

**Carbohydrate requirements to maintain stable glycaemia
during exercise performed under basal and high
insulinaemic conditions in individuals with type 1 diabetes**

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Thesis declaration

I, Vinutha Beliyurguthu Shetty, certify that:

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Written patient consent has been received and archived for the research involving patient data reported in this thesis.

The following approvals were obtained prior to commencing the relevant work described in this thesis:

1. Princess Margaret Hospital for Children Human Ethics Committee HREC number 1846/EP, 2013005EP and 2015009EP.

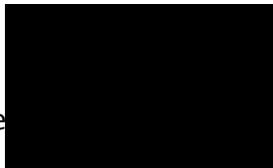
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Abstract

Achieving optimal glycaemic management in type 1 diabetes (T1D) during and after exercise is highly challenging as exercise can markedly increase the risk of hypoglycaemia. The resulting fear of hypoglycaemia is the greatest barrier to the adoption of a physically active lifestyle by people with T1D. Although regular blood glucose monitoring, carbohydrate supplementation, and insulin dose adjustment pre- and post-exercise are generally advocated to better manage blood glucose levels during and after exercise, only these first two strategies are suitable for unplanned exercise. Current exercise guidelines focus primarily on hypoglycaemia prevention, with little regard for achieving this goal while maintaining near euglycaemia. Hence, optimising carbohydrate intake to maintain near stable blood glucose levels during exercise is necessary if exercise is to contribute to optimal management of T1D. Given that the effects of exercise intensity and duration on the exogenous carbohydrate requirements to maintain blood glucose at stable, low or high levels under basal or hyperinsulinaemic conditions were still poorly understood at the time this thesis was initiated, our primary aims were to address these issues.

In order to estimate precisely the effects of exercise intensity and duration as well as those of plasma insulin levels on the exogenous glucose requirements to maintain stable blood glucose levels during and early after exercise in individuals with T1D, we adopted the use of euinsulinaemic-euglycaemic clamps, euinsulinaemic-hyperglycaemic clamps or hyperinsulinaemic-euglycaemic clamps. These different types of clamps allowed the precise quantification of the amount of exogenous glucose required to maintain stable glycaemia while exercising, and provided excellent experimental models to explore the glucoregulatory mechanisms involved by including the assays of key glucoregulatory hormones known to play important roles in glucose homeostasis together with the infusion of the stable [6,6-²H]glucose isotope to determine the rate of glucose appearance (Ra) and disappearance (Rd).

Two separate studies were undertaken to investigate the relationship between glucose requirement to maintain stable glycaemia and different exercise intensities under basal

and high insulin conditions. Nine young individuals with T1D were studied at 4 different exercise intensities (35, 50, 65 and 80% $\dot{V}O_2$ peak) following a randomised counterbalanced study design. On each occasion, they cycled for 40 min while undergoing a euinsulinaemic-euglycaemic (Study 1) or hyperinsulinaemic-euglycaemic clamp (Study 2) designed to achieve euglycaemia (5-6 mmol/l) while exposed to basal and high insulin levels, respectively. Our findings showed that under basal insulin conditions, the exogenous glucose requirements to maintain stable glycaemia were relatively low at all exercise intensities and followed an inverted U relationship with exercise intensity, with no exogenous glucose being required during high intensity aerobic exercise. In contrast, under hyperinsulinaemic conditions, more exogenous glucose was required to maintain stable glycaemia compared to basal insulinaemic conditions, and the relationship between exercise intensity and the glucose requirement to maintain stable glycaemia did not follow an inverted U relationship. Extra glucose was required to maintain stable glycaemia during early recovery from exercise performed under both basal and hyperinsulinaemic conditions. Of note, the glucose requirements to maintain euglycaemia during and early after exercise varied markedly between individuals.

The pattern of change in glucose Ra and Rd explains the inverted U relationship between exercise intensity and the amount of exogenous glucose required to maintain stable glycaemia under basal insulinaemic conditions. Our finding that glucose Rd was greater than glucose Ra during low to moderate intensity exercise explains why more exogenous glucose was required to maintain stable blood glucose levels at these exercise intensities. On the other hand, the marked increase in catecholamine levels together with the accompanying marked rise in glucose Ra matching glucose Rd associated with intense aerobic exercise (80% $\dot{V}O_2$ peak) may explain why no exogenous glucose was required to maintain stable glycaemia when high intensity exercise was performed under basal insulinaemic conditions.

Given the evidence that there is a delay before exogenous glucose requirements increase during exercise, irrespective of the levels at which plasma glucose levels are maintained, the final study was undertaken to evaluate the duration of this lag under basal

insulinaemic conditions, and to determine whether this lag is affected by the level at which glycaemia is maintained. For this study, eight participants with T1D underwent either a euglycaemic (5-6 mmol/L) or hyperglycaemic clamp (9-10 mmol/L) on separate days, and were infused with both insulin at a basal rate and [6,6-²H]glucose while cycling for 40 min at an intensity of 50% $\dot{V}O_2$ peak. We found that, irrespective of the levels at which glycaemia was maintained, there was a 20-minute period of low demand in exogenous glucose to maintain stable glycaemia. The absence of an increase in glucose infusion rate during these 20 min of exercise occurred despite elevated carbohydrate oxidation rates and no significant rise in glucose Rd, thus implying an increased reliance of skeletal muscles on the oxidation of their glycogen stores during that time. We also found that maintaining mild hyperglycaemia compared to euglycaemia before and during exercise increased the exogenous carbohydrate requirements to maintain stable glycaemia, but not during the post-exercise period.

Overall, our findings reinforce the recommendation that the safest approach to decrease the risk of exercise-mediated hypoglycaemia is to exercise while plasma insulin is at or near basal levels due to minimal changes in blood glucose levels and low exogenous glucose requirements as opposed to exercising under hyperinsulinaemic conditions. Although this thesis provides some evidence-based information to inform and improve future exercise guidelines for individuals with T1D, the oral glucose equivalent of our intravenous glucose infusion data should be established before recommending any glucose supplementation based on our findings.

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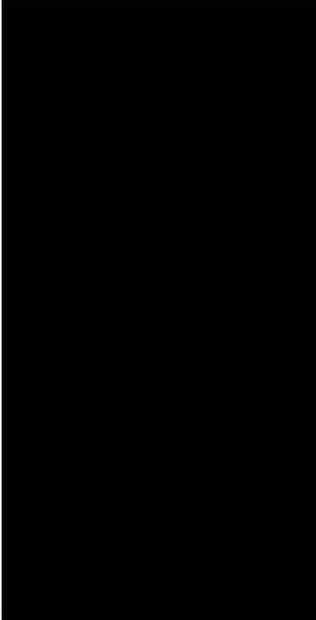
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List of abbreviations

<i>ADA</i>	<i>American Diabetes Association</i>
<i>ANOVA</i>	<i>Analysis of variance</i>
<i>APEG</i>	<i>Australian Paediatric Endocrine Group</i>
<i>BGL</i>	<i>Blood glucose level</i>
<i>BMI</i>	<i>Body mass index</i>
<i>CGM</i>	<i>Continuous glucose monitor</i>
<i>CHO</i>	<i>Carbohydrate</i>
<i>CI</i>	<i>Confidence interval</i>
<i>CSII</i>	<i>Continuous subcutaneous insulin infusion</i>
<i>DCCT</i>	<i>Diabetes control and complications trial</i>
<i>DirectNet</i>	<i>Diabetes research in children network</i>
<i>EDIC</i>	<i>Epidemiology of Diabetes Interventions and Complications</i>
<i>EDTA</i>	<i>Ethylene diamine tetra-acetic acid</i>
<i>ELISA</i>	<i>Enzyme-linked immunosorbent assay</i>
<i>F</i>	<i>Females</i>
<i>GH</i>	<i>Growth hormone</i>
<i>GIR</i>	<i>Glucose infusion rate</i>
<i>GI</i>	<i>Glycaemic index</i>
<i>[6,6-²H]glucose</i>	<i>Deuterated glucose</i>
<i>GLUT4</i>	<i>Glucose transporter 4</i>
<i>g/h</i>	<i>grams/hour</i>
<i>h</i>	<i>hour(s)</i>
<i>HbA1c</i>	<i>Glycated haemoglobin</i>

<i>IE</i>	<i>Isotopic enrichment</i>
<i>IHE</i>	<i>Intermittent high-intensity exercise</i>
<i>JDRF</i>	<i>Juvenile Diabetes Research Foundation</i>
<i>ISPAD</i>	<i>International Society for Pediatrics and Adolescent Diabetes</i>
<i>iv</i>	<i>Intravenous(ly)</i>
<i>LOPEH</i>	<i>Late onset post-exercise hypoglycaemia</i>
<i>NHMRC</i>	<i>National Health and Medical Research Council</i>
<i>M</i>	<i>Males</i>
<i>MDI</i>	<i>Multiple daily injections</i>
<i>min</i>	<i>minutes</i>
<i>R_a</i>	<i>Rate of glucose appearance</i>
<i>R_d</i>	<i>Rate of glucose disappearance</i>
<i>RER</i>	<i>Respiratory exchange ratio</i>
<i>RIA</i>	<i>Radioimmunoassay</i>
<i>sc</i>	<i>Subcutaneous(ly)</i>
<i>SE</i>	<i>Standard error</i>
<i>SG</i>	<i>Sensor glucose</i>
<i>TDD</i>	<i>Total daily dose</i>
<i>U</i>	<i>Units</i>
$\dot{V}O_2$	<i>Oxygen consumption rate</i>
$\dot{V}CO_2$	<i>Carbon dioxide production rate</i>
$\dot{V}O_{2\text{ peak}}$	<i>Peak oxygen consumption rate</i>
$\dot{V}O_{2\text{ max}}$	<i>Maximal oxygen consumption rate</i>

Publications arising from this thesis

1. Shetty VB, Davis EA. Effects of different types of exercise on blood glucose levels in type 1 diabetes. *Diabetes Management*, 47: 39-41, 2014 (Chapter 1).
2. Shetty VB, Fournier PA, Davey RJ, Retterath AJ, Paramalingam N, Roby HC, Cooper MN, Davis EA, Jones TW. Effects of exercise intensity on glucose requirements to maintain euglycaemia during exercise in type 1 diabetes. *Journal of Clinical Endocrinology and Metabolism*. 101(3): 972-980, 2016 (Chapter 4).
3. Shetty VB, Fournier PA, Davey RJ, Retterath AJ, Paramalingam N, Roby HC, Cooper MN, Davis EA, Jones TW. The time lag prior to the rise in glucose requirements to maintain stable glycaemia during moderate exercise in a fasted insulinaemic state is of short duration and unaffected by the level at which glycaemia is maintained in type 1 diabetes. *Diabetic Medicin*. 35(10):1404-1411, 2018 (Chapter 6).

Conference proceedings- National and International

1. Shetty VB, Fournier PA, Paramalingam N, Retterath AJ, Roby HC, Davey RJ, Davis EA, Jones TW. Glucose requirements for prevention of hypoglycaemia during exercise in individuals with Type 1 Diabetes Mellitus (T1DM). Young Investigator Award presentation, *Australian Paediatric Endocrine Group (APEG) Conference*, Sydney; July 2013
2. Shetty VB, Fournier PA, Paramalingam N, Retterath AJ, Roby HC, Davey RJ, Davis EA, Jones TW. Glucose requirements for prevention of hypoglycaemia during exercise in individuals with Type 1 Diabetes Mellitus (T1DM). *Royal Australasian College of Physicians (RACP) Annual Scientific Meeting*, Melbourne, 2013.

3. Shetty VB, Fournier PA, Davey RJ, Retterath AJ, Paramalingam N, Roby HC, Cooper MN, Davis EA, Jones TW. Glucose requirements for prevention of hypoglycaemia during exercise in individuals with Type 1 Diabetes Mellitus (T1DM). *European Association for the Study of Diabetes (EASD) Conference*, Barcelona; Sept 2013
4. Shetty VB, Fournier PA, Davey RJ, Retterath AJ, Paramalingam N, Roby HC, Cooper MN, Davis EA, Jones TW. Glucose requirements for prevention of hypoglycaemia during exercise in individuals with Type 1 Diabetes Mellitus (T1DM). *World Diabetes Conference*, Melbourne; Dec 2013
5. Shetty VB, Fournier PA, Davey RJ, Retterath AJ, Paramalingam N, Roby HC, Cooper MN, Davis EA, Jones TW. Effects of exercise intensity and ambient blood glucose levels on glucose requirements to maintain stable glycaemia during exercise in individuals with Type 1 Diabetes (T1D). Young Investigator Award presentation, *Combined Asia Pacific Paediatric Endocrine Society (APPES)- Australian Paediatric Endocrine Group Conference*, Darwin 2014.
6. Shetty VB, Fournier PA, Davey RJ, Retterath AJ, Paramalingam N, Roby HC, Cooper MN, Davis EA, Jones TW. Antidiuretic hormone as a mediator of the glycaemia-increasing effect of high intensity exercise in individuals with type 1 diabetes. Young Investigator Award presentation, *Combined International Society for Pediatric and Adolescent Diabetes (ISPAD)- Australian Paediatric Endocrine Group Conference (APEG) Conference*, Brisbane Oct 2015.
7. Shetty VB, Fournier PA, Paramalingam N, Roby HC, Soon W, Davis EA, Jones TW. Effects of exercise intensity on glucose requirements to maintain euglycaemia at high insulin levels in individuals with Type 1 Diabetes (T1D). Oral presentation, *Australian Paediatric Endocrine Group Conference*, Hobart; Nov 2017.

8. Shetty VB, Fournier PA, Paramalingam N, Roby HC, Soon W, Davis EA, Jones TW. Effects of exercise intensity on glucose requirements to maintain euglycaemia at high insulin levels in individuals with Type 1 Diabetes (T1D). Abstract accepted for oral presentation, *International Society of Paediatric and Adolescent Diabetes Conference*, Boston; Nov 2019.

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Chapter 1

Introduction

1.1 Overview of type 1 diabetes mellitus

1.1.1 Type 1 diabetes: aetiology and prevalence

Type 1 diabetes mellitus (T1D) is an autoimmune disease characterised by the progressive destruction of the insulin-producing β -cell of the pancreas, leading to an inability to secrete insulin (Association, American Diabetes. 2018). Since insulin promotes the storage of glucose as glycogen, fatty acids as triglycerides, and inhibits hepatic glucose and ketone body production, insulin deficiency leads to a marked activation of hepatic glycogenolysis, gluconeogenesis and ketone body production. The resulting hyperglycaemia causes polyuria, polydipsia, lethargy, polyphagia, all of which being common symptoms of insulin-untreated T1D (Association, American Diabetes. 2018). If left untreated, hyperglycaemia and the rise in blood ketone levels can lead to death by diabetic ketoacidosis (Balasubramanyam et al. 2008).

T1D affects 5 to 10% of the people who have diabetes worldwide. T1D accounts for more than 90% of childhood and adolescent cases of diabetes in most Western countries and less than half of people with T1D are diagnosed before the age of 15 years (Craig et al. 2009). T1D commonly develops during childhood and adolescence; however, it can develop at any time in life. The prevalence and incidence of T1D worldwide have been increasing during the last 20 years. The annual incidence rate for childhood T1D (0-14 years age group) varies between and within countries, and ranges from less than 1 per 100,000-person years to more than 60 per 100,000-person years (Bell et al. 2009; Patterson et al. 2009; 2006; Group 2006; Mayer-Davis et al. 2017). It also varies between different ethnic populations and age groups.

The highest incidence of T1D is in Finland, followed by Sweden (Group 2006; Berhan et al. 2011). Australia is ranked among the top 10 countries for T1D incidence globally (Craig et al. 2009), with an incidence of approximately 22 per 100,000 person years among 0–14-year-olds (Catanzariti et al. 2009), and with the highest incidence being in children aged 10-14 years (Australian Institute of Health and Welfare 2015). The annual rate of new

cases in Australia has remained stable over the last decade at around 10-13 per 100,000 person years (Australian Institute of Health and Welfare 2015). In particular, the incidence of childhood-onset T1D in Western Australia increased significantly from 11.3 per 100,000 in 1985 to 23.2 per 100,000 in 2002 (Haynes et al. 2004). Following this peak in 2003, the incidence of childhood T1D in Western Australia plateaued, with an annual incidence of 19.1/100,000 person years (Haynes et al. 2018).

1.1.2 Insulin therapy and management of type 1 diabetes

As yet, there is no cure for T1D, and this disease cannot be prevented. Fortunately, the discovery of insulin in 1921 has made it possible to treat T1D through the daily administration of insulin. Although insulin therapy has prolonged the life expectancy of people with T1D, a majority will develop complications resulting in increased morbidity and premature death (Livingstone et al. 2015; Secrest et al. 2010; Harjutsalo et al. 2011b; Laing et al. 2005). Microvascular pathologies including proliferative retinopathy and blindness, nephropathy, neuropathies and end-stage renal disease continue to be important sources of morbidity and premature mortality (Harjutsalo et al. 2011a; Klein et al. 2008; Pop-Busui et al. 2010; Abbott et al. 2011). In addition, T1D is associated with an increased risk of cardiovascular disease, coronary heart disease, stroke, and acute myocardial infarction (Orchard et al. 2006; Lind et al. 2011). Hence, compared to the general population, the lifespan of people with T1D is shorter by almost a decade (Dawson et al. 2008; Secrest et al. 2010a).

Although improved glycaemic control with newer insulins and intensified therapy has been shown to reduce the risk of micro and macrovascular complications (Wolfe et al. 1986; Nathan 2005; DCCT Research Group 1994; DCCT/EDIC Research Group 2005), patients with T1D with a glycated haemoglobin (HbA1c) level of $\leq 6.9\%$ have a risk of death from cardiovascular or other causes that is two times higher than that of matched non-diabetic individuals (Lind et al. 2014). Also, the findings from a large observational study in Sweden has shown that, independent of the duration of T1D, age at diagnosis was related to excess mortality and cardiovascular outcomes. Diagnosis of T1D before 10 years

of age resulted in men losing 14.2 life-years and women losing 17.7 life-years (Rawshani et al. 2018).

There is compelling evidence that chronic hyperglycaemia contributes to the aetiology of the aforementioned long-term complications by targeting vascular epithelial cells and neurons (Brownlee 2001; Kilpatrick et al. 2008). For this reason, the treatment of T1D has evolved since the discovery and implementation of insulin therapy, and now focuses on both maintaining tight blood glucose levels within close to physiological range and minimising glycaemic excursions in order to slow down or arrest the progression of long term complications (Orchard et al. 2015; Rewers et al. 2014b).

One important clinical tool that has enabled clinicians to evaluate the quality of the glycaemic control of their patients is the measurement of glycated haemoglobin (HbA1c) levels in blood. HbA1c level is so far considered a good marker for assessing long-term diabetes control since it reflects long-term average blood glucose levels (e.g. previous 2-3 months) (Saudek et al. 2006). This is because glucose can react and form an irreversible covalent bond with haemoglobin in red blood cells which have a life cycle of approximately 120 d. The haemoglobin is glycated slowly by glucose in a concentration-dependent manner. HbA1c levels thus reflect average blood glucose levels over the past 4–12 weeks, with more weightage toward the most recent 4 weeks, thus providing a measure of glycaemic control (Association, American Diabetes 2018; Rewers et al. 2014a; Saudek et al. 2006). The recommended target HbA1c level is <7.5% (Rewers et al. 2014b), with elevated HbA1c level being a predictor of long-term complications (DCCT Research Group 1993; 1994).

In order to achieve optimal glycaemic control, frequent blood glucose monitoring and insulin dose adjustment to carbohydrate intake and exercise are recommended. In this regard, it is noteworthy that Elliott P Joslin as early as the 1950s recognised the importance of insulin, diet and exercise in the management of T1D (Joslin 1953). The long-term benefits of such intensive insulin therapy are clearly shown by the results of the

Diabetes Control and Complications Trial (DCCT). This trial, performed in adults and adolescents with T1D, compared conventional management, consisting of administering one or two injections of insulin and one blood glucose level measurement per day, to intensive management with three or more insulin injections per day or continuous subcutaneous insulin infusion (CSII) along with measuring blood glucose levels \geq four times per day (DCCT Research Group 1993; 1994). There was clear evidence from the DCCT and other similar studies that intensive management in adults and adolescents is associated with better metabolic control, as indicated by a lower HbA1c level, and lesser and delayed microvascular complications (DCCT Research Group 1993; 1994; Nathan et al. 2009; White et al. 2001; Mohsin et al. 2005).

Currently, intensive diabetes management is delivered using either multiple (3 or more) daily subcutaneous injections of slow and rapid acting insulin or infusion of rapid-acting insulin with the help of a continuous subcutaneous insulin infusion (CSII) pump along with frequent self-monitoring of blood glucose (SMBG) levels using glucose meters or continuous glucose monitoring (CGM) systems (Rewers et al. 2014). Typically, SMBG using a personal blood glucose meter is performed, four to six times per day, most often before and after meals (DCCT Research Group 1993). Despite being a cornerstone of care in T1D, the use of glucose meters only gives a cross-sectional 'snapshot' of glucose levels. Hence, the undetected highs and lows in blood glucose levels, occurring between times of SMBG testing, can lead to incorrect decisions about insulin doses and carbohydrate consumption (Fiallo-Scharer 2005).

