugar is low?
_____ Never (R) _____ Rarely (R) _____ Sometimes (R) _____ Often (A)
_____ Always (A)

Four or more R responses = reduced awareness; 2 or fewer R responses = aware.

Appendix C. Reliability and validity of GlucoMen LX, Medtronic Ipro2 CGM, and Medtronic Paradigm Real Time CGM

Sample			Mean±SD	CV (%)	Validity against venous blood				
1	2	3			Mean bias ± SD	LOA	ICC	r^2	*p* value
Glucomen LX: blood glucose									
1.3	**1.3**	**1.2**	**1.3±0.04**	**5.6**					
3.2	**3.2**	**3.1**	**3.2±0.04**	**2.2**					
7.4	**7.6**	**7.9**	**7.6±0.18**	**2.8**	**0.4±0.37**	**-0.37 – 1.06**	**0.723**	**0.701**	**0.625**
13.1	**13.2**	**12.9**	**13.1±0.11**	**1.6**					
19.2	**19.4**	**19.9**	**19.5±0.25**	**1.8**					
Glucomen LX: blood ketone									
0.3	**0.4**	**0.3**	**0.3±0.06**	**17.3**					
0.6	**0.5**	**0.7**	**0.6±0.10**	**16.7**					
0.9	**1.1**	**1.1**	**1.0±0.12**	**11.2**	**-**	**-**	**-**	**-**	**-**
1.4	**1.2**	**1.3**	**1.3±0.10**	**7.8**					
1.1	**1.5**	**1.3**	**1.3±0.20**	**15.4**					
The Medtronic IPro 2 CGM									
2.2	**2.3**	**2.1**	**2.2±0.01**	**3.24**					
5.9	**5.8**	**6.2**	**6.0±0.01**	**5.00**					
9.9	**10.2**	**10.2**	**10.1±0.01**	**2.72**	**0.3±0.59**	**-0.85 – 1.47**	**0.802**	**0.801**	**0.401**
14.1	**14.3**	**14.2**	**14.2±0.01**	**1.79**					
19.0	**19.2**	**19.3**	**19.2±0.1**	**1.12**					
The Medtronic Paradigm Veo Real Time									
1.33	**1.27**	**1.25**	**1.9±0.00**	**4.5**					
14.70	**14.32**	**14.92**	**4.2±0.01**	**3.5**					
17.01	**16.96**	**16.67**	**8.4±0.01**	**1.7**	**0.23±0.60**	**-0.93 – 1.39**	**0.822**	**0.788**	**0.567**
20.09	**20.46**	**19.76**	**14.9±0.3**	**0.7**					
24.46	**24.17**	**23.92**	**22.0±0.01**	**0.8**					

Appendix D. Patient dietary and insulin administration recording record

DAY

Date: / /

Please use a separate line for each item. Please record in chronological order including insulin administration, and ketone measurement.

Time	Food/drink					Insulin administration	Ketone measurement	OFFICE USE
	Item	Brand	Description	Weight (gms)	Leftovers (gms)	Preparation, dose, site of injection		
GENERAL COMMENTS:								

Appendix E. Reliability of the Omron pedometer: Quantification of pre-and post-laboratory activity levels

Trial	Unit 1	Unit 2	Unit 3
1	100	101	102
2	100	102	102
3	101	101	101
4	101	101	101
5	100	100	100
6	100	100	101
7	101	101	100
8	101	102	101
9	101	100	101
10	100	100	101
Mean ± SEM	100.5±0.17	100.8±0.25	101.0±0.21
CV (%)	0.52	0.78	0.66

Appendix F. Blood glucose reliability testing

Sample			Mean±SEM ($mmol.l^{-1}$)	CV (%)
1	2	3		
1.33	1.27	1.25	1.28±0.02	3.24
2.34	2.36	2.27	2.32±0.03	2.03
3.20	3.01	2.90	3.04±0.09	5.00
4.35	4.12	4.23	4.23±0.07	2.72
7.04	7.14	6.89	7.02±0.07	1.79
9.68	9.52	9.41	9.54±0.08	1.42
11.78	11.60	11.89	11.76±0.08	1.25
12.23	12.01	12.45	12.23±0.13	1.80
14.70	14.32	14.92	14.65±0.18	2.07
17.01	16.96	16.67	16.88±0.11	1.09
20.09	20.46	19.76	20.10±0.20	1.74
24.46	24.17	23.92	24.18±0.16	1.12

Note: Samples were taken from venous whole blood of a male Type 1 Diabetes patient during experimental protocol.

Appendix G. Blood lactate reliability testing

Sample			Mean±SEM (mmol.l^{-1})	CV (%)
1	2	3		
0.65	0.6	0.59	0.61±0.02	5.24
1.54	1.42	1.39	1.45±0.05	5.47
2.87	2.85	2.61	2.78±0.08	5.21
3.32	3.26	3.22	3.27±0.03	1.54
4.01	3.99	3.95	3.98±0.02	0.77
4.89	4.84	4.88	4.87±0.03	0.54
5.43	5.32	5.35	5.37±0.03	1.06
6.01	6.35	5.96	6.11±0.12	3.48
6.74	6.53	6.59	6.62±0.06	1.63
7.43	7.54	7.42	7.42±0.04	0.89
8.67	8.59	8.49	8.58±0.05	1.05
9.01	9	8.91	8.97±0.03	0.61

Note: Samples were taken from venous whole blood of a male Type 1 Diabetes patient during experimental protocol.