The limitations associated with the use of personal glucose meters are not shared by CGM systems which, via the continuous measurement of glucose levels in the interstitial fluid, provide estimates of blood glucose levels more frequently. These systems thus allow the detection of glycaemic excursions not identified with conventional monitoring. These CGM devices may be particularly beneficial for T1D individuals with hypoglycaemic unawareness as these devices have provision for alarms that are triggered at specified glucose levels or during a rapid decrease in glucose levels (Rewers et al. 2014). Finally,

these systems have the advantage of providing an estimate of immediate glycaemic control by documenting average glucose levels as well as frequency, magnitude and duration of hyperglycaemic and hypoglycaemic episodes. This allows implementation of strategies to avoid out-of-range glucose values, thereby improving glycaemic outcomes (Rewers et al. 2014; Rodbard 2017).

1.2 Exercise and type 1 diabetes

1.2.1 Hypoglycaemia risk associated with exercise in type 1 diabetes

Although advanced technological therapies have improved the quality of life, morbidity and mortality outcomes of people with T1D, the main obstacle to achieving better and optimal glycaemic control has been the increased risk of hypoglycaemia associated with intensive management. Indeed, meticulous blood glucose management is accompanied by an increased risk of hypoglycaemia due to both glycaemia being maintained close to hypoglycaemia range (DCCT Research Group 1987; 1991) and the absence of natural feedback mechanisms whereby plasma insulin levels decrease as blood glucose levels fall (Group 2017; Cryer et al. 2009). Hence even though ketones are preferentially used by the brain in the absence of glucose, this lack of feedback mechanism in T1D, results in iatrogenic hypoglycaemia.

Hypoglycaemia occurs when blood glucose falls below physiological levels (Abraham et al. 2018; Cryer et al. 2009). Although there is no scientific consensus on a single numerical definition of hypoglycaemia, hypoglycaemia has recently been operationally defined as a blood glucose level at or less than 3.0 mmol/L (Abraham et al. 2018; Group 2017). When people are able to treat their hypoglycaemia themselves, it is considered to be mild. When help from a third party is required for hypoglycaemia treatment, it is considered as severe (DCCT Research Group 1993). There are individual variations in the rate of severe hypoglycaemia, with night-time hypoglycaemia contributing to almost fifty percent of the episodes of severe hypoglycaemia (Allen et al. 2003). There are some individuals with T1D

that never experience severe hypoglycaemia, while there are others who experience it several times a year (DCCT Research Group 1997).

Since the brain can neither synthesise glucose nor store more than a few minutes supply of glycogen, normal brain function depends almost entirely on a continuous supply of glucose from the circulation (Nehlig 1997; Mergenthaler et al. 2013). This explains why even a brief episode of severe hypoglycaemia can cause seizures, coma, profound brain dysfunction, and irreversible brain damage (McAulay et al. 2001). For these reasons, severe hypoglycaemia is an important cause for morbidity (Secrest et al. 2011; Weston and Gill 1999; Sovik and Thordarson 1999; Nishimura et al. 2001). In addition, by causing injury or precipitating cardiac events, severe hypoglycaemia can be life threatening (Cryer 2010). This contributes to the fear of hypoglycaemia experienced by people with T1D and their families (Anderbro et al. 2010; Barnard et al. 2010).

One of the factors increasing considerably the risks of hypoglycaemia in T1D is exercise (Davis et al. 1997a; Admon et al. 2005; Wasserman 2002; Jensen and Richter 2012; Zinman et al. 1977; Berger et al. 1979). Despite the recent availability of new medicines, demonstrable technological progress, provision of intensive patient education and psychosocial support, the risk of hypoglycaemia associated with exercise remains an ongoing challenge. This increased risk occurs not only while exercising, but also for many hours following exercise (McMahon et al. 2007; Tsalikian et al. 2005), particularly in those in optimal glycaemic control (Hopkins 2004).

Although there are several barriers to exercise like inadequate time, access to facilities, lack of motivation, body image issues, and lack of knowledge regarding exercise management (Jabbour et al. 2016; Lascar et al. 2014), fear of hypoglycaemia is one of the greatest barrier to exercise in individuals with T1D (Brazeau et al. 2008) and optimal HbA1c (Pearson 2008). This increased risk and fear of hypoglycaemia associated with an active lifestyle is partly responsible for the reluctance of many individuals with T1D to participate in sports and games (Kemmer 1992). Hence, it is not surprising to find reports

of low levels of exercise (Thomas et al 2004; Valerio et al. 2007; Bohn et al. 2015), and lower than average fitness levels (Gusso et al. 2008; Komatsu et al. 2005) in people with T1D. Indeed, the recommended levels of exercise are met by only a small percentage (10%) of young adults with T1D (Alman et al. 2014; Raitakari et al. 2003; Webber et al. 1991). This is unfortunate given the many health benefits associated with regular exercise as described in the following section. In part for these reasons, hypoglycaemia avoidance is a major endpoint target in the management of T1D (DCCT Research Group 1993).

1.2.2 Benefits of exercise for individuals with type 1 diabetes

The avoidance or minimisation of exercise is generally adopted by many people with T1D as a means to minimise the risk of exercise-mediated hypoglycaemia. This is unfortunate because regular exercise is associated with several physiological and psychological health benefits in the general population. Indeed, regular exercise is a significant feature of a healthy, balanced lifestyle to prevent, delay, or decrease the risk of a various common chronic pathologies (Vina et al. 2012) not only in the general population of adults, but also in younger populations (Strong et al. 2005). More specifically, the benefits of a regular exercise impact on a number of domains including body composition, cardiovascular risk, mental health, academic performance, bone mineral status and general wellbeing to list a few (D'Hooge et al. 2011; Laaksonen et al. 2000).

The physiological and psychological health benefits derived from regular exercise in the general population extend also to individuals with T1D (Waden et al. 2008; Association, 2004; Bohn et al. 2015). The effect of exercise on blood glucose levels was recognised by Lawrence MD in the 1920s (Lawrence MD 1926) and the importance of regular exercise in the management of T1D was recognised as early as in the 1950s when Elliott P Joslin advocated exercise as the third essential component in the management of blood glucose levels for people with T1D (Joslin 1953). The benefits of exercise for these individuals include improvement in insulin sensitivity (Hawley 2004; Stallknecht et al. 2000), cardiovascular function (Admon et al. 2005; Wasserman and Zinman 1994; Fuchsjager-

Mayrl et al. 2002), and vascular health including skin microvascular reactivity (Roche et al. 2008) and endothelial function (Seeger et al. 2011). Other than providing protective effects against several cardiovascular risk factors, regular exercise improves blood lipid profiles (Laaksonen et al. 2000; Lehmann et al. 1997; Ostman et al. 2018) and weight control (Lehmann et al. 1997), and increases self-esteem and psychological wellbeing (Riddell 2006b), thereby helping in improving quality of life of individuals with T1D (Steppel and Horton 2003).

Although the benefits of regular exercise on improving cardiovascular (Nocon et al. 2008; Trigona et al. 2010) and other risk factors are well documented, its benefits on glycaemic control in T1D have been controversial (Younk et al. 2009). Some studies have found small improvements in HbA1c with an increase in exercise (Beraki et al. 2014; Herbst et al. 2006), whereas others have found no such association (Aman et al. 2009) or even a worsening of HbA1c (Komatsu et al. 2005; Delahanty et al. 2009). A recent meta-analysis by MacMillan and colleagues found a small improvement in HbA1c only with longer (>12 weeks) and more frequent (>3 sessions per week) exercise intervention programs in adolescents with T1D (MacMillan et al. 2014). Similarly, a meta-analysis conducted by Tonoli and colleagues based on 33 studies that met their inclusion criteria out of 937 studies showed that regular exercise has a significant but small effect on glycaemic control by reducing HbA1c by approximately 0.3% (Tonoli et al. 2012). Finally, another recent meta-analysis of eleven studies in youth with T1D concluded that exercise has an overall benefit in improving HbA1c by 0.52% (Quirk et al. 2014).

1.2.3 Recommendations to maintain stable glycaemia during exercise

There are profound changes in glucose homeostasis that occur during exercise in people with T1D. This makes the task of preventing hypoglycaemia and hyperglycaemia highly challenging, with hypoglycaemia being the most severe acute complication of exercise for these individuals (Brazeau et al. 2008). Current strategies to decrease the risk of hypoglycaemia during and after exercise include regular blood glucose monitoring (Robertson et al. 2014), insulin dose reduction (Kemmer 1992; Rabasa-Lhoret et al. 2001;

Schiffrin and Parikh 1985; Bracken et al. 2012b; De Feo et al. 2003; Campbell et al 2013), and carbohydrate (CHO) supplementation before, during and after exercise (Kemmer 1992; Dube et al. 2005, 2006; Riddell et al. 1999; Grimm et al. 2004). Other strategies include the incorporation of a single or multiple bouts of high-intensity exercise (Guelfi et al. 2005a, 2007b) to moderate intensity exercise as these patterns of physical activity are conducive to a lesser fall in blood glucose levels (Purdon et al. 1993; Sigal et al. 1999; Marliss and Vranic 2002).

Altering insulin dose before exercise does significantly reduce the risk of hypoglycaemia during exercise (DirecNet Study 2006; Dube et al. 2005; Grimm et al. 2004; Mauvais-Jarvis et al. 2003; Rabasa-Lhoret et al. 2001; Riddell et al. 1999; Sonnenberg et al 1990; Schiffrin and Parikh 1985; Moser et al. 2015). However, this approach is suitable only for planned exercise when insulin dose adjustments can be made in advance. Hence consuming extra CHO is often the only strategy available to reduce hypoglycaemia risk during and after unplanned exercise, with this strategy being also often adopted for planned exercise (Kemmer 1992; Dube et al. 2005, 2006; Riddell et al. 1999; Grimm et al. 2004). The recommendations from the NHMRC 2011 guidelines, the Society for the Paediatric and Adolescent Diabetes (ISPAD 2018) guidelines, the American Diabetes Association (ADA) 2016 guidelines, and Lancet guidelines (Riddell et al 2017) and other key references will be discussed in the following chapter (chapter 2).

1.3 Statement of problem

Based on the evidence currently available, a number of recommendations have been proposed to prevent exercise-mediated hypoglycaemia using pre-exercise CHO intake as a management tool. The current body of evidence indicates that the pre-exercise ingestion of 10 to 20 g (~0.25 g/kg per hour of exercise) of CHO provides an effective means to prevent hypoglycaemia for low- to moderate-intensity aerobic activities lasting 30 to 60 min and undertaken when circulating insulin levels are at near basal levels (Riddell and Milliken 2011; Colberg et al. 2016; Adolfsson et al. 2018). In contrast, the amount of CHO

required to prevent hypoglycaemia for exercise performed after a bolus of insulin injection depends on the dose and proximity of the last insulin injection (Francescato et al. 2004). When insulin is at peak or near peak level, which is within 0.5-1.5 hours of insulin administration, one should consume 1.0-1.5 g of CHO per kg of body weight per hour of exercise, an amount which correspond to that which elicit maximum CHO entry into the blood stream (Riddell and Iscoe 2006a). However, if exercise is performed several hours after the insulin bolus dose, recommended CHO intake falls to 0.3-0.5 g CHO/kg per hour of exercise (Robertson et al. 2014). Finally, at >5.5 hours post-insulin bolus, at which time plasma insulin is back to near basal levels, a CHO intake of about 0.2 g/ kg per hour of exercise is recommended (Francescato et al. 2004).

One limitation with these guidelines and the current body of knowledge upon which they are based, is the absence of evidence-based information in key areas of blood glucose management. In particular, the effect of exercise intensity on the exogenous CHO requirements to maintain stable or near stable blood glucose levels under basal or hyperinsulinaemic conditions are questions still without a clear answer. Also, how these CHO requirements are affected over time by the level at which blood glucose is maintained is also a question without a clear answer. As discussed below, it will be our primary aims to address these unanswered questions.

The rate of blood glucose utilisation and rate of fall in glycaemia increase with exercise intensity (Kemmer 1992; Rabasa-Lhoret et al. 2001). Hence one would predict that the CHO intake required to maintain stable blood glucose levels should also increase with exercise intensity. This prediction does not hold when intense exercise (>80% $\dot{V}O_2$ peak) is performed under basal insulin conditions since, blood glucose level increases under such conditions (Mitchell et al. 1988; Marliss and Vranic 2002; Purdon et al. 1993; Sigal et al. 1999), with no CHO intake being required to prevent hypoglycaemia during exercise (Riddell and Iscoe 2006a; Robertson et al. 2008, 2009; ADA 2007; Guelfi et al. 2007; Group 2005). On the basis that the required amount of CHO increases during low and moderate intensity exercise and that no CHO is required to prevent blood glucose levels from falling

during and immediately after high intensity aerobic exercise when plasma insulin is at near basal level, it follows that an inverted U relationship rather than a linear one must exist between exercise intensity and the amount of glucose that must be ingested to maintain stable glycaemia under near basal insulinaemic conditions (Fig. 1.1, plot A). This implies that there must be an exercise intensity where the risk of hypoglycaemia is maximal and beyond which this risk decreases with exercise intensity.

One important factor likely to affect the aforementioned predicted inverted U relationship is plasma insulin concentration. Since the rate of CHO intake required to prevent hypoglycaemia during moderate intensity exercise increases with insulin concentrations (Chokkalingam et al. 2007a; Francescato et al. 2004) and because elevated plasma insulin levels inhibit hepatic glucose production and enhance peripheral glucose uptake (Camacho et al. 2005; Chokkalingam et al. 2007a; Zinman et al. 1977), plasma insulin levels would be expected to affect the low-moderate intensity end of the inverted U relationship by increasing the glucose requirements associated with exercise of light or moderate intensity. However, since mild hyperinsulinaemia is without any marked effect on the increase in the rate of hepatic glucose production during intense exercise (Sigal et al. 1996), maybe little extra glucose administration is required to maintain euglycaemia when high intensity exercise is performed under hyperinsulinaemic conditions, thus implying that our predicted inverted U relationship is maintained even under these conditions (Fig 1.1, plot B). Surprisingly, this predicted inverted U relationship between exercise intensity and the CHO requirements to maintain stable glycaemia and the glucoregulatory mechanisms underlying this relationship have never been investigated.

The duration of exercise and the extent to which blood glucose is maintained above euglycaemic levels are some other factors expected to increase the amount of exogenous glucose required to maintain stable glycaemia during exercise performed under near basal insulinaemic conditions. In this regard, Jenni and colleagues have shown that more glucose is required to maintain blood glucose at high and stable levels (Jenni et al. 2008). They also showed that irrespective of the levels at which glycaemia is maintained, the

exogenous glucose requirements to maintain stable glycaemia are low early during moderate intensity exercise, but elevated 1.5-2.0 hours later (Jenni et al. 2008). This suggests that following the onset of exercise, there is a time period during which hypoglycaemia risk is reduced when exercise is performed under near basal insulinaemic conditions, thus making exercise safer. The temporal pattern of increase in glucose requirements with increasing duration of exercise has not been described so far.

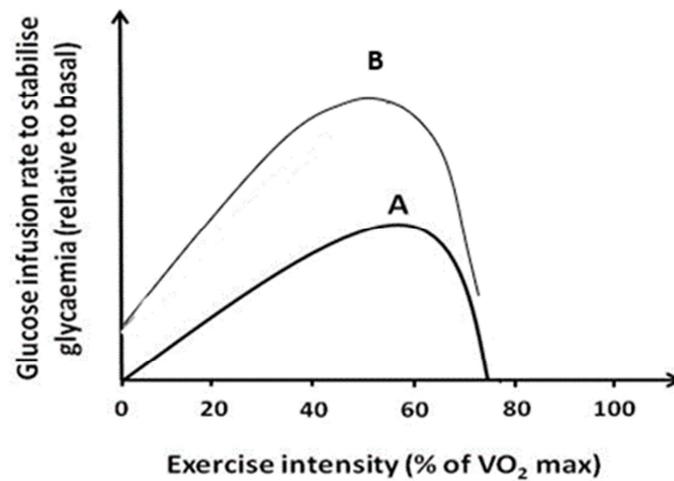


Figure 1.1: Predicted relationship between exercise intensity and glucose required to maintain stable blood glucose levels under (A) basal insulin and (B) high insulin conditions.

1.4 Aims, hypotheses and significance

1.4.1 General aim

Since the relationship between exercise intensity and the amount of CHO required to maintain near stable glycaemia under basal and hyperinsulinaemic conditions has never been systematically investigated, the first two aims of this thesis are to determine the relationship between exercise intensity and the intake of CHO required to maintain stable glycaemia under both basal and high insulin levels. Our next aims are to determine the extent to which maintaining blood glucose at high levels affect the exogenous glucose requirements to maintain stable glycaemia and the temporal pattern of change in exogenous glucose requirements.

More specifically, our aims are to test the following clinically important hypotheses in order to assist in the provision of more practical and useful guidelines for preventing exercise-mediated hypoglycaemia.

1.4.2 Research hypotheses

Hypothesis I: Under basal insulin levels, the amount of exogenous glucose required to maintain stable plasma glucose levels during and early after exercise follows an inverted U relationship with exercise intensity (Fig 1.1, plot A) rather than a linear one, with no exogenous glucose required during high intensity exercise. It is also predicted that during low/moderate intensity exercise, the amount of glucose required to stabilise blood glucose level increases due to a more rapid rise in endogenous rate of glucose utilisation relative to hepatic glucose production rate, but with the converse during high intensity exercise.

Hypothesis II: Under hyperinsulinaemic conditions, there is also an inverted U relationship between exercise intensity and the exogenous glucose requirements to maintain stable glycaemia, but with increased glucose administration being required when exercise is

performed at low and moderate intensities compared to rest with no or little extra glucose required at high intensity (Fig 1.1, plot B).

Hypothesis III: Under near basal insulinaemic conditions, there is a short time period during which the exogenous glucose requirement to maintain stable glycaemia is reduced after the start of moderate intensity exercise, with this low glucose demand period not being affected by the level at which glycaemia is maintained.

1.4.3 Specific aims

Specifically, this thesis aims to:

1. Investigate the relationship between exercise intensity and the rate of exogenous glucose infusion required to prevent blood glucose levels from falling during and early after exercise when plasma insulin is at near basal levels.
2. Elucidate the glucoregulatory mechanisms relating exercise intensity and glucose infusion rate.
3. Investigate the relationship between exercise intensity and the rate of exogenous glucose infusion required to prevent blood glucose levels from falling during and early after exercise performed under hyperinsulinaemic conditions.
4. Determine how long is the low exogenous glucose demands period following the start of moderate intensity exercise performed under basal insulinaemic conditions, and how the duration of this period is affected by the level at which glycaemia is maintained.

1.4.4 Significance

The significance of encouraging people with T1D to exercise regularly cannot be understated given the adverse effects of sedentary behaviour coupled with the

atherogenic nature of diabetes. Intensive management of T1D with the goal of achieving tight glycaemic control has become the core of current diabetes care. A strong and consistent message from consumer focus groups has been the lack of confidence young people with T1D have about exercising safely due to the increased risk of hypoglycaemia and hyperglycaemia associated with exercise. Given the many limitations with current guidelines, the information gained from this thesis should contribute to the body of evidence to inform guidelines for the prevention of exercise-mediated hypoglycaemia in individuals with T1D and help them exercise safely. These studies focus on young people with T1D, who are establishing lifetime habits of exercise and general T1D management. Hence this research, once published, may lead to the eventual translation of its findings to clinical practice, and directly impact clinical care and improve the wellbeing of people with T1D.

1.5 Organisation and structure of the thesis

This thesis is presented as an introduction and review of the literature (chapter 1 and 2) followed by a series of published/unpublished manuscripts, with two already published chapters (chapter 4 and 6). A third and fourth manuscripts (chapter 2 and 5) are being prepared for publication. Chapter 7 aims to integrate the findings of this thesis with current knowledge, and with directions for future research.

Chapter 1 of this thesis provides a background to understand the basis of the series of research questions to be addressed in this project. The gap in the literature particularly with respect to carbohydrate supplementation strategies during exercise in T1D was identified following a comprehensive review of the existing literature which is described in the chapter 2. The description of the experimental approaches adopted to conduct the studies in this research is described in chapter 3. Chapters 4, 5 and 6 describe the first, second and the third study undertaken in this project respectively.

Chapter 2

Literature review

Foreword

Regular blood glucose monitoring, carbohydrate (CHO) supplementation, and insulin dose adjustment pre- and post-exercise are generally advocated to better manage blood glucose levels during and after exercise. One of the main clinical challenges with using CHO supplementation to manage blood glucose level is one of ingesting enough CHO before, during and after exercise to prevent hypoglycaemia without causing excessive hyperglycaemia. This challenge is seldom met despite the marked glycaemia lowering effect of most types of exercise. It is the overconsumption of CHO in an effort to avoid hypoglycaemia that is likely responsible, at least in part, for the small impact regular exercise has on improving glycaemic control. Hence, optimising CHO intake to maintain near stable blood glucose levels during exercise is necessary if exercise is to contribute to optimal management of T1D.

There are gaps in the current body of knowledge upon which the guidelines for glycaemic management during exercise in T1D are recommended. This gap in the literature particularly with respect to CHO supplementation strategies during exercise in T1D was identified following a comprehensive review of the existing literature which is described in this chapter. Most of the evidence with regards to this key area comes from studies undertaken almost two decades ago, and it is not clear whether the findings from these earlier studies on CHO supplementation are relevant to current diabetes management around exercise. This chapter provides a comprehensive review of evidence existing for different strategies recommended for the management of blood glucose levels around exercise in T1D, with particular emphasis on CHO intake strategies. In order to understand the basis of current CHO supplementation recommendations, this chapter also provides a review of the physiological response to different types of exercise in individuals with and without T1D, various factors that affect the glycaemic response to exercise, as well as the current evidence for the different strategies recommended to prevent hypoglycaemia during and after exercise.

2.1 Physiological response to exercise in individuals without type 1 diabetes

2.1.1 Hormonal regulation of glucose metabolism during exercise

The regulation of hepatic glucose production during exercise is controlled by a number of circulating hormones including insulin, glucagon, catecholamines, cortisol, and growth hormone and by autonomic nerve impulses to the liver (Wahren et al. 1971; Hartley et al. 1972; Hartley 1975; Bak et al. 1991). The relative contributions of these regulatory mechanisms to the activation of hepatic glucose production depend on exercise duration and intensity.

The hormonal changes that causes an increase in hepatic glucose production during exercise include a fall in insulin levels (Wahren et al. 1971) and rises in the levels of glucagon, catecholamines, cortisol, and growth hormone (Hartley et al. 1972; Camacho et al. 2005; Marliss and Vranic 2002). The magnitude of these changes increases with exercise intensity (Felig et al. 1972) and duration (Felig et al. 1972; Ahlborg et al. 1974).

The decrease in insulin levels is mediated by the activation of the sympathetic nervous system, which via the α -adrenergic stimulation of the pancreatic beta cells inhibits insulin secretion (Wahren et al. 1971; Hermansen et al. 1970; Aarnio et al. 2001). This suppression of insulin secretion is more pronounced with a rise in exercise intensity due to the greater catecholamine-mediated inhibition of insulin secretion. Decreases in insulin levels are required for activation of glycogenolysis in the liver (Wasserman et al. 1989b), whereas an increase in glucagon levels, a potent activator of hepatic glucose production, is necessary for both increased hepatic glycogenolysis and gluconeogenesis (Wasserman et al. 1989c; Rivera et al. 2010).

The fall in plasma insulin levels is favourable to the activation of hepatic glucose production during moderate intensity exercise because insulin inhibits both hepatic glycogenolysis and gluconeogenesis, with a more potent effect on glycogenolysis (Edgerton et al. 2001; Petersen et al. 1998). Insulin also suppresses hepatic

gluconeogenesis indirectly by inhibiting lipolysis, thus decreasing the delivery and extraction of gluconeogenic precursors such as glycerol (Adkins et al. 2003; Sonksen and Sonksen 2000). A fall in insulin levels also acts indirectly by increasing the activation of hepatic glucose production by glucagon as the liver is more sensitive to the actions of glucagon when insulin levels are low (Lins et al. 1983).

Though most studies report significant changes in plasma insulin and glucagon levels during moderate intensity exercise, some studies report no change or just minor changes in the levels of these hormones, in particular glucagon levels (Wasserman et al. 1993, 2009). However, it is important to note that, ultimately, it is the insulin-to-glucagon ratio (Richter and Galbo 1986) and the portal levels of these hormones that determine their effects on hepatic glucose production (Wasserman et al. 1989c). And even a small increase in plasma glucagon levels can have a significant effect on hepatic glucose production since exercise enhances the potency of a given glucagon level at stimulating hepatic glucose production (Lins et al. 1983; Wasserman et al. 1989c, 2009). Of note, the minimal changes in glucagon levels often measured in blood during exercise are due to the hepatic extraction of glucagon which leads to an underestimation of the physiological importance of glucagon in blood glucose regulation (Wasserman et al. 1993, 2009).

Since the rise in plasma glucagon levels can account for only about 60% of total splanchnic glucose output under certain conditions of submaximal intensity exercise, this indicates that other factors, such as catecholamines, must stimulate hepatic glucose production (Wasserman 1995; Hargreaves and Spriet 2006). The role catecholamines plays in the stimulation of hepatic glucose production during mild to moderate intensity exercise has been the object of some controversies (Wasserman et al. 1990; Coker et al. 2005).

A role for an adrenergic-mediated stimulation of hepatic glucose during moderate intensity exercise is challenged by the observations that hepatic glucose production is not reduced when exercise is performed together with combined alpha and beta adrenergic blockade in dogs (Coker et al. 1997b) and humans (Marker et al. 1991), and a normal

increase in hepatic glucose output is observed during moderate exercise in adrenalectomized humans (Howlett et al. 1999). Also, direct adrenergic stimulation at physiological doses has little effect on the rate of hepatic glucose production during moderate intensity exercise in dogs even in the absence of changes in plasma glucagon and insulin levels (Coker et al. 2002). However, a role for catecholamines is supported by the observation that both epinephrine and norepinephrine can increase hepatic glucose output by enhancing hepatic glycogenolysis, with epinephrine being significantly more potent than norepinephrine at stimulating hepatic glucose output (Connolly et al. 1991). Catecholamines can also act indirectly by stimulating lipolysis which leads to increased levels of circulating gluconeogenic precursors like glycerol (Issekutz 1978; Wahrenberg et al. 1987; Wasserman, Lacy, et al. 1989).

In response to high intensity aerobic exercise (>80% $\dot{V}O_2$ peak), the stimulation of hepatic glucose production and associated rise in plasma glucose levels are mediated by catecholamines rather than changes in insulin-glucagon ratio. This is supported indirectly by both the rapid and marked elevation in circulating plasma catecholamine levels (Calles et al. 1983; Marliss et al. 1992, 1991; Purdon et al. 1993), with plasma norepinephrine increasing 18 fold and epinephrine 14 fold, and the close relationship between the rise in hepatic glucose production during this type of exercise and increases in catecholamines levels (Purdon et al. 1993; Sigal et al. 1996; Manzon et al. 1998) as opposed to the absence of change in circulating insulin-to-glucagon ratio (Sigal et al. 1996; Marliss and Vranic 2002). Also, when epinephrine and norepinephrine are infused during moderate intensity exercise so as to reproduce the pattern of catecholamine response observed during intense aerobic exercise, hepatic glucose production is activated to an extent comparable to that which occurs during intense exercise (Kreisman et al. 2003). In addition, epinephrine also inhibits insulin-stimulated glucose uptake especially in the skeletal muscle (Han and Bonen 1998), thus contributing further to the rise in blood glucose levels associated with high intensity exercise. Of note, however, a role for catecholamines as the primary mediator of the activation of hepatic glucose production during high intensity aerobic exercise is challenged by the work of Cocker and colleagues

who reported that under conditions where glucagon, insulin, and plasma glucose levels are matched, net hepatic glucose output responses to heavy exercise is unaffected by both hepatic adrenergic receptor blockade (Coker et al. 1997a, 1997b) and attenuation of sympathetic nerve stimulation of the liver and adrenal medulla (Kjaer et al. 1993, 1995).

During recovery from high intensity aerobic exercise, plasma catecholamines return rapidly to pre-exercise levels. This period is also associated with a hyperglycaemia-mediated transient increase in plasma insulin levels, with this rise in insulin concentrations being favourable to the fall and normalisation of blood glucose levels as recovery progresses (Wasserman et al. 1989a; Calles et al. 1983; Yale et al. 1989; Marliss et al. 1992). It has been proposed that the hyperglycaemia and hyperinsulinaemia occurring post-exercise are favourable to the replenishment of the muscle glycogen stores mobilised during exercise (Marliss et al. 1992; Marliss and Vranic 2002).

The other hormones involved in regulation of glucose production during and after exercise include growth hormone (GH) and cortisol. These hormones appear to play little or no role in the regulation of glucose homeostasis during short-duration exercise of moderate or high intensity, although GH and cortisol concentrations during moderate-intensity exercise can increase by up to tenfold and twofold, respectively (Tsintzas et al. 1995, 1996). However, as the duration of exercise increases, high GH and cortisol levels stimulate the release of free fatty acids and glycerol into the circulation by stimulating whole body lipolysis, thus increasing indirectly hepatic gluconeogenesis (Hartley 1975; Bak et al 1991).

Of note, although the role catecholamines plays in the stimulation of hepatic glucose production during exercise of different intensities has been the object of some controversies, it is well established that these hormones play an important role in the activation of both hepatic gluconeogenesis and glycogenolysis under resting conditions. For instance, when epinephrine is infused for 90 min in resting individuals under conditions of basal circulating insulin and glucagon concentrations, this results in a

biphasic increase in hepatic glucose production. During the first hour of epinephrine infusion, hepatic glycogenolysis accounts for most of the increase in glucose production, whereas the rate of hepatic glucose production decreases later on, with a 2.5-fold increase in hepatic gluconeogenesis accounting for 80% of glucose production (Dufour et al. 2009).

2.1.2 Counterregulatory responses to hypoglycaemia during exercise

During exercise in healthy individuals without diabetes, blood glucose levels are normally maintained within a narrow physiological range. When aerobic exercise is prolonged, the glucose taken up from the circulation by the working skeletal muscles is replaced by the glucose arising from hepatic glycogen breakdown and gluconeogenesis, thereby preventing hypoglycaemia (Camacho et al. 2005). However, during prolonged exercise performed in the fasted state and continued to the point of fatigue, blood glucose levels may fall as a result of the depletion of liver glycogen stores, causing a mismatch between hepatic glucose production and muscle glucose utilisation rates.

Changes in blood glucose levels and hypoglycaemia are detected by a number of highly specialised regions in the body including the beta cells of the pancreas, ventromedial hypothalamus, and glucose sensing neurons in the mouth, gut, portal/mesenteric vein and carotid body (McCrimmon 2009; Watts and Donovan 2010). A fall in blood glucose levels triggers complex but highly effective physiological mechanisms known as counterregulatory responses that aim to reverse falling blood glucose levels and restore euglycaemia (Kronenberg et al. 2008). Factors that determine the magnitude of such counterregulatory responses are the depth and duration of hypoglycaemia as well as age, gender, rate of decline in blood glucose levels before the onset of hypoglycaemia, antecedent exercise (Galassetti et al. 2001b; Sprague and Arbelaez 2011; Mitrakou et al. 1993), and antecedent hypoglycaemia (Davis et al. 1997b, 2000).

In response to a fall in blood glucose concentration in resting individuals, there is a series of counterregulatory responses that occur to elevate blood glucose levels within normal

range or prevent a further decrease in blood glucose level. When blood glucose levels fall to ~4.4 mmol/l under resting conditions, there is a reduction in insulin secretion (Fanelli et al. 1994; Cryer et al. 2003) that contributes to decrease peripheral glucose disposal rate and activate hepatic glucose production (Bolli and Fanelli 1999). At blood levels of ~3.8–3.9 mmol/l, there is a marked rise in glucagon and epinephrine levels (Felig et al. 1972), and at ~3.6–3.7 mmol/l, norepinephrine and growth hormone levels also experience a rise. When blood glucose levels fall to ~3.0 mmol/l, cortisol secretion is increased. At ~3.2 mmol/l autonomic symptoms develop, and at glucose levels between 2.8 and 3.0 mmol/l cognitive deterioration occurs (Mitrakou et al. 1991; Heller and Macdonald 1996).

During exercise, insulin, glucagon, and catecholamines levels also respond in a hierarchical fashion to activate hepatic glucose production and prevent exercise-induced hypoglycaemia. When hypoglycaemia occurs during exercise in individuals without diabetes, the normal counterregulatory hormone (catecholamines, glucagon, cortisol, and GH) response to exercise is amplified (Sotsky et al. 1989). This stimulatory effect of exercise contributes to increasing hepatic glucose output and limiting peripheral glucose utilisation, altogether opposing hypoglycaemia during exercise (Sotsky et al. 1989).

It is important to note that the counterregulatory response to exercise or hypoglycaemia described above is blunted by prior exercise or hypoglycaemia in healthy individuals without diabetes. Indeed, the counterregulatory hormone response to exercise is blunted by pre-exercise hypoglycaemia (Davis et al. 2000) and the counterregulatory hormone response to hypoglycaemia is blunted by prior exercise (Galassetti et al. 2001b). Almost a 50% reduction in the counterregulatory hormone response to moderate intensity exercise is found in healthy individuals exposed to two prior 120-min episodes of moderate hypoglycaemia (~2.9 mmol/l) the previous day (Davis et al. 2000). A similar reduction in the counterregulatory hormone response to a 2-h bout of moderate hypoglycaemia (~3.0 mmol/l) also occurs when preceded the day before by two 90-min bouts of moderate intensity exercise separated by a 3-h rest (Galassetti et al. 2001b). The similarity between these two responses suggests a common underlying mechanism which has been the

object of much research effort (Galassetti et al. 2001b; Davis et al. 1997b; Veneman et al. 1994).

2.2 Physiological response to exercise in individuals with type 1 diabetes

2.2.1 Factors affecting blood glucose level response to exercise: overview

In T1D, the pattern of blood glucose response to physical activity is generally difficult to predict as exercise may cause hypoglycaemia, hyperglycaemia or even no change in blood glucose levels. This poor predictability is due to the complexity of managing exogenous insulin administration in a physiological manner so as to compensate for the absence of feedback loop to control plasma insulin levels during exercise (Randle et al. 1963). As a result, a number of factors have the potential to affect the glycaemic response to exercise.

Factors affecting the glycaemic response to exercise include- the intensity and duration of exercise, the type of exercise, the levels of circulating “on board” insulin during and after exercise, the type of insulin, the nutritional status (pre- or post-prandial), the time of the day when exercise is performed, blood glucose level, age, sex, fitness and stress levels of the individuals, insulin sensitivity, the prevailing concentrations of the counter regulatory hormones, and the availability of both supplemental and stored CHO (Bally et al. 2016b; Rabasa-Lhoret et al. 2001; Yardley et al. 2018; Eshghi et al. 2019). Of note, the glycaemic response to exercise varies considerably between individuals even after controlling for all of the above factors (Riddell, et al. 2017; Temple et al. 1995).

In the following subsections, we will examine how the intensity of exercise and insulinaemic state affect blood glucose levels because these variables are the main factors affecting the glycaemic response to exercise. A particular emphasis will be placed on hypoglycaemia risk associated with exercise as this is a widely recognised risk for individuals with T1D. Other factors known to affect blood glucose response to exercise will also be briefly described.

2.2.1.1 Aerobic exercise of submaximal intensity

The measurements of maximum rate of oxygen consumption ($\dot{V}O_2$ max) and lactate threshold (LT) have been used to define relative exercise intensity. At low to moderate exercise intensity, lactate production and clearance are matched, and blood lactate levels do not increase significantly. However, as exercise intensity increases, there is an intensity, defined as lactate threshold or anaerobic threshold (Davis et al. 1983), at which blood lactate level begins to increase. Although exercise intensity is generally expressed relative to $\dot{V}O_2$ max or $\dot{V}O_2$ peak (Goodman et al. 1988), exercise intensity is also expressed relative to lactate threshold (Katch et al. 1978), with high intensity aerobic exercise corresponding to exercise intensity above lactate threshold.

Aerobic exercise can be maintained for prolonged periods through oxidative metabolism of various fuel sources including CHOs, fats and some protein. Although the mix of fuel utilisation during exercise in individuals with T1D is similar to that of healthy individuals (Krzentowski et al. 1981; Ramires et al. 1997; Riddell et al. 2000; Francescato et al. 2004; Robitaille et al. 2007), some but not all studies have reported that exercise is associated with an increased reliance on fat compared to CHO oxidation in individuals with T1D (Riddell et al. 2000; Raguso et al. 1995). There is some evidence that plasma glucose oxidation rates may be lower and muscle glycogen utilisation rates higher during prolonged exercise in individuals with T1D than in individuals without diabetes (Chokkalingam et al. 2007b), but the impact this may have on blood glucose levels remains to be investigated.

Aerobic activities of submaximal intensity (<80% $\dot{V}O_2$ peak) have a small or no effect on blood glucose levels when performed under basal or near basal insulin conditions in individuals with T1D (Martin et al. 1982), thus making blood glucose level response to exercise generally predictable. In support of this view, Nathan and colleagues (1985) found that individuals with T1D who exercise for 45 min on a cycle ergometer at moderate intensity after a 10 hour overnight fast and while in a basal insulinaemic state, experienced stable plasma glucose levels during exercise followed by a progressive fall in

blood glucose levels later after exercise (Nathan et al. 1985). These findings were subsequently corroborated by the work of Soo and colleagues (1996; Table 1), who found that 45 min of moderate intensity exercise at 60% $\dot{V}O_2$ peak while under basal insulin conditions was associated with only a small fall in blood glucose levels during exercise.

The prevailing insulin level is an important factor that determines the glycaemic response to moderate intensity exercise (Campbell et al. 2014a; Dube et al. 2006), with plasma insulin levels being related to the insulin dose and the timing of exercise relative to the last insulin bolus administration. There is potential for a marked fall in blood glucose level when exercise is performed within few hours after a meal-time insulin bolus, particularly if not enough CHO are ingested (Francescato et al. 2004). However, this fall in blood glucose levels may not necessarily result in hypoglycaemia since post-meal glucose levels tend to be higher than pre-meal glucose levels (Mallad et al. 2015).

When moderate intensity exercise is performed under hyperinsulinaemic conditions, both muscle contraction and insulin stimulate muscle glucose utilisation (Guelfi et al. 2007b). This increased glucose utilisation in combination with insulin-mediated inhibition of hepatic glucose production (Guelfi et al. 2007b) increases the rate of fall in blood glucose level both during and after exercise (Guelfi et al. 2005a, 2007a, 2005a; West et al. 2011b; Bussau et al. 2007). In addition, deficiencies in the release of counterregulatory hormones during exercise may also impair hepatic glucose production (Camacho et al. 2005; Adolfsson et al. 2012; Sprague and Arbeláez 2011; Tsalikian et al. 2009). The resulting elevated risk of hypoglycaemia during exercise is increased further when exercise intensity is moderate/vigorous or when exercise duration is prolonged since glucose uptake into skeletal muscle increases with the intensity of muscle contraction and with exercise duration (Kemmer and Berger 1983; Rabasa-Lhoret et al. 2001; van Loon et al. 2003; Richter and Hargreaves 2013).

In practise, the hypoglycaemic risk associated with exercise is difficult to predict due to the challenge of evaluating one's insulinaemic state which can vary markedly over time

following a bolus insulin injection (Francescato et al. 2004). The risk of hypoglycaemia increases as soon as within about 45 min of starting aerobic exercise (Tansey et al. 2006; Riddell et al. 1999; Garcia-Garcia et al. 2015). Altogether, these factors explain why hyperinsulinised individuals with T1D are at increased risk of hypoglycaemia during and after exercise unless a source of exogenous CHO is provided (DCCT Research Group 1994; Admon et al. 2005; Ruderman et al. 2002; Guelfi et al. 2007a; Mallad et al. 2015; Francescato et al. 2004). In this respect, however, excessive pre-emptive consumption of CHO made before exercise can result in severe hyperglycaemia (Campbell et al. 2014a).

Of note, moderate intensity exercise has the potential to increase blood glucose levels and cause ketoacidosis if performed by under-insulinised individuals with T1D who either do not give themselves enough insulin or omit their insulin doses (Taplin et al. 2010). Indeed, although basal insulin rate suspension during exercise can reduce the rate of fall in glycaemia during exercise for those on insulin pump therapy (Tsalikian et al. 2006), aggressive reductions in insulin administration or a skipped insulin dose can cause hyperglycaemia before and during aerobic exercise (Taplin et al. 2010). This is because a low plasma insulin level in itself increases hepatic glucose production rates and decreases exercise-mediated muscle glucose utilisation (Ramires et al. 1997; Wahren et al. 1975). In addition, insulin deficiency promotes lipolysis and increases hepatic ketogenesis to an extent that may be conducive to ketoacidosis (Wahren et al. 1984). These effects of low plasma insulin levels are further exacerbated by the sensitising effect low insulin has on the activation of hepatic gluconeogenesis and ketogenesis by glucagon, thus increasing further the risk of severe hyperglycaemia and ketoacidosis (Lins et al. 1983).