Appendix H. Calculation of plasma volume shifts

Blood volume (BV)

$$BV^a = 100\%$$

$$BV^b = BV^a * (haemoglobin^b / haemoglobin^a)$$

Red cell volume (CV)

$$CV^a = BV^a * (haematocrit^a / 100)$$

$$CV^b = BV^a * (haemotcrit^b / 100)$$

Plasma volume (PV)

$$PV^a = BV^a – CV^a$$

$$PV^b = AV^b – CV^a$$

Percentage changes

$$BV\ \%\ change = 100 * (BV^b – BV^a) / BV^b$$

$$CV\ \%\ change = 100 * (CV^b – CV^a) / CV^b$$

$$PV\ \%\ change = 100 * (PV^b – PV^a) / PV^b$$

Appendix I. Summary of assays used for the quantification of hormones, metabolites and cytokines across studies

Analyte	Sample	Method	Product no.	Product name	Manufacturer	Sensitivity	Intra-assay reliability (%)
Insulin	Serum	Quantitative sandwich enzyme immunoassay	IV2-001 / 101	Invitron	Invitron Ltd, Monmouth, UK	0.35 mU/l	7.7%
Glucagon	Plasma	Competitive Enzyme Immunoassay	RAB0202	Glucagon EIA	Sigma Aldrich, MD, USA	0.97 pg/ml	7.2%
Adrenaline	Plasma	Quantitative sandwich enzyme immunoassay	RE59242	CatCombi	IBL, Europe Ltd	10 pg/ml	7.1%
Noradrenline	Plasma	Quantitative sandwich enzyme immunoassay	RE59242	CatCombi	IBL, Europe Ltd	20 pg/ml	7.4%
Cortisol	Serum	Quantitative sandwich enzyme immunoassay	KGE008, SKGE008, PKGE008	Paramter Cortisol Assay	R&D Systems, Minneapolis, USA	0.071 ng/ml	6.3%
NEFA	Serum	Enzymatic colourimetric assay	FA115	Randox NEFA	Randox Laboratories, UK	0.01 mmol.l	1.7%
B-hydroxybutyrate	Serum	Enzymatic colourimetric assay	RB1007	Randox B-hydroxybutyrate	Randox Laboratories, UK	0.07 mmol.l	1.7%
IL-6	Plasma	Quantitative sandwich enzyme immunoassay	HS600B, SS600B, PHS600B	Quantikine HS ELISA Human IL-6 Immunoassay	R&D Systems, Minneapolis, USA	1.6 pg/ml	4.9%
TNF-α	Plasma	Quantitative sandwich enzyme immunoassay	DTA00C, STA00C, PDTA0C	Quantikine ELISA Human TNF-α Immunoassay	R&D Systems, Minneapolis, USA	1.6 pg/ml	4.9%
GLP-1 Total	Plasma	Quantitative sandwich enzyme immunoassay	RE53131	Glucagon-Like Peptide-1 total Elisa	IBL International, Hamburg, Germany	0.6 pmol.l	7.7%

Note: Sensitivity and intra-assay reliability derived from manufacturers information

Appendix J. Calculation and conversion of analyte concentrations

Glucagon $C_{153}H_{225}N_{43}O_{349}S$

C = 12.0107	x 153	= 1837.6371	(g.mol)	
H = 1.0794	x 225	= 226.7865	(g.mol)	
N = 14.0067	x 43	= 602.288	(g.mol)	
O = 15.9994	x 349	= 783.9706	(g.mol)	
S = 32.0655	x 1	= 32.0655	(g.mol)	
				+
=	3482.748	(g.mol)	*	sample value = pg.ml

Adrenaline $C_9H_{13}NO_3$

C = 12.0107	x 9	= 108.0963	(g.mol)	
H = 1.0794	x13	= 13.1032	(g.mol)	
N = 14.0067	x 1	= 14.0067	(g.mol)	
O = 15.9994	x 3	= 47.9982	(g.mol)	
				+

= 183.2044 (g.mol) / ((pg.ml) /1000) = nmol.l

Noradrenaline $C_8H_{11}NO_3$

C = 12.0107	x 8	= 96.0856	(g.mol)	
H = 1.0794	x11	= 11.0873	(g.mol)	
N = 14.0067	x 1	= 14.0067	(g.mol)	
O = 15.9994	x 3	= 47.9982	(g.mol)	
				+

= 169.1778 (g.mol) / ((pg.ml) /1000) = nmol.l

Cortisol $C_{21}H_{30}NO_5$

C = 12.0107	x 21	= 252.2247	(g.mol)	
H = 1.0794	x 30	= 30.2382	(g.mol)	
O = 15.9994	x 5	= 79.9970	(g.mol)	
				+

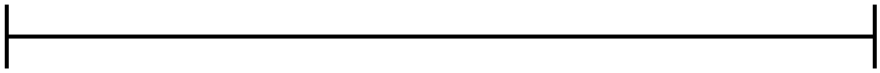
= 362.4599 (g.mol) / ((pg.ml) /1000) = nmol.l

Appendix K. Visual Analogue Scales (VAS)

Appetite Visual Analogue Scale

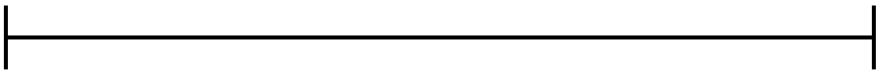
Time point 1:

How hungry do you feel?



I have never felt so hungry — I have never felt so full

How satisfied do you feel?



I feel completely empty — I cannot eat another bite