2.2.1.2 High intensity aerobic exercise and maximal sprint effort

High intensity aerobic exercise corresponds to aerobic exercise at an intensity above lactate threshold. The pattern of blood glucose response to either high intensity aerobic exercise or a maximal sprint effort performed under basal insulin conditions in individuals with T1D is markedly different from that of moderate intensity exercise in that it is

typically associated with an increase in blood glucose levels. This has been thoroughly investigated for over more than 10 years by the team of Marliss and Vranic (Mitchell et al. 1988; Marliss et al. 1992; Marliss and Vranic 2002; Purdon et al. 1993; Sigal et al. 1994a; 1994b; 1996; 1999). They showed that ten to fifteen min of high-intensity aerobic exercise (>80% of maximal rate of oxygen consumption) performed under basal insulinaemic conditions causes a rise in blood glucose levels that remain elevated during recovery in individuals with and without T1D (Marliss and Vranic 2002; Purdon et al. 1993; Sigal et al. 1994; 1996). They also provided evidence that this exercise-mediated rise in blood glucose level is mediated by a large increase in plasma catecholamine levels resulting in a disproportionate activation of endogenous glucose production rates relative to the increase in peripheral glucose utilisation rates (Tran and Galassetti 2014; Mitchell et al. 1988; Marliss and Vranic 2002; Purdon et al. 1993; Sigal et al. 1999), but not all studies support such a role for catecholamines (Coker and Kjaer 2005).

During recovery from high intensity aerobic exercise, blood glucose levels remain elevated for at least 2 hours in individuals with T1D (Marliss and Vranic 2002). This is in marked contrast to individuals without T1D in whom hyperglycaemia post-exercise is short-lasting as a result of being opposed by an increase in insulin secretion (Marliss and Vranic 2002). That insulin deficiency might explain the sustained post-exercise rise in blood glucose level in individuals with T1D is supported by the work of Purdon and colleagues who showed that the markedly increased glucose production rate and associated hyperglycaemic state post-exercise can be normalised more rapidly to pre-exercise levels by the injection of insulin (Sigal et al. 1994b). Overall, these findings thus support the view that the marked increase in glucose rate of appearance (Ra) during exercise and the rapid decrease of Ra during recovery are probably regulated by catecholamines, whereas post-exercise hyperinsulinemia is required to return plasma glucose to pre-exercise levels (Purdon et al. 1993).

A short duration (~10 sec) bout of a maximal sprint effort performed under basal insulinaemic conditions can also cause a rise (1-2 mmol/L) in blood glucose concentrations

that can be sustained for a few hours after exercise (Davey et al. 2014; Fahey et al. 2012). However, the mechanism underlying this rise differs from that associated with high intensity aerobic exercise in that the increase in blood glucose levels after a short maximal sprint effort results primarily from a transient exercise-mediated fall in peripheral glucose utilisation rates (Fahey et al. 2012). Rapid intramuscular glycogenolysis during this type of exercise has been proposed to inhibit muscle glucose utilisation rate via a glucose 6-phosphate-mediated inhibition of hexokinase (Wasserman 1995).

Since a short maximal sprint effort performed under basal insulin conditions has the capacity to increase blood glucose levels, the effect of combining a short sprint with moderate intensity exercise on blood glucose levels was examined in moderately hyperinsulinaemic individuals with T1D. It was found that performing a 10-second sprint immediately before or following moderate-intensity exercise results in a lesser rate of fall in blood glucose level during recovery than only resting post-exercise (Bussau et al. 2006, 2007). Additionally, the rate of fall in blood glucose levels under mildly hypersulinaemic conditions is not as rapid if continuous moderate intensity exercise is interspersed with several short 4-sec sprints (Guelfi et al. 2005a, 2005b, 2007a, 2007b). These findings have been explained on the basis that the metabolic and hormonal responses to sprinting, such as elevated lactate, catecholamine and growth hormone levels, are conducive to opposing a fall in blood glucose levels, with intermittent sprinting resulting in a more favourable balance between glucose rate of appearance and disposal during exercise and recovery (Guelfi et al. 2007b).

It remains to be determined if high intensity aerobic exercise or maximal sprint effort performed under hyperinsulinaemic conditions is also conducive to a post-exercise lesser fall in blood glucose levels. Since insulin and muscle contraction have an additive effect on muscle glucose utilisation rates (Goodyear and Kahn 1998) and given the inhibitory effect of insulin on hepatic glucose production during exercise (Camacho et al. 2005; Chokkalingam et al. 2007a; Zinman et al. 1977), one would expect the glycaemia rising

effect of high intensity exercise to be blunted under hyperinsulinaemic conditions and for high intensity exercise to be less effective at opposing a lesser fall in blood glucose levels.

2.2.1.3 Intermittent high-intensity exercise and resistance exercise

As mentioned above, intermittent high-intensity exercise typical of team sports is associated with a lesser rate of fall in blood glucose levels compared to moderate intensity exercise (Guelfi et al. 2005a), with a marked fall taking place if this exercise type is prolonged (Campbell et al. 2015a; Adolfsson et al. 2012).

Resistance exercise also results in more stable glucose levels both during and after exercise than continuous moderate intensity aerobic exercise (Turner et al. 2015, 2016a; Yardley et al. 2012b; 2013c), with resistance exercise even causing a modest rise in glycaemia in some individuals under fasting conditions (Turner et al. 2015). Of note, hypoglycaemia risk may be increased after this type of exercise due to depleted glycogen stores (Yardley et al. 2012b). Resistance exercise also tends to attenuate the decrease in glycaemia when done before aerobic exercise (Yardley et al. 2012a). These benefits of resistance exercise are possibly due to the increased concentrations of counter regulatory hormones which stimulate gluconeogenesis and inhibit muscle glucose uptake (Bally et al. 2016b).

2.2.1.4 Other factors affecting blood glucose response to exercise

The glycaemic response to exercise and associated risk of hypoglycaemia also varies depending on the time of day when exercise is performed since the prevailing insulin levels change throughout the day depending on meal times (Eshghi et al. 2019). The risk of hypoglycaemia is minimal when exercise is done early in the morning before breakfast while basal insulin conditions are prevailing (Nathan et al. 1985; Martin et al. 1982) and higher when exercise is performed soon after meals (Francescato et al. 2004). The risk of overnight and next day hypoglycaemia increases when mild to moderate activity is performed in the afternoon or evening (Metcalf et al. 2014; Tsalikian et al. 2005).

The prevailing blood glucose level at the start of exercise is another factor that affects the risk of hypoglycaemia (Riddell et al. 1999). The risk of hypoglycaemia increases when blood glucose levels are <6.7 mmol/L prior to exercise (Tansey et al. 2006; Riddell et al. 1999), but is lower when blood glucose levels at the start of exercise are >8.3 mmol/L and plasma insulin levels not elevated (Tansey et al. 2006). Accordingly, the recommendation for a reasonably safe starting blood glucose level prior to performing aerobic exercise lasting up to an hour ranges between 7–10 mmol/L for conditions where plasma insulin levels are not excessive (Riddell et al. 2017). Of note, when blood glucose levels prior to exercise are excessively high because of insulin omission, further increases in blood glucose levels are likely to occur during exercise (Berger et al. 1977).

The glycaemic response to exercise may vary depending on fitness level (Al Khalifah et al. 2016). Greater fall in blood glucose levels during aerobic exercise have been reported in trained individuals with T1D compared to individuals with reduced physical fitness (Al Khalifah et al. 2016). Of note, some of these differences may be due to the higher work rate in trained individuals rather than the result of their higher aerobic fitness levels *per se* (Riddell et al. 2017). There are data to suggest that trained individuals experience lower glycaemic variability than those who are aerobically unconditioned (Singhvi et al. 2014).

There is evidence that the glycaemic response to exercise also varies depending on the stress levels experienced during exercise. Psychological stress of competition may cause a rise in blood glucose levels even when pre-exercise blood glucose levels are normal (Riddell et al. 2006b). Activities involving vigorous aerobic exercise that cause hypoglycaemia on regular training or practice days may cause hyperglycaemia on the day of the competition (Riddell et al. 2006b). It has been proposed that this response may be due to a high anticipatory stress just prior to exercise, increasing the counterregulatory hormones levels which in turn may cause hyperglycaemia by increasing hepatic glucose production.

Finally, the availability of both supplemental and stored CHO can influence the glycaemic response during and after exercise. When insulin adjustments are not done prior to exercise, lack of CHO supplementation during aerobic exercise can cause a fall in blood glucose concentrations (Mallad et al. 2015). On the other hand, CHO overconsumption for fear of hypoglycaemia during exercise can result in high glucose levels (Fahey et al. 2012). Poor glycaemic control can reduce the capacity to mobilise liver glycogen stores during exercise and can result in hypoglycaemia (Urban et al. 2013; Bischof et al. 2001).

2.2.2 Counterregulatory responses to exercise in type 1 diabetes

As discussed earlier, an increase in glucagon and a fall in insulin concentrations in the portal vein help maintain blood glucose homeostasis during exercise in individuals without T1D. Such a decrease in insulin levels also helps to sensitize the liver to increased glucagon concentrations, increasing further hepatic glucose production rate (Camacho et al. 2005). In T1D, hepatic glucose production is impaired because the insulin in the portal circulation does not fall during exercise (Camacho et al. 2005), and the inability to reduce exogenous insulin levels during exercise is the key factor increasing the risk of exercise-mediated hypoglycaemia as the levels of insulin are fixed and cannot fall during exercise (Zinman et al. 1977). In fact, due to increased subcutaneous blood flow favourable to accelerated absorption of injected/infused insulin and reduced insulin disposal during exercise (Marliss and Vranic 2002; Sigal, et al. 1994b), the insulin levels may even rise significantly (Mallad et al. 2015), thus increasing the risk of hyperinsulinaemia and hypoglycaemia (Berger et al. 1979; Frid et al. 1990). Indeed, insulin levels during aerobic exercise in individuals with T1D can be two- to threefold above those in people without diabetes (Riddell et al. 2000; Zinman et al. 1977).

There is evidence that an impaired counterregulatory response to both exercise and hypoglycaemia may contribute to the risk of hypoglycaemia associated with exercise in these individuals (Adolfsson et al. 2012; Sprague and Arbeláez 2011; Tsalikian et al. 2009). This blunted response to hypoglycaemia is present even when individuals with T1D maintain good glycaemic control (Tsalikian et al. 2009). In particular, the effect of

glucagon on hepatic glucose production is hampered by high insulin levels, thereby leading to decline in blood glucose levels.

Other factors contributing to the hypoglycaemia risk during exercise in patients with T1D include the loss of glucagon response to developing hypoglycaemia (Gerich et al. 1973), impaired stimulation of hepatic glucose output in response to glucagon secretion (Orskov et al. 1991), impaired adrenergic responses to exercise when hypoglycaemic (Schneider et al. 1991), low level of hepatic glycogen content in poorly controlled T1D (Cline et al. 1994) and/or reduced gluconeogenesis and/or increased peripheral glucose disposal due to unopposed hyperinsulinemia (Chokkalingam et al. 2007b). Finally, individuals with T1D with intact glucagon response to exercise (Schneider et al. 1991) develop deficient glucagon response during exercise if preceded several hours earlier by some hypoglycaemic episodes (Galassetti et al. 2003). In addition to the glucagon response affected this way, the counterregulatory responses of other hormones to hypoglycaemia are also impaired in a similar way with a prior recent episode of either exercise or hypoglycaemia (Davis et al. 2014).

Although hypoglycaemia is generally associated with a rise in cortisol, growth hormone and norepinephrine levels in children with T1D exposed to moderate intensity exercise, this counterregulatory response has been shown to be insufficient to increase or maintain stable glucose levels (Admon et al. 2005). In adolescents and young adults experiencing hypoglycaemia during moderate intensity exercise, norepinephrine and growth hormone levels show a significant rise, but with no change in cortisol and glucagon levels, suggesting the impaired responses of these latter hormones might have contributed to hypoglycaemia (Tansey et al. 2006). Of note, the counterregulatory responses to high intensity aerobic exercise (80% $\dot{V}O_2$ peak) in 11-15 year-old individuals with T1D is such that the glucagon response is impaired (Galassetti et al. 2006b), but given the evidence that the glycaemia rising effect of this type of exercise is mediated by catecholamines (Marliss and Vranic 2002; Purdon et al. 1993; Sigal et al. 1999; Kreisman et al. 2003), the significance of this finding remains to be determined.

2.2.3 Effect of exercise on the risk of early and late onset post-exercise hypoglycaemia (LOPEH)

Moderate-to-vigorous activity can increase the risk of hypoglycaemia not only during exercise, but also early after exercise as well as the following evening and even the next day, with such a risk increasing further with increasing the volume of exercise (Harmer et al. 2007). This risk of late onset post-exercise hypoglycaemia (LOPEH) is also greater with late-day exercise as compared to morning exercise (Association, American Diabetes 2015).

Many factors contribute to LOPEH including impaired counterregulatory response to hypoglycaemia (MacDonald 1987), increased glucose disposal by skeletal muscles to restore glycogen stores, and heightened insulin sensitivity post-exercise (MacDonald 1987; Peirce 1999; Tamborlane 2007). Following afternoon or early evening exercise, a biphasic pattern of change in insulin sensitivity is observed with heightened insulin sensitivity during and early after exercise and again 7–11 hours later during sleep (Yardley et al. 2012b; McMahon et al. 2007). Insulin sensitivity post-exercise is increased for approximately 11h when exercise is performed earlier in the day (Franc 2015) and for up to 24-48 hours if the stores of muscle and hepatic glycogen are not promptly replenished (Teich and Riddell 2016; Bogardus et al. 1983; Mikines et al. 1988), thus increasing the risk of hypoglycaemia late after exercise (Gomez et al. 2015; Teich and Riddell 2016; Admon et al. 2005). This post-exercise enhanced insulin sensitivity serves to increase glucose disposal to restore glycogen stores (Teich and Riddell 2016; Bogardus et al. 1983; Mikines et al. 1988).

In addition, the counterregulatory hormone response to hypoglycaemia is impaired if exercise is prolonged and causes marked glycogen depletion, increasing further the risk of post-exercise hypoglycaemia (Adolfsson et al. 2012; Sprague and Arbeláez 2011; Tsalikian et al. 2009). Under these conditions, CHO supplementation is required to prevent blood glucose from falling (Tansey et al. 2006). Of note, although sprinting reduces the risk of hypoglycaemia immediately after exercise, it has no glucoregulatory benefits few hours after sprinting and does not reduce the risk of LOPEH (Davey et al. 2013b). In fact, the risk

of LOPEH is greater after intermittent high-intensity exercise compared to moderate exercise (Maran et al. 2010).

2.3 Evidence-based strategies and recommendations to maintain stable glycaemia during exercise

With the help of evidence-based information and expert opinion, recommended blood glucose monitoring, insulin dose adjustments, CHO supplementation protocols and other strategies such as sprinting have been published for the prevention of hypoglycaemia by different learned societies and organisations (Sherr et al. 2018; Riddell et al. 2017; Colberg et al. 2016; Craig et al. 2011). The recommendations from the National Health and Medical Research Council (NHMRC) 2011 guidelines, the Society for the Paediatric and Adolescent Diabetes (ISPAD 2018) guidelines, the American Diabetes Association (ADA) 2016 guidelines, and Lancet guidelines (Riddell et al 2017) and other key references will be discussed in the subsequent sections.

2.3.1 Blood glucose monitoring and the prevention of hypoglycaemia

To exercise safely, monitoring of blood glucose levels before, during and after physical activity is the approach of choice to prevent hypoglycaemia (Adolfsson et al. 2018; Robertson et al. 2014; Wasserman and Zinman 1994) and should form an integral part of exercise planning. It is generally recommended that blood glucose levels should be measured before engaging in any form of exercise as well as during and after exercise (Adolfsson et al. 2018; Riddell et al. 2017) by self-monitoring blood glucose (SMBG) levels using multiple daily finger sticks combined with a personal glucose monitor or continuous glucose monitor (CGM) systems.

Although SMBG can reduce the risk of hypoglycaemia associated with exercise (Adolfsson et al. 2008), the frequency of blood glucose levels monitoring by most youths with T1D is minimal (Adolfsson et al. 2008) as they measure mainly premeal blood glucose levels during the day and rarely measure glucose levels during the evening and night which is the

time of greatest vulnerability to hypoglycaemia (Porter et al. 1997). Glucose profiles provided by SMBG often miss some cases of glycaemic excursions since SMBG only gives brief glimpses into the 24 hour profile (Boland et al. 2001). In addition, frequent SMBG is difficult to adhere to since it requires a pause in activity, a limitation that is not shared with the use of CGM (Riddell and Perkins 2009). Consequently, the recent development of devices for the continuous monitoring of extracellular glucose levels as a proxy for plasma glucose levels is an important advance for the management of T1D, especially with respect to detecting the unpredictable glycaemic excursions associated with exercise.

CGM devices are inserted subcutaneously, and the sensor component of these CGM devices measures interstitial fluid glucose levels at 5- to 15-minute intervals (Olczuk and Priefer 2017). The CGM devices which are currently used employ enzyme-tipped electrodes or fluorescence technology to measure the glucose levels (Sherr et al. 2018; Olczuk and Priefer 2017). Although the accuracy of these sensors is acceptable (Bally et al. 2016a; Yardley et al. 2013b; Laffel 2016; Wadwa et al. 2018), accuracy can be affected by the rapidly changing glucose levels during exercise and the lag time required for blood glucose to equilibrate with the interstitial space. This can result in overestimation of blood glucose level when it is rapidly falling and underestimation when the blood glucose concentration is rising (Davey 2010; Taleb et al. 2016).

There are advantages with using CGM compared to SMBG (Houlder and Yardley 2018). Conventional glucose testing misses the significant day-to-day variations in plasma glucose from high to low values that constitute the glycaemic response to exercise in T1D. These glycaemic variations missed by SMBG can be detected by CGM (Boland et al. 2001). The use of CGM has made blood glucose monitoring during exercise easier (Bally et al. 2016a; Yardley et al. 2013b) and has the potential to help prevent exercise mediated hypoglycaemia by providing detailed information on the rate of change of blood glucose levels and real-time trends (Robertson et al. 2014; Adolfsson et al. 2011; Adolfsson and Lindblad 2002; Riddell and Milliken 2011) particularly during activities where self-monitoring of blood glucose is challenging (Adolfsson et al. 2008).

The comprehensive information provided by CGM devices has empowered people with T1D with the capacity to make clinical decisions during exercise, thereby increasing their self-efficacy. The ability of CGM sensors to transmit signals to the 'cloud' has, in addition, allowed for remote monitoring and the option of adding followers. These followers can view glucose levels in real time and receive alerts on their own devices, such as smartphones, tablets, and smart watches. This has enabled caregivers to view the blood glucose level tracing from CGM of the people they are following and alert them when their blood glucose levels fall out of an acceptable range while playing sports (Sherr et al. 2018; Olczuk and Priefer 2017; Cengiz 2013; Adolfsson et al. 2018). Recently in a study done by Breton et al (2018), a CGM-based decision support system consisting of an insulin titration tool, bolus advisor informed by CGM and an exercise advisor was found to improve safety around exercise in terms of improving the time spent below 3.9 mmol/l (Breton et al. 2018).

Insulin pumps are currently available that have insulin pump delivery and CGM data integrated in a single device. Managing glucose levels during exercise has become easier with the availability of these sensor augmented pump therapies which allow insulin delivery to be altered based on sensor glucose readings, thus enabling more advanced features to be used such as low-glucose suspend (LGS) and predictive low glucose management (PLGM) (Sherr et al. 2018). LGS interrupts insulin delivery for 2 hours when sensor glucose readings reach a pre-defined low sensor threshold. After 2 hours, the pump then automatically resumes insulin delivery regardless of sensor glucose levels readings (Garg et al. 2012; Bergenstal et al. 2013; Ly et al. 2013; Holder et al. 2011; Danne et al. 2011; Agrawal et al. 2011). The use of insulin pumps with these advanced features has been shown to help prevent exercise-mediated hypoglycaemia (Tsalikian et al. 2006).

PLGM systems differ from LGS systems in that the insulin pumps with PLGM feature interrupt insulin delivery if the sensor glucose reading is predicted to reach a level 1.1 mmol/L (20 mg/dL) above the pre-set low glucose limit within 30-min (Buckingham et al. 2015; Calhoun et al. 2016). Once hypoglycaemia is corrected, the pump then

automatically resumes basal insulin delivery (Abraham et al. 2017; Sherr et al. 2018; Buckingham et al. 2017, 2018; Battelino et al. 2017; Scaramuzza et al. 2017b).

The recent advancement in pump technology in addition to having features of LGS and PLGM systems discussed above, can also increase insulin delivery based on sensor glucose values. Most of these automated insulin delivery systems use a “hybrid” approach. In this approach, basal insulin delivery is controlled by an algorithm, but the user needs to manually deliver an insulin bolus during meals by entering the blood glucose values and estimated CHO intake. Automated insulin delivery systems have been shown to be safe and effective, particularly in response to real world challenges including exercise simulated in clinic studies (Van Bon et al. 2012; de Bock et al. 2017; Sherr et al. 2013).

The use of these technologies described above has a significant role to play in preventing hypoglycaemia during and after exercise (Adolfsson et al. 2018). Details of blood glucose levels in real time with a predictive element can help inform appropriate measures to avoid early and late hypoglycaemia post-exercise (Scaramuzza et al. 2017a). However, when sensor glucose values do not match symptoms and when sensor values change rapidly, it is recommended to measure blood glucose levels using blood glucose meters. This is because real time CGM tend to overestimate blood glucose levels under conditions where blood glucose is falling rapidly such as in response to exercise performed under hyperinsulinaemic conditions (Adolfsson et al. 2018).

2.3.2 Insulin dose adjustment and the prevention of hypoglycaemia

The inability of the pancreas to regulate plasma insulin levels in T1D implies that changing the magnitude and timing of insulin dose provide the only means to adjust plasma insulin levels prior to exercise. Due to the multiple factors that affect glycaemia during exercise, it is highly challenging to recommend precisely the amount of insulin reduction required to avoid hypoglycaemia without increasing the risk of insulin-deficiency that may increase the risk of hyperglycaemia and cause marked glycaemic excursions.

Hypoglycaemia prevention during exercise can be achieved by reducing bolus or basal insulin or both. The glucoregulatory benefits of reducing insulin dose prior to exercise are supported by a number of studies which have demonstrated lower risk of hypoglycaemia when exercise is performed when the prevailing insulin concentration is low (Schiffrin and Parikh 1985; Sane et al. 1988). Under such conditions, muscle glucose uptake is enhanced (Borghouts and Keizer 2000) due to the increased activation of the non-insulin sensitive glucose transporter 4 GLUT-4; (Gulve and Spina 1995; Thorell et al. 1999) which translocates to the cell surface and increases the glucose uptake even when insulin levels are low (Thorell et al. 1999).

When exercise is performed in the presence of high insulin levels, muscle glucose uptake increases further, and hepatic glucose production is inhibited and hence cannot counterbalance the increase in muscle glucose uptake driven by exercise (Steppel and Horton 2003; Wasserman et al. 1992; Edgerton et al. 2001; Sindelar et al. 1998). This mismatch between muscle glucose uptake and hepatic glucose production is further increased by antecedent exercise (Galassetti et al. 2001a) and antecedent hypoglycaemia (Galassetti et al. 2003, 2006a; Davis et al. 2000) due to a blunted counterregulatory response, thus enhancing further the risk of hypoglycaemia. Hence the strategy of decreasing insulin bolus dose prior to or during exercise can minimise the risk of hypoglycaemia (Kemmer 1992; De Feo et al. 2003). Hypoglycaemia risk has been reported to be lower under low insulin levels (Ruegemer et al. 1990), with muscle glycogen stores being used as the main source of CHO for energy production (Francescato et al. 2004). However, due to the multiple factors that affect glycaemia during exercise, it is difficult to recommend precisely by how much to reduce insulin dose and this remains a challenge.

2.3.2.1 Basal insulin dose reduction prior to exercise

One approach to decrease the risk of exercise-mediated hypoglycaemia is to reduce basal insulin dose, but only to such an extent that excessive hyperglycaemia is avoided. Basal insulin dose can be reduced before or after exercise to help increase hepatic glucose

production and reduce peripheral glucose utilisation rates. Performing such changes in basal insulin dose is challenging for multiple daily injections (MDI)-treated people, particularly for those who are treated with very slow release insulin such as glargine and degludec (Heise et al. 2016). This is because basal insulin dose reduction with these types of preparations has to take place up to 24 hours before exercise, depending on the time elapsed between basal insulin injection and exercise. For this reason, the use of insulin preparations with shorter duration of action like intermediary acting insulin preparations (e.g. NPH insulin) or the slow release insulin detemir might be advantageous since these are associated with a lower risk of exercise-mediated hypoglycaemia compared to insulin glargine (Arutchelvam et al. 2009).

For MDI treatment, the benefits of reducing basal insulin dose to minimise the risk of exercise-mediated hypoglycaemia risk is supported by only limited research (Campbell et al. 2015b; Moser et al 2019a.). The ISPAD and ADA guidelines recommend a 20% basal analogue (e.g. insulin glargine, detemir, neutral protamine Hagedorn [NPH]) dose reduction on the day of exercise to reduce the risk of hypoglycaemia (Adolfsson et al. 2018; Colberg et al. 2016). It is important to note that aggressive reductions in insulin doses can result in low insulin concentrations resulting in hyperglycaemia prior to and during exercise (Zander et al. 1983), leading to ketosis even with mild activity (Berger et al. 1977).

The challenges with changing basal insulin dose prior to exercise are easier to meet for individuals treated with continuous subcutaneous insulin infusion (CSII) pumps. This is because insulin pumps offer the flexibility to modify basal insulin delivery, with the effect of changing basal insulin delivery rate on plasma insulin levels and glycaemia being detectable within ~1–2 hours (Heinemann et al. 2009). In this regard, a 50% reduction in basal insulin infusion rates 60 min prior to exercise has been reported to be not enough to decrease insulin levels sufficiently upon the start of exercise (McAuley et al. 2016). Hence, basal insulin rate reductions are recommended 60–90 min before the start of exercise

(Robertson et al. 2014; Riddell et al. 2017; Chu et al. 2011; Adolfsson et al. 2018; Colberg et al. 2016; Zahrieva et al. 2019).

For patients on CSII therapy, the pump may also be disconnected or suspended to reduce basal insulin levels (Adolfsson et al. 2018), particularly prior to certain types of activities such as contact sports. Although suspending insulin infusion during exercise is opted by few individuals to prevent hypoglycaemia, hypoglycaemia can still occur for several hours after the end of the activity once basal insulin is resumed (Admon et al. 2005; Sonnenberg, et al. 1990). Of note, however, this strategy is generally not recommended as excessively low insulin levels increase the risk of both severe hyperglycaemia (Tsalikian et al. 2006) and ketoacidosis if the insulin pump is inactivated for too long. In this respect, an 80% basal insulin dose reduction at the onset of exercise has been shown to be more effective than basal insulin suspension (Franc et al. 2015) at preventing both hypoglycaemia during and after exercise and hyperglycaemia post-exercise (Franc et al. 2015). For those who still prefer to suspend their insulin infusion prior to exercise, a time limit of less than 2 hours for pump suspension is advised, based on rapid-acting insulin pharmacokinetics, to prevent the risk of severe hyperglycaemia and ketosis (Heinemann et al. 2009).

2.3.2.2 Bolus of fast acting insulin dose reduction prior to exercise

The currently used rapid acting insulin analogues result in plasma insulin levels that peak at around 30-90 min post-administration, with the risk of hypoglycaemia being highest when exercise coincides with these insulin peaks (Tuominen et al. 1995). Given that the hypoglycaemia risk associated with exercise under hyperinsulinaemic conditions is markedly increased if exercise is performed within 2-3 hours of injecting a fast acting insulin (Chokkalingam et al. 2007a; Francescato et al. 2004), current strategies to decrease such a risk is to reduce bolus insulin dose (Kemmer 1992; Rabasa-Lhoret et al. 2001; Schiffrin and Parikh 1985; Bracken et al. 2012b; De Feo et al. 2003; Franc et al. 2015;

Mauvais-Jarvis et al. 2003). The intensity and duration of exercise dictates the extent to which mealtime insulin dose is reduced.

The insulin dose reduction recommended before moderate intensity exercise ranges from 25% (Rabasa-Lhoret et al. 2001) to 66% (Schiffrin and Parikh 1985), with a reduction of up to 75-90% being recommended for more intense and longer duration exercise (Rabasa-Lhoret et al. 2001; Mauvais-Jarvis et al. 2003; Adolfsson et al. 2018; Moser et al. 2015). Based on these studies, it is recommended (Adolfsson et al. 2018; Colberg et al. 2016; Riddell et al. 2017) that when continuous, moderate to vigorous intensity aerobic activities are performed in an hyperinsulinaemic state (within 90 min of meal insulin bolus), premeal rapid acting insulin bolus should be reduced by 25 to 50% for activities lasting 30 to 45 min and by 50 to 75% for activities lasting more than 45 min. The recommended reduction of premeal insulin bolus before mixed aerobic and anaerobic burst activities (e.g. hopping, skipping, dance, gymnastics, tag, dodgeball, field and team sports, individual racquet sports, etc.) is approximately 25% for activities lasting 30 to 45 min and 50% for activities lasting more than 45 min. The rapid acting insulin bolus should also be reduced by up to 50% for meals after such activities.

Of note, implementing pre-exercise insulin dose reduction strategy to prevent hypoglycaemia may in many instances result in higher plasma glucose levels (West et al. 2010; Rabasa-Lhoret et al. 2001). This strategy also does not always ensure nil hypoglycaemia risk post-exercise (Grimm et al. 2004; Francescato et al. 2011). Finally, as discussed later, CHO supplementation in addition to reducing pre-exercise insulin dose is often required to prevent hypoglycaemia (Grimm et al. 2004; West et al. 2011b).

2.3.2.3 Insulin dose adjustments during exercise

Disconnecting the insulin pump or reducing insulin dose prior to exercise may result in hyperglycaemia, particularly during competitions and high intensity exercise or if accompanied with excessive CHO intake. Hyperglycaemia during exercise may be treated

by giving a small additional dose of rapid-acting insulin during (e.g. at half-time) or after exercise (Zaharieva and Riddell 2015). For example, 50% of the usual correction bolus may be given when blood glucose levels are >14mmol/L (Adolfsson et al. 2018).

The use of CGM devices allows insulin dose to be adjusted during exercise to attenuate or avoid hypoglycaemia. As discussed earlier, the use of LGS and PLGM functions allows suspension of insulin delivery during exercise when blood glucose reaches a pre-set threshold level, which offers additional protection against hypoglycaemia (Danne et al. 2014). The threshold glucose level at which the predictive component of PLGM is activated can be individually set before, during and following exercise.

The benefit of using such technology is supported by the finding that sensor augmented pump therapy used in conjunction with moderate intensity exercise reduces the number of hypoglycaemic events (Abraham et al. 2016). Similarly, the use of closed-loop automated insulin delivery system during and after unannounced physical activity in young people with T1D has been promoted to enable them to maintain blood glucose levels mostly within target range, without an increased risk of hypoglycaemia (Dovc et al. 2017).

2.3.2.4 Insulin dose reduction after exercise to decrease the risks of LOPEH

Reduction of circulating basal insulin concentrations during recovery can reduce hypoglycaemia risk which can persist for up to 24 hours post-exercise (MacDonald 1987; Tsalikian et al. 2005; McMahon et al. 2007). Due to increased flexibility at altering basal insulin infusion rates, insulin pumps are preferred over MDI in the management of early (Yardley et al. 2013a) and late onset hypoglycaemia post-exercise (Taplin et al. 2010). The current recommendation is to reduce basal insulin post-exercise by approximately 20% by either decreasing the overnight long-acting/basal insulin dose for people on MDI (Campbell et al. 2015b) or reducing basal rate for those treated with insulin pump (Taplin et al. 2010) or reducing the subsequent mealtime boluses (Adolfsson et al. 2018; Riddell et

al. 2017). Reduction by about 50% of the bolus insulin dose administered prior to a meal after exercise is recommended to address the elevated insulin sensitivity post-exercise (Riddell et al. 2017).

Insulin dose reduction can be supplemented by the consumption of low glycaemic index snacks after exercise (Adolfsson et al. 2018; Campbell et al. 2014b). To prevent nocturnal hypoglycaemia following evening exercise, a 25 to 75 % reduction in the rapid acting insulin analogue dose before the evening meal together with the consumption of 10-15 grams of fast acting carbohydrate before evening exercise is also recommended (Adolfsson et al. 2018).

Advanced features now available in the newer generation of pumps described above can help with the prevention of post-exercise nocturnal hypoglycaemia, although threshold settings may need to be adjusted if a recent bout of hypoglycaemia has occurred (Garg et al. 2014). Also, the access to glucose data in real time with the predictive element offered by these technologies makes it possible to take appropriate measures to avoid early and delayed post-exercise hypoglycaemia. In this respect, automated insulin delivery systems have shown to reduce nocturnal hypoglycaemia following afternoon physical activity (Sherr et al. 2013).

2.3.2.5 Limitations with current insulin dose reduction recommendations

There are a number of limitations with implementing pre-exercise insulin dose reduction to improve blood glucose profile during exercise. For instance, if the insulin dose reduction is excessive, it may result in a severe exercise-mediated rise in plasma glucose levels (West et al. 2010; Rabasa-Lhoret et al. 2001). Insulin dose reduction does not necessarily prevent hypoglycaemia risk post-exercise (Grimm et al. 2004; Francescato et al. 2011). This approach is also impractical for unplanned exercise as insulin dose reduction must be performed at least 1-2 hours prior to exercise to achieve target blood insulin levels. Under circumstances where insulin dose cannot be modified ahead of time,

CHO supplementation provides the only effective means to prevent exercise-induced hypoglycaemia (Riddell et al. 1999; Kemmer 1992).

Another limitation with insulin dose reduction relates to the increased risk of excessive hyperglycaemia that may arise in response to high intensity exercise. High intensity aerobic exercise as well as exercise involving maximal sprint efforts are associated with a modest rise in blood glucose levels when exercise is performed under basal or near basal insulinaemic conditions (Marliss and Vranic 2002; Purdon et al. 1993; Sigal et al. 1994, 1996). This rise in blood glucose level is likely to be far more pronounced for exercise performed under low insulin conditions. There is limited evidence to inform the amount of extra insulin that should be administered to normalise blood glucose levels post-high intensity exercise (Aronson et al. 2019).

One issue of concern with such post-exercise administration of fast acting insulin to normalise blood glucose level is the expected increase in insulin sensitivity post-exercise, implying that the correction dose should consider a lower insulin-to-carbohydrate ratio. For this reason, the ISPAD guidelines recommend that high blood glucose levels after intense exercise may be opposed by administering a small additional dose of rapid-acting insulin at half-time or immediately after exercise– namely a 50% correction bolus when levels are >14 mmol/L (Robertson et al. 2014; Adolfsson et al. 2018; Riddell et al. 2017). Of note, such a recommendation is not evidence-based. Also, since the rise in blood glucose associated with high intensity exercise is generally modest (<2 mmol/L), such a mild hyperglycaemia may not warrant administering insulin post-exercise as this may increase the risk of post-exercise hypoglycaemia. There are limited studies investigating insulin dose corrections to minimise post exercise hyperglycaemia (Turner et al. 2016b; Aronson et al. 2019) and the precise insulin dose adjustments required to maintain stable blood glucose levels during and after different intensities of exercise still remain an unresolved challenge.

2.3.3 Carbohydrate supplementation for the prevention of hypoglycaemia

Altering insulin dose before exercise does significantly reduce the risk of hypoglycaemia during exercise (Diabetes Research in Children Network Study et al. 2006; Dube et al. 2005; Grimm et al. 2004; Mauvais-Jarvis et al. 2003; Rabasa-Lhoret et al. 2001; Riddell et al. 1999; Sonnenberg 1990; Schiffrin 1985; Moser et al. 2015). However, this approach is suitable only for planned exercise when insulin dose adjustments can be made in advance. Hence, consuming extra CHO is often the only strategy available to reduce hypoglycaemia risk during and after unplanned exercise, with this strategy being also often adopted for planned exercise (Kemmer 1992; Dube et al. 2005, 2006; Riddell et al. 1999; Grimm et al. 2004).

Some have recommended that a meal containing CHO, fats and protein should be consumed approximately 3 to 4 hours before exercise (Sawka et al. 2007; Thomas 2016); however, such a timing is impractical for many. Hence it is important to ensure that quick acting CHO are readily available prior to and during exercise, with exercise to be avoided otherwise. In order to exercise safely, the recommended type, amount and timing of CHO intake should aim at providing fuel for exercise while maintaining euglycaemia during and after exercise.

The amount of CHO required to maintain near stable blood glucose level is affected by the intensity and duration of the exercise, with many physical activities such as soccer, cycling, jogging, and swimming requiring extra CHO not only before, but also during and often after the activity (Robertson et al. 2014; Adolfsson et al. 2018). In contrast, short duration high-intensity anaerobic activities or very high intensity aerobic exercise ($>80\%$ $\dot{V}O_2$ peak) such as weight lifting, sprints, board diving, may not require any prior CHO intake particularly if performed while plasma insulin is at near basal levels, but extra CHO intake afterwards may be needed (Robertson et al. 2014; Adolfsson et al. 2018; Riddell et al. 2017). As discussed in the following sections, recommended CHO intake for the prevention of hypoglycaemia should consider not only the intensity and duration of exercise, but also, and very importantly, prevailing blood insulin levels.

2.3.3.1 Impact of pre-exercise blood glucose level on recommended carbohydrate intake for the prevention of hypoglycaemia

The optimal glycaemic range for optimal exercise performance is unclear (Riddell, Gallen, Smart, Taplin, Adolfsson, Lumb, Kowalski, Rabasa-Lhoret, McCrimmon, Hume, et al. 2017). However, limited data from the literature suggest that maintaining blood glucose levels between 6–8 mmol/L might be ideal since hypoglycaemia can compromise exercise performance and cognition (Kelly et al. 2010) and sustained hyperglycaemia can impact work capacity by impairing many metabolic and circulatory processes (Galassetti and Riddell 2013).

The amount of ingested CHO recommended to prevent hypoglycaemia depends, in part, on blood glucose levels at the start of exercise (Moser et al. 2019b). The most recent ADA guidelines recommend that pre-exercise target range of blood glucose levels should be between 5 and 13.9 mmol/L (Colberg et al. 2016). In comparison, more recent guidelines stipulate that blood glucose levels should be between 7–10 mmol/L for aerobic exercise lasting up to an hour to minimise the risk of hypoglycaemia (Riddell et al. 2017). This range is preferable during competitive sports where performance is a major goal and where stress-induced hyperglycaemia may be a challenge to overcome (Hargreaves et al. 1996). However, given the difficulties in achieving and maintaining blood glucose levels within the range of 7–10 mmol/L, pre-exercise blood glucose levels higher than 7–10 mmol/L is an option if added protection against hypoglycaemia is needed.

Since blood glucose levels tend to remain relatively stable and fall to a lesser extent or even rise marginally during anaerobic exercise and high intensity exercise compared to continuous aerobic exercise when plasma insulin are approaching basal levels, a lower pre-exercise blood glucose level at near 5-7 mmol/L may be suitable (Riddell et al. 2017). Although recent ISPAD guidelines (Adolfsson et al. 2018) do not provide any recommendation on pre-exercise blood glucose levels target range, they recommend strategies related to different pre-exercise blood glucose levels which are similar to the strategies suggested by Riddell and colleagues (2017), but with few minor differences. For

instance, Riddell and colleagues (2017) suggest ingesting 10 g instead of 10 to 20 g of glucose before starting aerobic exercise when pre-exercise blood glucose levels are between 5.0-6.9 mmol/L, but with additional CHO if aerobic exercise lasts longer than 30 min.

It is important to stress that if pre-exercise blood glucose levels are above 14-15 mmol/L and accompanied with low to high ketone bodies levels, ISPAD guidelines recommend not to start any physical activity until actions are taken to correct blood ketones if >0.6 mmol/L since blood ketone levels >0.5 mmol/L in children with diabetes are abnormal (Samuelsson and Ludvigsson 2002; Laffel et al. 2006). In contrast, Riddell and colleagues' guidelines (2017) do not exclude short duration (<30 min) light intensity exercise for modestly elevated ketones up to 1.4 mmol/L if ketosis is diet-induced but not if due to insulin withdrawal. They also suggest a small corrective insulin dose before starting exercise if needed. These different recommendations are due to the ISPAD guidelines focussing particularly on children whereas the ADA and the guidelines of Riddell and colleagues (2017) focus on the adult population. It must be stressed, however, that all current guidelines agree that exercise is to be avoided and insulin administration should be performed if blood ketones levels are ≥ 1.5 mmol/L or urine ketone levels are ≥ 2 mmol/L. If blood ketones levels are 3.0 mmol/L or more, management by a health-care professional is recommended.

Current guidelines recommend avoiding high intensity exercise if pre-exercise blood glucose levels are high >14 mmol/L with any evidence of elevated ketone levels. Exercise is not recommended under these conditions for fear that hyperglycaemia combined with mild ketosis may reflect a state of severe insulin deficiency conducive to severe ketoacidosis that may be exacerbated by physical activity. An insulin bolus using half the usual correction factor should be administered in the setting of high glucose and high ketone levels and exercise postponed until ketonemia has cleared (Riddell et al. 2017; Adolfsson et al. 2018). The strategies suggested by the ADA guidelines, which are adapted from Zaharieva and Riddell (Zaharieva and Riddell 2015), are less specific compared to the

ISPAD guidelines as shown in Table 1 and also targets different range of blood glucose levels where action has to be taken (Colberg et al. 2016).

The main limitations with the recommendations listed here is that they overlook the duration of exercise, insulinisation state and blood glucose trend of the participants prior to exercise. This latter limitation is particularly problematic for people not wearing a CGM as a single measurement of blood glucose level for MDI-treated individuals provides no information about blood glucose trend. Two blood glucose level measurements should thus be taken at least 10-15 min apart by these individuals to identify such trends. The aforementioned recommendations also overlook the limitations shared by all CGM, with their accuracy, as discussed before, being poor under conditions of rapidly changing blood glucose levels prior to exercise (Davey 2010; Taleb et al. 2016).

Table 2.1 Comparison of ADA (2016) and ISPAD (2018) guidelines on blood glucose concentrations before exercise commencement and recommended glucose management strategies

ADA Guidelines		ISPAD Guidelines	
Blood Glucose	Carbohydrate and Glucose management strategies	Blood Glucose	Carbohydrate and Glucose management strategies
<5 mmol/L	Ingest 15–30 g of fast-acting carbohydrate at start of exercise.	<5 mmol/L	Ingest 10–20 g of CHO before starting exercise. Delay exercise until blood glucose is > 5 mmol/L.
5-8.3 mmol/L	Ingest 0.5–1.0 g CHO/kg/ hour at the start of exercise, depending on the type of exercise and the amount of active insulin	5-6.9 mmol/L	Ingest 10 to 20 g of CHO before starting aerobic exercise.
8.3-13.8mmol/L	Exercise should be initiated and the consumption of carbohydrate delayed until blood glucose levels are < 8.3 mmol/L	7-10 mmol/L	Aerobic and anaerobic exercise can be started.
13.9-19.4 mmol/L	Test for ketones. Do not perform any exercise if moderate-to-large amounts of ketones are present. Initiate mild-to-moderate intensity exercise. Intense exercise should be delayed until glucose levels are <13.8 mmol/L.	10.1-14 mmol/L	Aerobic and anaerobic exercise can be started.
>19.4 mmol/L	Test for ketones. Do not perform any exercise if moderate-to-large amounts of ketones are present. If ketones are negative (or trace), consider conservative insulin correction before exercise Initiate mild-to-moderate exercise and avoid intense exercise until glucose levels decrease.	>14 mmol/L	If hyperglycaemia is unexplained check blood ketones. If blood ketones are >0.6 mmol/L, actions are required before any exercise is started.

2.3.3.2 Pre-exercise carbohydrate intake for the prevention of exercise-mediated

hypoglycaemia: basal insulinaemic state

The basal insulinaemic state refers to a condition where no exogenous CHO is required to maintain stable blood glucose at rest. Exercising under such a condition reduces the risk of acute hypoglycaemia as circulating insulin levels are typically low (Ruegamer et al. 1990). Hence the ISPAD guidelines recommend that CHO may not be required prior to mild to moderate intensity exercise lasting less than 30 min if exercising under a basal or near basal insulinaemic state.

When the duration of the bout of low to moderate intensity/aerobic exercise is greater than 30 min, 0.2-0.5 grams/kg/hour may be required to maintain euglycaemia. However, to improve exercise performance, an optimal level of 1 gram/kg/hour may be required. The guidelines by Riddell and colleagues (2017) also incorporate exercise duration and pre-exercise blood glucose levels in their CHO recommendation for exercise performed under basal insulin levels. In particular, they recommend the ingestion of a high glycaemic index meal or snack providing 10 to 20 g CHO if blood glucose level is <5mmol/L immediately before exercise done under a near basal insulinaemic state. Similarly, the American Diabetes Association recommends an intake of 10 to 15 g of carbohydrate for low to moderate intensity aerobic activities lasting 30 to 60 min when insulin levels are close to basal levels, with a trial and error approach being recommended to adjust CHO needs at different exercise intensities (Colberg et al. 2016).

The adequacy of these recommendations is supported by the work of Nathan and colleagues (1985) who showed that the ingestion of 13 g CHO, 15 min before 45 min of exercise performed under basal insulin conditions was effective at preventing post-exercise hypoglycaemia without causing hyperglycaemia during exercise. The suitability of this dose was further supported by the work of Soo and colleagues (1996; Table 2.2] who showed that the ingestion of 30 g of either simple or complex CHO prior to 45 min of moderate intensity exercise at 60% $\dot{V}O_2$ peak performed under basal insulinaemic conditions produced a moderate to substantial elevation in plasma glucose levels (mean

rise in plasma glucose 5.1 ± 0.8 mmol/l). For this reason, and in agreement with the earlier work of Nathan and colleagues, they recommended reducing the amount or even omitting CHO as a pre-exercise supplement for exercise performed in the morning before the morning insulin dose (Soo et al. 1996). However, only one exercise intensity was tested in these studies, thus leaving unanswered the question of the extent to which these recommendations are likely to be affected by exercise intensity.

2.3.3.3 Pre-exercise carbohydrate intake for the prevention of exercise-mediated

hypoglycaemia: hyperinsulinaemic state

The required pre-exercise CHO intake for the prevention of hypoglycaemia during exercise are elevated if pre-exercise insulin levels are high at the start of exercise. A number of strategies have been proposed to quantify one's CHO needs to prevent hypoglycaemia during and after exercise performed under hyperinsulinaemic conditions. One such approach is to estimate the energy cost of the exercise and calculate the CHO requirements on the assumption that 60% of total energy is provided by CHO. Some recent guidelines (Riddell et al. 2017) proposed that such an approach could provide an individualised strategy based on total energy utilisation (Frankenfield et al. 2005). Unfortunately, this approach overlooks the fact that muscle glycogen is the preferred CHO oxidised during exercise and not blood glucose (Robitaille et al. 2007).

Another approach to estimate how much dietary CHO is required to minimise the risk of exercise-mediated hypoglycaemia is to match the amount of ingested CHO as closely as possible to that oxidised during exercise (Riddell and Iscoe 2006a). In this respect, Riddell et al (1999; Table 2.3) found that glucose ingestion at a rate equal to total CHO oxidation rate decreases the magnitude of the fall in glucose level during exercise in adolescents with T1D. In this study, 20 adolescents with T1D performed 60 min of moderate intensity exercise 100 min after breakfast and insulin administration, and ingested during (every 10 min) and after exercise (10 min after) either water (control trial) or a 6-8% glucose solution (glucose trial), with the total amount of administered CHO for each participant

being equal to the total amount of oxidised CHO (average of 1.46 ± 0.08 g/min). This intervention resulted in a lower rate of hypoglycaemia compared to the water trial, which was expected given the large amount of ingested CHO. The results from this study forms the basis for the recommendations by the ADA, ISPAD, NHMRC and JDRF that a carbohydrate intake of 1.0-1.5 g per kg of body mass per hour of strenuous and long duration exercise should be adopted to minimise the risk of hypoglycaemia when exercise is performed during peak insulin action (Colberg et al. 2016; Sherr et al. 2018; Craig et al. 2011; Riddell et al. 2017). These recommended intakes are expected to be effective at preventing hypoglycaemia as they correspond to those shown to result in the highest rate of gastrointestinal glucose absorption (Duchman et al. 1997), with no additional benefit to be expected with higher CHO intake.

The main limitation with using CHO oxidation rate to determine how much CHO to ingest to prevent hypoglycaemia is that this approach has the capacity to overestimate markedly the extent to which blood glucose is oxidised during exercise as muscle glycogen rather than blood glucose is by far the preferred CHO oxidised during exercise (Robitaille et al. 2007), with glycogen oxidation being little affected by ambient insulin levels (Chokkalingam et al. 2007a). This may explain, at least in part, why recommended CHO intakes based on CHO oxidation rates are significantly greater than those reported by other researchers to be required to maintain euglycaemia during moderate intensity exercise (McMahon et al. 2007), and hence conducive to marked hyperglycaemia. In part for these reasons, the most recent ADA guidelines recommend the ingestion of 30 to 60 g of CHO per hour of exercise for activities performed under conditions of modest hyperinsulinaemia (Colberg et al. 2016).

Of note, the limitations with the experimental approaches described above are not shared by the work of Robitaille and colleagues (2007; Table 2.3) who measured the response not only of substrate oxidation to exercise, but also that of exogenous glucose, glucose released from liver, and muscle glycogen oxidation using indirect calorimetry combined with tracer technique in individuals with and without T1D. All participants with T1D in this

study received their usual insulin dose along with a breakfast 3 hours before exercise. Then, they received a 30 g glucose load 15 min before engaging in 60 min of moderate intensity exercise. During exercise, plasma glucose levels in T1D participants decreased by only 2 mmol/L (from 9.6 to 7.5 mmol/l), and they experienced a lower plasma glucose oxidation rate compared to healthy individuals without diabetes. In addition, total CHO oxidation rate was not significantly different from that of healthy subjects, indicating a larger oxidation rate of muscle glycogen (Robitaille et al. 2007). These results thus suggest that individuals with T1D rely more on muscle glycogen and less on plasma glucose oxidation than healthy subjects when glucose is consumed before exercise performed under moderately elevated plasma insulin levels.

In agreement with the findings of Robitaille, the work of Dube and colleagues (2005) concluded that a pre-exercise liquid supplement of 40 g of glucose ingested 15 min prior to exercise performed 3 hours after a meal and insulin bolus should provide an effective means to maintain safe blood glucose levels during 60 min of late postprandial moderate intensity exercise (Dube et al. 2005). In this study, the participants were randomly assigned to ingest 0, 15 or 30 g of a liquid glucose supplement 15 min before exercise. The quantity of ingested glucose required to prevent hypoglycaemia during exercise was plotted against the three pre-exercise glucose supplements, and based on the resulting regression equation thus obtained, a glucose supplement of 40 g was estimated to be required in order to maintain blood glucose levels within the normal range during and 1 hour after exercise (Dube et al. 2005).

One limitation shared by all the aforementioned studies is that each investigated the benefit of ingesting CHO without any adjustment in insulinaemic state. This limitation is not shared by the work of Grimm and colleagues (Grimm et al. 2004) who compared different treatment strategies to prevent hypoglycaemia associated with exercise, and reported that it is possible to prevent almost all cases of hypoglycaemia simply by adequately adjusting CHO with or without any insulin dose adjustments (Grimm et al. 2004). In their study, 67 participants with T1D were assigned to four treatment strategies

to prevent hypoglycaemia during and after exercise, namely exercise with and without CHO intake and/or with and without insulin dose reduction. Participants performed mild intensity long lasting (>4h) exercise (<60% of maximal heart rate), 1 hour after breakfast. They were allocated into four treatment arms referred to as group A, B, C and D. Group A consumed 10-20 g/h CHO and did not modify their usual insulin regimen, group B consumed 10-20 g/h CHO and reduced their daily insulin dose by 10%, group C reduced their daily insulin dose by 10% and did not consume additional CHO, while group D did not perform any changes in insulin dose and did not ingest any additional CHO. This study showed that group A and B who ingested the recommended CHO before, during and after exercise experienced a lower rate of hypoglycaemia than the other 2 groups who did not take any additional CHO. The average extra CHO ingested was 18 ± 1 and 19 ± 2 g/h for group A and B, respectively.

Another limitation shared by all the aforementioned studies is that each investigated only one exercise intensity with the exception of Grimm and colleagues (2004), who in a subsequent sub-study reallocated the same participants involved in the study above into 9 subgroups with three intensities of exercise (mild, moderate and high intensity) and three different exercise durations (<20 min, 20-60 min and >60 min). Unfortunately, the insulinaemic state of their participants was not reported. Every single participant was advised to consume additional CHO and/or adjust their insulin as per the findings of the main study described above. They found only 6 hypoglycaemic events out of 265 exercise sessions, with hypoglycaemia occurring during prolonged exercise (> 4 hours). The average amount of CHO ingested was 26 ± 1 g/h for mild (<60% of maximal heart rate), 72 ± 2 g/h for moderate (60-75% of maximal heart rate), and 106 ± 3 g/h for high intensity exercise (>75% of maximal heart rate). On the basis of their results, the authors designed a CHO intake table for different physical activities, exercise duration and exercise intensity, with recommended CHO intake ranging from 15 to 100 g/hr exercise (Grimm et al. 2004). As expected, the amount of extra CHO needed to prevent hypoglycaemia was higher when no insulin dose adjustments were performed. The authors concluded that without insulin reductions for long duration exercise, exercising individuals are forced to

compensate by ingesting an amount of CHO in the upper range, which could predispose them to weight gain. This extra amount of energy intake may thus be counter-productive particularly when a holistic approach to diabetes management is the goal as nutritional advice for hypoglycaemia prevention should not contribute to weight gain (Bracken et al. 2012).

2.3.3.4 Recommended carbohydrate intake during exercise for the prevention of hypoglycaemia

Although several studies have focused on the amount of CHO to be given before exercise, only a few studies have attempted to estimate how much CHO should be ingested during exercise to prevent hypoglycaemia. This is not an issue for short duration exercise. However, longer duration aerobic activities such as soccer (often described as a mixture between aerobic and anaerobic exercise), cycling, jogging, and swimming may require extra CHO not only before, but also during and often after the activity (Robertson et al. 2014; Adolfsson et al. 2018).

CHO can be consumed every 10-20 min throughout exercise rather than all at the beginning of exercise (Riddell and Iscoe 2006a; Matyka and Annan 2012). When circulating insulin levels are high at the start of exercise, ISPAD guidelines recommend the ingestion of up to 1.5 g CHO per kilogram of body mass per hour of long duration exercise (Riddell et al. 1999). The recommended amount of ingested CHO during exercise falls to 0.3-0.5 g CHO/kg/h if prolonged exercise is performed several hours after the insulin bolus dose has been given (Robertson et al. 2014a). This hourly amount falls to approximately 0.2 g/kg/h by ~5.5 hours post-insulin bolus (Francescato et al. 2004). The recommendation by Riddell and colleagues (Riddell et al. 2017) is more specific than those of the ISPAD guidelines as they incorporate blood glucose levels and duration of exercise in their carbohydrate recommendation for exercise performed under low and hyperinsulinaemic conditions. For aerobic exercise of low to moderate intensity performed under low insulinaemic conditions, they recommend the ingestion of high glycaemic index CHO providing 10-15 g

CHO per hour of exercise, but 15-30 g CHO every 30 min under hyperinsulinaemic conditions, depending on exercise intensity and the blood glucose concentration measured during the activity.

For high intensity exercise, a CHO supplementation of 10-20 g during exercise is recommended by Riddell and colleagues (2017) only when the blood glucose levels measured during the activity is less than 5 mmol/L. When the duration of exercise is 60 to 150 min, they recommend 30-60 g CHO per hour under low insulinaemic conditions and up to 75 g CHO per hour under hyperinsulinaemic conditions to prevent hypoglycaemia and enhance performance. When duration of exercise exceeds 150 min, they recommend an intake of 60-90 g/h CHO (Riddell et al. 2017).

2.3.3.5 Carbohydrate requirements for competitive athletes

A different approach to CHO supplementation may be required in cases of elite athletes with T1D whose goal is to ingest enough CHO not only to achieve optimal glycaemic management, but also to optimise endurance performance by providing their muscles with an adequate fuel supply. To maximise performance and glycaemic outcomes, individualised meal planning is essential (Riddell et al. 2017). The timing of exercise should be taken into account while planning the daily intake of CHO, fat and protein to maximise liver and muscle glycogen stores for exercise and replenishment in the recovery period (Thomas et al. 2016). Most of the advice for optimal nutrition and CHO requirements for competitive athletes are based on studies done in highly trained individuals without diabetes (Thomas et al. 2016), with very limited studies performed in T1D individuals.

In a randomised control trial involving 18 athletes with T1D, Murillo and colleagues (2015) found that a pre-exercise CHO supplement of 0.35 g/kg was more effective at stabilising glycaemic levels during competitive running lasting 50-60 min than a higher dose of 55 g (0.7 g/kg); (Murillo et al. 2015). However, others found that a high CHO supplementation of 75 g/h during prolonged aerobic exercise lasting >3 hours is effective at maintaining

euglycaemia (Adolfsson et al. 2015). Of note, such high intakes of CHO (60-70 g/hour) have been shown by most but not all studies (Wong et al. 2009) in healthy individuals without diabetes to be favourable to endurance performance (Moore et al. 2009; Stephens et al. 2008), particularly if lasting more than 2.5 hours (Currell and Jeukendrup 2008; Jeukendrup 2010; Ahlborg and Felig 1977; Devlin et al. 1986), and to improve physical perception of effort (Holtz et al. 2008). There is evidence that this is also the case for individuals with T1D.

Ramires et al (1997) found that glucose ingestion equivalent to 1g/kg 30 min before exercise increased the endurance capacity of those individuals with T1D whose blood glucose levels decreased during exercise. These findings thus suggest that the ingestion of large amounts of CHO prior to endurance events may also be beneficial to individuals with T1D without causing marked hyperglycaemia. These findings have been recently corroborated by those of Bally and colleagues (2017; Table 2.2) who compared the metabolic effects of co-ingesting glucose-fructose compared to only glucose during 90 min of moderate intensity exercise in 15 male individuals with T1D. They found that the co-ingestion of glucose and fructose during exercise was as effective as ingesting glucose alone in stabilising glycaemia, and had a beneficial impact on fuel metabolism by inducing a shift towards fat oxidation with a concomitant glycogen sparing effect in the working muscle (Bally et al. 2017). Recently, Gray et al (2016; Table 2.2) examined the influence of ingesting a high molecular weight carbohydrate waxy barley starch (WBS; GI = 98) compared to glucose on glycaemia and endurance performance in individuals with T1D. They found that WBS resulted in similar hyperglycaemic responses to glucose, but a greater rate of carbohydrate use at rest and improvement in endurance performance (Gray et al. 2016).

As mentioned earlier, the risk of hypoglycaemia is lessened when reduction in insulin doses are made during competitive sports (Sane et al. 1988). This practice may predispose athletes to severe hyperglycaemia (Ebeling et al. 1995). In part for this reason, ISPAD guidelines recommend extra CHO supplementation just before exercise is a better

strategy than insulin reduction for optimising exercise performance (Adolfsson et al. 2018).

The recommendations by Riddell and colleagues (2017) for endurance exercise performance in athletes with and without diabetes are more specific. Their recommendation is to consume a low GI and low-fat meal with a CHO content equivalent to 1 g/kg bodyweight before and after exercise. For aerobic exercise of low to moderate intensity lasting 30 to 60 min, they recommend the ingestion of 10-15 g/h carbohydrate to enhance performance. For exercise duration of 60 to 150 min, they recommend the ingestion of 30-60 g carbohydrate per hour. When exercise duration exceeds 150 min, they recommend an intake of 60–90 g/h CHO from mixed CHO sources spread across the activity (e.g. 20-30 g carbohydrate every 20 min) to enhance performance (Riddell et al. 2017). In addition, they recommend to balance the insulin dose to CHO intake. For instance, one such a strategy includes reducing the bolus dose pre-exercise by 30-50% up to 90 min before exercise (Franc et al. 2015).

2.3.3.6 Recommended carbohydrate intake for the prevention of LOPEH

Managing CHO intake after exercise to maintain euglycaemia and prevent late onset post-exercise hypoglycaemia (LOPEH) is essential to achieve tight glycaemic control, overcome the fear of exercise-mediated hypoglycaemia, and ensure good compliance with regular physical activity. Given the close association between the risk of LOPEH and the state of depletion of muscle and hepatic glycogen stores, it is crucial to replenish these stores as soon as possible after exercise so as to prevent LOPEH.

In order to replenish rapidly muscle and hepatic glycogen stores to decrease LOPEH risk post-exercise, it is generally recommended to consume meals with high CHO content immediately after exercise (Adolfsson et al. 2018; Riddell et al. 2017; Colberg et al. 2016). There is very limited research describing the type and amount of CHO that should be

ingested after exercise to prevent LOPEH and nocturnal hypoglycaemia following late afternoon or evening exercise.

One of the first studies to test the benefits of CHO consumption and insulin dose adjustment for the prevention of LOPEH in individuals with T1D was published by Campaigne and colleagues (1987). They assessed the role of additional CHO intake and reduction in insulin dose on blood glucose profile 12 hrs after an evening bout of 45 min exercise at 60% $\dot{V}O_2$ peak performed 2 hours post-dinner in nine men with T1D who were on two daily insulin injections with a combination of intermediate acting and soluble insulin dose. Despite the 50% insulin dose reduction or ingestion of extra CHO (~53 g), 6 out of their nine participants experienced hypoglycaemia within 5 hours of completing the exercise, independently of prior reduction in insulin dose or post-exercise feeding. Hence, it was concluded that neither insulin dose reductions nor additional caloric intake is totally effective at preventing post-exercise hypoglycaemia (Campaigne et al. 1987).

Further to the work of Campaigne and colleagues, a number of studies have examined how best to adjust insulin dose and/or increase CHO intake post-exercise to prevent LOPEH and associated nocturnal hypoglycaemia. In the late 1980s, a few studies reported that nocturnal hypoglycaemia in children (Bergada et al. 1989) and adults (Vervoort et al. 1996) with T1D can be reduced by a bedtime snack.

More recently, Hernandez and colleagues (2000; Table 2.3) investigated the effectiveness of different snacks at preventing LOPEH in individuals with T1D. The amount of CHO in the snack was calculated so that the total energy content of the snack was equivalent to 150% of the energy likely to be derived from CHO during exercise. They compared the effectiveness of an isoenergetic amount of whole milk (CHO 39.8 ± 12 g), skim milk (CHO 66.4 ± 20 g), and two commercially available sports drinks (CHO [121 ± 24 g], electrolytes) and (CHO [74 ± 18 g], fat and protein) at preventing LOPEH in seven individuals with T1D (Hernandez et al. 2000). These drinks were given in equal boluses 15 min before moderate intensity exercise performed at 60% $\dot{V}O_2$ peak in the late afternoon, during mid-exercise,

and 20 min after exercise. Most drinks, except whole milk, resulted in moderate hyperglycaemia (11 to 15 mmol/L) for up to 3 hours post-exercise. Persistent hyperglycaemia (11 to 17 mmol/L) from the end of exercise to 4 hours post-exercise was observed with the sports drink that contained fat and protein along with CHO. Of note, LOPEH occurred in 7 of the 28 trials, showing that in general the snacks were effective, but still fallible. Since the rate of decline in glycaemia in the evening, 4 to 8 hours after exercise did not differ between the whole milk and sports drink trials, the authors recommended that in order to prevent LOPEH 60 to 120 grams of CHO should be consumed before, during and after exercise in the form of whole milk or sports drink (Hernandez et al. 2000).

The studies referred to above showed that reduction or elimination of post-exercise nocturnal hypoglycaemia risk can be achieved by only relying on CHO supplementation, but this is more challenging to achieve without adjusting insulin dose (Campbell et al. 2014b). For this reason, basal and bolus insulin dose reductions combined with CHO intake are generally recommended to prevent LOPEH and nocturnal hypoglycaemia. For instance, the ISPAD guidelines recommend that individuals on MDI should ingest a CHO snack equivalent to 0.4 g/kg CHO at bedtime combined with a 20% reduction of basal analogue (e.g. insulin glargine, detemir, neutral protamine Hagedorn [NPH]) to decrease their risk of nocturnal hypoglycaemia (Taplin et al. 2010). If the basal analogue has a prolonged and more stable effect (e.g. insulin degludec or glargine), the dose reduction may have to be initiated before exercise.

For individuals treated with CSII, current guidelines also recommend the ingestion of CHO together with a 20% reduction in basal insulin infusion rate for 6 hours at bedtime to decrease the risk of nocturnal hypoglycaemia (Campbell et al. 2015b; Taplin et al. 2010). For prolonged activities, such as long distance walking or unusual activities (e.g. sports camps), Riddell and colleagues (2017) recommend a 30 to 50% reduction in basal insulin infusion rate all day and overnight or a 30-50% reduction of long-acting insulin the night before and on the day of the activity (Toni et al. 2006). With respect to bolus insulin dose

reduction, current guidelines recommend to reduce post-exercise meal insulin bolus by approximately 50% together with the intake of a low glycaemic index (GI) snack at bedtime (Campbell et al. 2014b).

Since hypoglycaemia risk is lower when bedtime blood glucose level is >7.0 mmol/L (Whincup and Milner 1987; Tansey et al. 2006), additional care is required when bedtime blood glucose level is <7.0 mmol/L, but no specific bedtime glucose value protects against nocturnal hypoglycaemia. For individuals with a history or recurrent hypoglycaemia, additional preventive measures are recommended, including glucose checks overnight, monitoring night time blood glucose levels with CGM with low glucose level alarms turned on, and the use of insulin pumps with PLGM or LGS features (Garg et al. 2014; Wilson et al. 2008).

There is evidence that adding protein in the post-exercise meal or snack provides an effective means to prevent LOPEH. Although the benefits of adding a small amount of protein to a bedtime snack for the prevention of LOPEH has been questioned previously (Hess and Beebe 1999; Franz 2000), some studies have shown that for an equivalent amount of energy and less CHO, protein-rich snack are equally effective as a standard CHO snack at preventing nocturnal hypoglycaemia in individuals with T1D, particularly when bedtime blood glucose levels are <7 mmol/L (Hess and Beebe 1999; Kalergis et al. 2003), a condition of high hypoglycaemia risk (Kalergis et al. 2001; Group 1991; Pramming et al. 1985; Schiffrin and Suissa 1987).

There is also evidence that protein intake causes a late increase in blood glucose levels (Hess and Beebe 1999; Peters and Davidson 1993; Saleh and Cryer 1997; Winiger et al. 1995; Smart et al. 2013), including night time in T1D (Hess and Beebe 1999; Saleh and Cryer 1997; Winiger et al. 1995). In addition, increasing the intake of protein (12.5 to 75 g) in a low-fat meal (<0.3 g), with equivalent amount of CHO (30 g), decreases glucose excursions in the early (0-60 min) postprandial period and increases blood glucose levels between 150 and 300 min post-meals in a dose-dependent manner (Paterson et al. 2017).

Similarly, the glycaemic effect of 75-100 g of proteins consumed alone without CHO or fat in individuals with T1D results in a delayed and sustained postprandial increase in blood glucose levels between 3 and 5 hours after the meal (Paterson et al. 2016).

Although, the exact mechanism underlying the glycaemia rising effect of protein remains to be elucidated, the most likely causes include amino acids-mediated stimulation of plasma glucagon levels and/or other counter regulatory hormones (Sherwin and Felig 1981; Brown et al. 2008; Palmer et al. 1976; Slag et al. 1981; Hedo et al. 1977; Wylie-Rosett 1988) and the gluconeogenic conversion of the amino acids derived from proteins to glucose (Linn et al. 1996; Felig et al. 1977).

2.3.3.7 Combining carbohydrate ingestion and insulin dose reduction

All forms of exercise, when performed under hyperinsulinaemic conditions, are likely to cause a decrease in blood glucose levels. When exercise is performed under hyperinsulinaemic conditions, combining CHO ingestion and insulin dose reduction pre-exercise are often recommended to preserve euglycaemia during exercise (Adolfsson et al. 2018). In particular, some guidelines (Riddell et al 2017) recommend a 75% reduction of the pre-exercise meal insulin bolus combined with the ingestion of a low GI snack or meal in preparation for exercise performed ~30 min after a meal. This recommendation is based on the work of West and colleagues (2011b).

In addition to showing that bolus insulin dose reduction together with CHO intake decreases the risk of hypoglycaemia if exercise is performed ~30 min after a meal, West and colleagues (2011b) also demonstrated that the fall in blood glucose levels during exercise was less for the 30 min condition compared with exercising 120 min after a meal and matched insulin bolus. Since there were no cases of hypoglycaemia when exercise was performed 30 min after a meal combined with the administration of a bolus of rapid-acting insulin, these authors questioned the recommendation of avoiding the injection of rapid acting insulin less than 2 hours before exercise (De Feo et al. 2006). On the basis of

these findings, some have recommended that protection against hypoglycaemia during and after exercise can be achieved by combining a 75% reduced dose of rapid-acting insulin (West et al. 2010; Rabasa-Lhoret et al. 2001) with the ingestion of low GI CHO (West et al. 2011a) 30 min before exercise.

One factor with the potential to affect the efficacy of insulin dose reduction combined with CHO intake at reducing hypoglycaemia risk is the glycaemic index (GI) of the CHO consumed. The GI provides a ranking of CHO-containing foods based on their acute glycaemic impact over time compared to that of a reference standard glucose solution (Wolever et al. 1991). Hence, GI is used as a means for classifying CHO containing foods according to their blood glucose responses post-ingestion (Wolever et al. 1991).

CHO with a low GI either contain a high proportion of fructose or galactose (low GI CHO) or are being digested at a slower rate than CHO with a high GI (Asp 1996). Lower GI food sources include wholegrain breads, legumes, pasta, and wholegrains such as oats, barley and quinoa. Other low GI foods include many fruits (temperate, citrus, most stone fruit and berries) and dairy foods. High GI CHO foods, such as white bread, rice and potatoes, cause a rapid increase in blood glucose concentrations as opposed to the more gradual increase and lower peaks in blood glucose levels following the ingestion of low GI CHO (Foster-Powell, Holt, and Brand-Miller 2002).

In order to determine whether the GI of the ingested CHO has to be controlled for when CHO intake is combined with insulin dose reduction prior to exercise, Bracken and colleagues (2012a; Table 2.2) compared the effect of ingesting isomaltulose or glucose alongside a 50% reduction in rapid-acting insulin 2 hours before an incremental run test followed by a 10-min distance trial exercise in seven T1D individuals. They found a 50% lower peak in blood glucose levels before running after the consumption of isomaltulose compared with glucose (Bracken et al. 2012a), and concluded that the consumption of a low GI CHO improves glycaemia to an extent similar to that elicited using high GI CHO (Bracken et al. 2012b).

These findings are corroborated by another study (Campbell et al. 2014b) where 10 male participants were subjected to a 75% rapid-acting insulin dose reduction prior to a pre-exercise meal ingested 60 min before 45 min of treadmill running (70% $\dot{V}O_2$ peak). Then, 60 min after exercise, participants in that study were given either a low GI or high GI isoenergetic meal with a 50% reduction in rapid-acting insulin dose followed 3 hours after exercise by a low GI or high GI snack. Consuming low GI food with a reduced rapid-acting insulin dose after evening exercise prevented postprandial hyperglycaemia, and both treatments prevented hypoglycaemia for 8 hours post-exercise (Campbell et al. 2014b). Of note, however, half the participants experienced nocturnal hypoglycaemia with both types of interventions (Campbell et al. 2014b).

In a subsequent study, Campbell and colleagues (2015b) showed that a 20% reduction in the dose of long-acting basal analogue on the day of exercise combined with a low GI meal was effective at preventing nocturnal hypoglycaemia. In this study, 10 male participants received either their normal daily basal insulin dose or 80% of that dose, and the following morning after an overnight fast they were exposed to a 75% rapid-acting insulin dose reduction prior to ingesting a pre-exercise meal 60 min before 45 min of treadmill running (70% $\dot{V}O_2$ peak). Then, 60 min after exercise, they were administered a low GI meal with a 50% reduction in rapid-acting insulin dose followed 3 hours later by a low GI snack. This strategy, involving a 20% reduction in basal insulin dose combined with reduced prandial bolus insulin dose and a low GI carbohydrate meal, prevented hypoglycaemia during and for 24 hours following evening exercise without causing any marked hyperglycaemia (Campbell et al. 2015b).

2.3.4 Factors affecting the efficacy of carbohydrate supplementation at preventing hypoglycaemia

2.3.4.1 Types of carbohydrates

The amount and type of CHO ingested can influence the rate of appearance of glucose in the circulation. There are different types of CHO including monosaccharides (e.g. glucose, fructose, galactose), disaccharides (e.g. sucrose, lactose) and polysaccharides (e.g. starch,

starch-derived maltodextrin); (Bracken et al. 2012b). Following ingestion, these CHO influence glucose rates of appearance in the circulation to different extents. This is due to differences in the rate of gastric emptying (Leiper et al. 2000; Schvarcz et al. 1993; Schvarcz et al. 1997) and the use of different transport systems by the enterocytes in the small intestine, namely the sodium/glucose co-transporter 1 (SGLT1) for glucose and galactose, and GLUT5 for fructose (Wood and Trayhurn 2003). Glucose entry into the bloodstream is rate-limited since the transport of glucose by SGLT1 is saturable and somewhat independent of the ingested glucose load when above saturation level. However, when a mixture of glucose and fructose is ingested, CHO transport rate into the bloodstream is enhanced due to the involvement of more than one transport system (Lina et al. 2002; Jeukendrup and Jentjens 2000).

In addition to the amount and type of CHO found in food, the type of food processing as well as the presence of protein, fat and fibre in food are all factors altering the rate at which glucose and fructose released from digested CHO-containing food enters into the blood (lafusco 2010). However, irrespective of the CHO being ingested, glucose maximum rate of appearance into the blood after its absorption for the gastrointestinal tract is approximately 1.0-1.1 g per minute. Hence, the maximum amount of CHO that can be absorbed per hour to maintain normal blood glucose levels during exercise is approximately 60-66 grams (Bracken et al. 2012b). This amount is comparable to the highest CHO intake recommended by the ADA for the prevention of hypoglycaemia in individuals exercising while plasma insulin is at a peak level.

As indicated above, the GI of CHO-containing food can also influence the rate of appearance of glucose into the circulation following its absorption. Lower daily mean blood glucose concentrations (Nansel et al. 2008), and reduced incidence of hypoglycaemia, and reductions in HbA1c levels (Brand et al. 1991; Gilbertson et al. 2001), are some of the many benefits of including low GI CHO in the diet of T1D individuals. Hence, dietary recommendations for healthy individuals with T1D promote healthy low-fat, low GI CHO choices (Bracken et al. 2012b). Low GI CHO are also recommended for

consumption well before (3 to 4 h) the start of exercise to maximise hepatic and muscle glycogen stores (Bracken et al. 2012b). However, high GI CHO is recommended just before and during exercise because they are rapidly absorbable CHO (Nathan et al. 2009).

A number of studies support the notion that the type of CHO as well as their combinations with other macronutrients can impact blood glucose response to exercise. For instance, Nathan and colleagues (1985) compared the glycaemic benefit of ingesting orange juice, whole milk and skim milk, each containing 13 g CHO, prior to 45 min of cycling at moderate intensity while under the influence of basal insulin. They showed that whole milk, which provided a sustained source of CHO release, was a better pre-exercise snack than rapid-acting CHO sources, such as orange juice and skim milk, at preventing post-exercise hypoglycaemia without causing hyperglycaemia during exercise (Nathan et al. 1985). More recently, Soo and colleagues (1996) found that supplementation with 30 grams CHO before moderate intensity performed in a basal insulinaemic state resulted in a marginally greater elevation of plasma glucose levels with glucose than with bread. The lack of marked differences between conditions is not surprising given that bread has a GI close to that of glucose.

This limitation, however, is not shared by the work of West and colleagues (2011a) who compared blood glucose responses following an overnight fast to the consumption of 75 g of either a low (isomaltulose; GI=32) or high GI CHO (dextrose; GI=96) 2 hours before a 45-min treadmill run in individuals with T1D. They showed that peak blood glucose response was twice as elevated post-exercise after the high compared to the low GI carbohydrate, with blood glucose area under the curve being 21% lower after ingesting the low GI CHO for the entire 180-min period after exercise. In addition, CHO oxidation rate was lower in the isomaltulose trial, thus making this treatment favourable to endurance performance due to the CHO sparing associated with it. On this basis, the authors concluded that the intake of a low GI CHO source may be beneficial for maintaining blood glucose within normal ranges after exercise (West et al. 2011a).

These findings are corroborated by the work of others (Bracken et al. 2012a; Table 2.2) who, as described above, compared the ingestion of isomaltulose or glucose alongside a 50% reduction in rapid-acting insulin 2 hours before an incremental run test followed by a 10-min distance trial exercise. This study reported a 50% lower peak in blood glucose concentration before running after the consumption of isomaltulose compared with glucose. Attenuation of absolute glycaemic response was found before, during and after exercise following isomaltulose consumption compared with glucose (Bracken et al. 2012a)

Only a few studies have examined the effect of ingesting different types of CHO on blood glucose profile for prolonged endurance events in individuals with T1D. Bally and colleagues (2017) compared the metabolic effects of co-ingesting glucose and fructose compared with only glucose prior to 90 min of moderate intensity exercise (50% $\dot{V}O_2$ max) performed under basal insulinaemic conditions few hours after breakfast. The glucose-fructose mixture or glucose was given only during the 90-minute exercise session to maintain stable glycaemia. The insulin levels were identical between the two conditions. This study showed that the co-ingestion of glucose-fructose resulted in a similar glycaemic profile, with higher fat oxidation and lower CHO oxidation rates during exercise being consistent with the sparing of glycogen. The increase in lactate levels during exercise was higher with the glucose-fructose co-ingestion although the exercise protocols were similar for the two conditions. This suggests that fructose was partially converted to lactate and hence fructose-derived lactate could provide another fuel during exercise while stable and similar glycaemia is maintained (Bally et al. 2017).

More recently, and as discussed earlier, Gray and colleagues (2016; Table 2.2) examined the influence of ingesting a high molecular mass carbohydrate waxy barley starch (WBS; GI = 98) on glycaemia in T1D compared with glucose. They found that WBS and glucose resulted in a similar hyperglycaemic responses, but a greater rate of CHO oxidation for WBS at rest (Gray et al. 2016).

2.3.4.2 Concentration of carbohydrates

Since the consumption of CHO drinks is generally recommended to prevent hypoglycaemia, this raises the issue of the optimal concentration of the CHO drinks that is most likely to prevent hypoglycaemia. At the origin of this issue is the well-established observation that drinks with a high CHO concentration reduce gastric emptying rates in healthy individuals without diabetes (Davis et al. 1990; Maughan and Leiper 1999; Murray et al. 1997), with a 6% (w/v) oral solutions of high GI CHO appearing to be the best in this regard (Riddell and Iscoe 2006a) since such a solution provides better gastric emptying rate compared to beverages which are more concentrated such as juice or carbonated drinks that delay gastric emptying and cause gastric upset.

Given the evidence that CHO drink concentrations affect gastric emptying rate, the efficacy of different concentrations of CHO drinks at preventing exercise-mediated hypoglycaemia has been compared in individuals with T1D. Perrone and colleagues (2005; Table 2.3) compared the effect of consuming a 8% (w/v) versus a 10%(w/v) CHO solution before and during cycling for 60 min at 55-60% $\dot{V}O_2$ peak 3 hrs after the last bolus insulin administration, and found the 10% (w/v) CHO treatment to be better at maintaining higher glycaemia and preventing hypoglycaemia (Perrone et al. 2005). However, these findings are highly questionable since insulin levels were not measured to assess whether they were matched between trials, and 25% more CHO was ingested with the 10% (w/v) compared to the 8% (w/v) CHO trial, thus making it impossible to determine whether the benefit of the 10% (w/v) CHO drink resulted from the concentration of this drink or the amount of CHO it provided as both variables were not matched between treatments.

2.3.4.3 Levels at which blood glucose is maintained during exercise

One factor that affects the amount of exogenous CHO required to maintain stable blood glucose levels during exercise is the level at which blood glucose is maintained during exercise. In this regard, Jenni and colleagues (2008) found that more exogenous glucose is required to maintain blood glucose at high and stable levels during exercise (Jenni et al.

2008), but with these glucose requirements being low early during moderate intensity exercise and elevated 1.5-2 hours later (Jenni et al. 2008). Indeed, although individuals with higher pre-exercise blood glucose concentrations have to make smaller adjustments to CHO intake to prevent exercise-induced hypoglycaemia (Kemmer and Berger 1983), the rate of glucose utilisation is enhanced at higher blood glucose concentrations (Jenni et al. 2008; Riddell et al. 1999), and more CHO must be administered to prevent blood glucose levels from falling when maintained stable at higher levels (Jenni et al. 2008). This is because, for a given plasma insulin level, the rate of peripheral glucose uptake increases with blood glucose concentrations (Jenni et al. 2008). In addition, hepatic glucose production during moderate intensity exercise is inhibited by high blood glucose levels (Jenni et al. 2008; Marliss and Vranic 2002).

Table 2.2 Summary of the literature investigating the types of carbohydrate (CHO) supplementation to manage blood glucose levels during exercise in T1D

Reference	Subjects	Timing of exercise	Exercise intensity and duration	Carbohydrate supplementation	Main findings
<i>Nathan et al (1985)</i>	5 adults with T1D (3M/2F)	Following an overnight fast	45 min of moderate intensity exercise after a 10 hour overnight fast	Three pre-exercise snacks: orange juice, whole milk and skim milk, each containing 13 g CHO ingested 15 min before exercise	Whole milk was found to be a better pre-exercise snack than orange juice and skim milk in preventing post-exercise hypoglycaemia without causing hyperglycaemia during exercise
<i>Soo et al (1996)</i>	9 adults with T1D (8M/1F)	Following an overnight fast	45 min of moderate intensity exercise at 60% $\dot{V}O_{2peak}$	30 g of CHO administered either as oral glucose 5 min prior or white bread 20 min before exercise	30 g CHO produced a moderate to substantial elevation of plasma glucose, slightly greater with glucose than with bread
<i>West, Morton et al (2011)</i>	8 adults with T1D (7M/1F)	Following an overnight fast and 2 hours after consumption of CHO	45-min treadmill run at 80% $\dot{V}O_{2peak}$	75 g of either isomaltulose (GI=32) or dextrose; (GI=96) 2 hours before exercise	Peak BGL responses were twice as great after the high GI dextrose compared with the low GI isomaltulose. Blood glucose area under the curve was 21% lower after low GI CHO ingestion for 3 h after exercise
<i>Bracken et al (2012a)</i>	7 adults with T1D	Following an overnight fast and 2 hours after consumption of CHO	Discontinuous incremental run to 80% - $\dot{V}O_{2peak}$ followed by a 10-min all-out performance test	0.6 g·kg ⁻¹ body mass of either dextrose (DEX; glycaemic index = 96) or isomaltulose (ISO; glycaemic index = 32), 2 hours before exercise	There were a 50% lower peak BGLs before running after consumption of isomaltulose compared with dextrose while maintaining the high-intensity running performance
<i>Bally et al (2017)</i>	15 male adults with T1D	3.5 hours after breakfast and insulin dose	90 min iso-energetic continuous cycling session at 50% $\dot{V}O_{2max}$	Glucose-fructose (GLUFRU) or glucose alone (GLU) were provided based on personalized CHO-intake regimens which were pre-determined during the familiarisation period	Total CHO needs to maintain stable BGLs during exercise were similar: 34g GLUFRU and 38 g GLU. Glucose-fructose co-ingestion advised due to a beneficial impact on fuel metabolism by increasing fat oxidation whilst sparing glycogen

<i>Gray et al (2016)</i>	7 adults with T1D (2M/5F)	Following an overnight fast and 2 hours after consumption of CHO	26 min of incremental treadmill run finishing at 80% VO_{2peak} followed by a 10 min performance run	0.6g/kg of dextrose (DEX) or waxy barley starch (WBS) 2 hours before exercise	WBS resulted in similar high BGL responses to dextrose, but a greater rate of CHO use at rest
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Table 2.3 Summary of the literature investigating carbohydrate (CHO) supplementation without insulin adjustment during exercise in T1D

Reference	Subjects	Timing of exercise	Exercise intensity and duration	Carbohydrate supplementation	Main Findings
<i>Riddell et al (1999)</i>	20 adolescents with T1D	100 min after breakfast and insulin dose	60 min of moderate intensity exercise	Ingested water (control trial) or 6-8% glucose solution (glucose trial) before and during exercise. The amount of glucose equal to the individual total CHO utilisation ingested in 6 divided doses 10 min before and each 10 min after the start of exercise	In the control trial, 9 participants as compared to only 3 in the glucose trial had BGL fall <4 mmol/l during exercise. Hence the amount of glucose ingested which was equal to total CHO utilisation prevented the fall in BGL during exercise. The total CHO utilisation averaged 1.46 ± 0.08 g/min during exercise in the water trial. The amount of glucose ingested was 87.3 ± 5.1 g (46-127 g) during the entire glucose trial
<i>Hernandez et al (2000)</i>	7 adults with T1D (6M/1F)	Late noon	60-min cycling at 60% - $\dot{V}O_{2peak}$. After 30 min, a 10-min rest period was carried out for fluid ingestion	Water (0 g CHO), whole milk (40 g), skim milk (66 g), sports drink A (121 g), and sports drink B (74 g) consumed in thirds immediately before, during, and after exercise. BGL monitored 12 hours post-exercise	No trial completely prevented hypoglycaemia. Milk trials had \downarrow pre-bed BGLs. During milk trials, no early morning incidents of hypoglycaemia; there was 1 incident under sports drink B. Consumption of CHO beverage before, during, and after exercise was advised, with the amount depending on level of glycogen depletion
<i>Perrone et al (2005)</i>	16 adults with T1D (10M/6F)	3 hrs after insulin dose	60-min continuous cycling at 55 to 60% $\dot{V}O_{2max}$	Participants consumed an 8% (5.4 g glucose; 2.6 g fructose per 100 mL) or 10% (6.7 g glucose; 3.3 g fructose per 100 mL) before and during exercise	4 incidents of hypoglycaemia during exercise under 8% and none with 10%. 60-min post-exercise BGL \downarrow under 8%, but \leftrightarrow under 10%. A 10% CHO solution recommended to avoid hypoglycaemia during exercise

<i>Dubé et al (2005)</i>	9 adults with T1D (6M/3F)	3 hours after breakfast	60-min continuous cycling at 50% $\dot{V}O_{2max}$	Ingested either 0, 15, or 30 g of glucose immediately before exercise	30 g delayed the time before glucose needed to be infused more than 15 g. 7 of 9 needed glucose infusion under 0 g, 4 of 9 under 15 g and 3 under 30 g. A glucose supplement of 40 g was estimated in order to maintain blood glucose levels within the normal range during and 1 hour after exercise
<i>Robitaille et al (2007)</i>	16 participants: 8 controls and 8 participants with T1D.	3 hours after insulin dose along with the breakfast	60 min moderate intensity exercise	30 g glucose load 15 min before exercise	Plasma glucose levels in participants with T1D dropped from 9.6 to 7.5 mmol/l and they showed a lower plasma glucose oxidation rate compared to healthy individuals. However, the total CHO and fat oxidation was not significantly different, indicating a larger oxidation of glucose derived from muscle glycogen
<i>Murillo et al (2015)</i>	18 athletes with T1D		10 km run lasting between 50 and 60 min	Testing a pre-exercise carbohydrate supplement of 0.7g/kg or 0.35 g/kg.	The lower dose of CHO studied 30 g (0.35g/kg) proved to be more effective for regulating glycaemic levels during exercise than a higher recommended dose of 55 g (0.7 g/kg).

2.3.5 Use of sprinting as a means to prevent hypoglycaemia during and after moderate intensity exercise

There are other strategies that have been proposed to reduce the risk of hypoglycaemia during and after exercise. Over a decade ago, it was reported that moderate-intensity exercise interspersed with several bouts of high-intensity sprints is associated with a lesser fall in blood glucose levels in comparison with continuous moderate intensity exercise alone (Guelfi et al. 2005a; Robertson et al. 2014; Iscoe and Riddell 2011; Campbell et al. 2015b; Maran et al. 2010). Multiple studies have also found since then that short sprints added to aerobic exercise can reduce the risk of hypoglycaemia during exercise and shortly thereafter (Tonoli et al. 2012; Fahey et al. 2012).

A 10-second maximal intensity sprint performed before (Bussau et al. 2007) or after (Bussau et al. 2006) a moderate-intensity exercise session has the potential to decrease the risk of hypoglycaemia during exercise. These results obtained in controlled laboratory settings are important on clinical grounds as they imply that the decline in blood glucose levels associated with sustained exercise of moderate intensity, such as jogging or light cycling, is reduced if this type of exercise is interspersed with several short 3-4 second sprints or combined with a 10-second sprint. These counter-intuitive findings that the rate of fall in glycaemia associated with exercise can be decreased by exercising even more vigorously (i.e. performing a sprint) are now included in some clinical practice consensus guidelines (Robertson et al. 2014; Adolfsson et al. 2018; Riddell et al. 2017; Colberg et al. 2016).

However, before advocating sprinting for reducing the risk of exercise-mediated hypoglycaemia, it is important to determine whether these in-clinic data are applicable for all insulin conditions. This is because the beneficial effect of repeated high intensity sprinting has been tested only under marginally elevated insulin conditions. Though the current guidelines advocate sprinting as a strategy for hypoglycaemia prevention during exercise, they do not specify the insulin conditions where this strategy will work, and the

populations of individuals who may not benefit from this strategy (e.g. elderly, obese individuals).

2.4 Conclusion

Based on the evidence currently available and described in the previous sections, a number of recommendations have been proposed to prevent exercise-mediated hypoglycaemia using pre-exercise CHO intake as a management tool. The literature review provided in this chapter was helpful in identifying the gaps in this key area and future research direction in order to inform exercise guidelines.

Following the literature review, a series of research questions listed in the previous chapter (chapter 1) were identified for investigating in this project. Given that the effects of exercise intensity and duration on the exogenous CHO requirements to maintain blood glucose at stable low or high levels under basal or hyperinsulinaemic conditions were still poorly understood at the time this thesis was initiated, our primary aims were to address these issues.

Chapter 3

Methods

Foreword

This chapter provides a general overview of the rationale of the experimental approaches adopted to conduct the studies undertaken in this research. In order to estimate precisely the effects of exercise intensity and plasma insulin levels on the exogenous glucose requirements to maintain stable blood glucose levels during exercise, this thesis has adopted the use of glucose clamp technique, which is considered to be the “gold standard” for assessing insulin action. The different types of clamps performed in the studies described in this thesis allow the precise quantification of the amount of exogenous glucose required to maintain stable glycaemia while exercising, and provide excellent experimental models to explore the glucoregulatory mechanisms involved. The details of this methodology of glucose clamp techniques and the incremental maximum oxygen uptake ($\dot{V}O_2$ peak) test performed to grade exercise intensity will be described in this chapter. To avoid repetition of content, the description of common methods provided in this chapter will be referred to in the relevant section of later chapters.

3.1 Rationale of the experimental approach adopted to achieve our aims

Given the main aim of the thesis was to determine the relationship between exercise intensity and the exogenous glucose infusion rate required to maintain euglycaemia during and early after exercise under basal and high insulin conditions, we had to find out the amount of glucose that is needed to maintain stable glucose levels at various exercise intensities. In order to achieve this, we performed a glucose clamp, which is considered to be the “gold standard” for assessing insulin action (DeFronzo et al. 1979) and graded the different exercise intensities by determining the incremental maximum oxygen uptake ($\dot{V}O_2$ peak) test.

The use of a glucose clamp provides an effective means to determine the relationship between exercise intensity and the exogenous glucose requirements to maintain stable glycaemia under basal and high insulinaemic conditions. The benefits of this experimental approach include the precision with which the exogenous glucose requirements to maintain euglycaemia are measured, and the opportunity it offers to explore the mechanism underlying the effect of exercise intensity and insulin levels on those requirements. In particular, this design allows the simultaneous determination of the rate of glucose appearance (Ra) and disappearance (Rd) obtained by infusing the non-radioactive stable isotope [6,6-²H]glucose, and the roles glucose Ra and Rd play in determining glucose requirements. For these reasons, this experimental approach was adopted to estimate the amount of glucose required to maintain stable glycaemia over a range of exercise intensities.

3.1.1 Exercise clamp studies

Glucose clamp is a technique that places the plasma glucose concentration under the investigator’s control. This technique, which is in routine use in our laboratory (Davey et al. 2013; McMahon et al. 2007; Guelfi et al. 2007), allows precise quantification of the amount of exogenous glucose required to maintain stable glycaemia during exercise. This is achieved by infusing insulin and glucose intravenously to maintain stable blood glucose

and insulin levels prior to exercise. Once this is achieved, exercise is initiated without changing the insulin infusion rate while varying the rate of glucose infusion to maintain blood glucose at a stable level. This glucose infusion rate (GIR) is then used to calculate the exogenous glucose requirements to maintain euglycaemia.

Since our aim was to maintain euglycaemia (defined here as blood glucose levels between 5 to 6 mmol/L), at basal and high insulin levels, the two types of glucose clamp we adopted were an euinsulinaemic-euglycaemic clamp and a hyperinsulinaemic-euglycaemic clamp, where stable blood glucose levels was maintained while infusing insulin at basal and high insulin rates respectively.

Euinsulinaemic-euglycaemic clamp. In this technique, stable blood glucose levels were maintained by infusing insulin at a basal rate. One approach to standardise insulin levels for the euinsulinaemic treatment across participants is to adopt for each participant the insulin infusion rate at which hepatic/renal glucose production is matched by the rate of peripheral glucose utilisation, with no exogenous glucose required to maintain euglycaemia (Marliss and Vranic 2002; Galassetti et al. 2003). This insulin infusion rate, operationally referred to as basal insulin infusion rate, results in plasma insulin levels comparable to those found in T1D individuals when their blood glucose is stable at euglycaemic level. Since individual insulin requirements vary, the insulin infusion rate required was determined by the participant's lowest basal insulin rate, if on insulin pump, or the dose of the long acting insulin (Glargine) if on a multiple dose insulin regimen.

To achieve this, the insulin pump (CSII) of each participant was disconnected and insulin (Humalog, Eli Lilly Australia Pty Ltd, Australia), at a concentration of 0.1 unit of insulin per ml infusate was infused using an Alaris Asena[®] GH syringe pump (Alaris Medical Systems, United Kingdom). Then stable glucose levels were maintained between 5 to 6 mmol/L by titrating the variable rate glucose infusion based on 5 to 15-minute glucose measures. The glucose assays were done using a YSI analyser (YSI, Yellow Springs, Ohio). The rate of insulin infusion was then adjusted, depending on the blood glucose value. This process

required minor changes to the rate of insulin infusion as well as small bolus amounts in some instances. This procedure was accompanied by a waiting period during which the effect of changing insulin infusion rate was observed and recorded. Once blood glucose level was stable, insulin infusion rate was decreased progressively in a stepwise fashion until insulin infusion rate reached a level where no exogenous glucose was required to maintain a stable glycaemia between 5 to 6 mmol/L. Insulin infusion rate was considered to have reached the basal level when the target range of blood glucose levels did not change for a period of 30 minutes despite no glucose being infused. Once this insulin rate was achieved, this rate remained constant for the duration of the experiment. This experimental approach has been used previously in our laboratory (McMahon et al. 2007).

Hyperinsulinaemic-euglycaemic clamp.

The hyperinsulinaemic-euglycaemic clamp technique adapted here is based on that performed by De Fronzo et al (1979). In this clamp technique, exogenous insulin is infused to create a hyperinsulinaemic plateau of plasma insulin concentration, while plasma glucose is maintained constant at euglycaemic levels by infusing exogenous glucose at variable rates.

Exogenous short-acting insulin (Humalog, Eli Lilly Australia Pty Ltd, Australia), at a concentration of 1 unit of insulin per ml infusate, was infused using an Alaris Asena[®] GH syringe pump (Alaris Medical Systems, United Kingdom) at a continuous rate of 30 mU/m²/min after receiving a priming dose of the same insulin during the initial 10 min in a logarithmically decreasing manner so as to raise plasma insulin acutely to attain serum insulin levels resembling those encountered after a meal (Bonora et al. 2000; Mallad et al. 2015). Since mean pre-prandial and peak therapeutic insulin levels in individuals on basal bolus insulin regimen are typically 120-180 and 420-480 pmol/L, respectively (Nielsen et al. 1995; Gulan et al. 1987), and thus within the physiological range observed after the ingestion of a large CHO-rich meal (270-600 pmol/L; (Lee and Woleve 1998; Chokkalingam et al. 2007a), our aim was to target plasma insulin levels of ~300-550 pmol/L.

We estimated that an insulin infusion rate of 30 mU/m²/min should enable us to attain such plasma insulin levels. This estimate was based on the work of others who showed that primed continuous rates of 20 and 40 mU/m²/min results in plasma insulin concentrations of 304 ± 42 pmol/l (Bonora et al. 1989) and 728 ± 35 pmol/L (DeFronzo et al. 1979), respectively. The insulin infusion rate adopted here was also chosen on the basis that it is associated with an increased hypoglycaemia risk due to plasma insulin reaching levels known to suppress completely hepatic glucose production in individuals with and without T1D (Nyholm et al. 1996; Heptulla et al. 2003; Rizza et al. 1981).

During the clamp procedure, the glucose levels were monitored every 5 to 10 mins, and the samples for determining insulin levels were collected at regular intervals. The rate of infusion of a 20% (w/v) dextrose solution was adjusted during the procedure following the published algorithm of De Fronzo and colleagues (1979) and blood glucose levels were maintained between 5-6 mmol/L. Steady state was attained 2 hours after initiation of the clamp. Once the desired insulin infusion rates were achieved, participants were required to cycle at a predetermined intensity while glucose was infused simultaneously to maintain stable blood glucose levels. The glucose infusion rate (GIR) thus obtained provided a minute to minute estimate of the exogenous glucose requirements to maintain stable glucose levels at a given exercise intensity. This information could not have been obtained using an experimental protocol where glucose is administered orally due to the challenging difficulty of maintaining stable blood glucose levels under this condition.

3.1.2 Determination of $\dot{V}O_2$ peak and grading of exercise intensity

The four different intensities of exercise tested in this project were determined by performing an incremental maximum oxygen uptake ($\dot{V}O_2$ peak) test during the first familiarisation session with the participant. During this test, we measured the peak rate of oxygen uptake ($\dot{V}O_2$ peak) and lactate threshold achieved on a cycle ergometer (Lode Corival Ergometer) connected to a computer running the Cyclemax software (UWA, Australia).

Participants cycled at an initial workload of 25 watts followed by subsequent progressive 25 watts increase of workload every 3 minutes until exhaustion, with blood sampled at each step for lactate assay. Breath by breath analysis was conducted using a Vmax spectra analysis system (SensorMedics Corporation, USA). Using 30-second epochs, a plateau in oxygen consumption rate (an increase of $< 150 \text{ ml kg}^{-1}\text{min}^{-1}$) and/or a respiratory exchange ratio greater than 1.15 during the last minute of exercise were the criteria for the achievement of $\dot{V}O_2$ peak. The results thus obtained were used to calculate the workloads corresponding to 35, 50, 65 and 80% of $\dot{V}O_2$ peak. These exercise intensities were chosen on the grounds that they correspond to light, moderate, and vigorous (low and upper) intensities (Norton et al. 2010). The lactate threshold was determined by calculating the power output corresponding to the greatest perpendicular distance from a regression line of lactate to workload as well as a straight line formed by the first and last points of the regression line (Cheng et al. 1992).

3.2 Elucidating glucoregulatory mechanisms

To understand the glucoregulatory mechanisms underlying the various glycaemic responses to different intensities of exercise, we infused the non-radioactive stable isotope $[6,6\text{-}^2\text{H}]$ glucose in order to determine the rate of glucose appearance (Ra) and disappearance (Rd). This enabled us to determine the extent to which the changes in glucose infusion rate (GIR) required to maintain stable blood glucose level during exercise resulted from an imbalance between the rates of glucose production and utilisation.

To achieve this, a blood sample was drawn for the determination of background enrichment of $[6,6\text{-}^2\text{H}_2]$ glucose once the basal insulin rate was achieved (3.2.1). Then, a priming bolus dose of 3.3 mg.kg^{-1} of the stable isotope $[6,6\text{-}^2\text{H}_2]$ glucose was administered. This was followed by the commencement of a constant infusion of $[6,6\text{-}^2\text{H}_2]$ glucose at the rate of $2.4 \text{ mg.kg}^{-1}.\text{h}^{-1}$ until the end of the experiment (Finegood et al. 1987; Guelfi et al. 2007b). Blood sampling to determine steady enrichment was conducted regularly at 15-minute intervals from 15 minutes prior to reaching isotopic equilibrium till the end of

exercise. Isotopic equilibrium was reached approximately 150 minutes after the start of the [6,6-²H₂]glucose infusion. During the recovery period, blood sampling to determine steady enrichment was conducted at 30 to 45-minute intervals.

Expired air was collected using an indirect calorimetry system (V Max Spectra; Sensor Medics Corp) starting 10 minutes before exercise, throughout exercise and for 15 minutes after exercise at the 45 minute recovery time to determine rates of O₂ consumption and CO₂ production.

3.2.1 Measurement of [6,6-²H₂]glucose enrichment and use of indirect calorimetry

The [6,6-²H₂]glucose enrichment was determined by gas chromatography-mass spectrometry (Bio analytical Mass Spectrometry Facility, The University of NSW, Sydney, Australia). The readings obtained were corrected for background enrichment of naturally occurring [6,6-²H₂]glucose, and the rates of endogenous glucose appearance (Ra) and disappearance (Rd) were calculated from the changes in glucose enrichment using the single compartment, non-steady-state model of Steele (Wolfe 2005), as modified by Finegood et al (Finegood and Bergman 1983).

The concomitant measurement of the rates of O₂ consumption ($\dot{V}O_2$) and CO₂ production ($\dot{V}CO_2$) by indirect calorimetry, made it possible to calculate the amount of carbohydrate oxidised during exercise. Exercise-mediated CHO oxidation rates were calculated from the $\dot{V}O_2$ and $\dot{V}CO_2$ measurements (Frayn 1983) using the non-protein respiratory quotient determined with the help of the equation $4.585 \dot{V}CO_2 - 3.226 \dot{V}O_2$ (Peronnet and Massicotte 1991). Fat oxidation rates and energy production during exercise were calculated using appropriate non-protein respiratory quotient stoichiometric equations that assumed negligible protein oxidation (Frayn 1983; Peronnet and Massicotte 1991). The information thus obtained could help explain differences between experimental treatments. A similar experimental approach has been used previously by our team to quantify the rate of exogenous glucose administration required to maintain blood glucose

at stable levels during exercise in participants with T1D (McMahon et al. 2007; Guelfi et al. 2007).

3.2.2 Measurement of glucoregulatory hormones

A cannula was inserted in the dorsum of one hand, and this hand was placed in a Hotbox (Omega CN370, Sydney, Australia) at approximately 60°C for the sampling of arterialised venous blood. Arterialised venous blood samples were sampled for the analyses of blood glucose and lactate levels using a YSI analyser (Yellow Springs Instrument, Yellow Springs, Ohio). Heparinized plasma was assayed for free insulin using a non-competitive immunoassay (Architect i2000SR; Abbott Laboratories, Abbott Park, IL USA). The lower limit of detection was <1µu/L. The reproducibility data with the mean/coefficients of variation (CV%) obtained from the quality control materials for this assay were 5/4.5; 14/30; 50/2.5 and 146/2.7%.

Heparinized plasma treated with sodium metabisulphite was used to determine epinephrine and norepinephrine levels by High Performance Liquid Chromatography (HPLC, UltiMate 2000; Thermo Fisher Scientific, Melbourne, VIC, Australia). The catecholamines were separated by reversed-phase chromatography on a C18 column (ESA HR-80; particle size 3 µm, dimension 80 x 4.0 mm) using a 0.22 µm (Durapore Membrane Filters 0.22 µm GV) filtered isocratic mobile phase (ESA Cat-A-Phase). Samples were placed in a temperature controlled sample carousel at 4°C. Column temperature was maintained at a constant temperature of 30°C. The mobile phase was maintained at 0.8 ml/min with a pump (UltiMate 3000 SD Pump) and sampler (UltiMate 3000 SL Autosampler) to inject 80 µl of extracted sample or standards. The electrochemical detector (ESA Cuolochem III) was used to detect and quantify extracted catecholamines in oxidation mode. The HPLC and data acquisition was under the control of the Chromeleon 7 software, with the potentials set to +200 mV for the conditioning cell, +100 mV for the analytical cell – electrode 1 and -100 mV for the analytical cell – electrode 2. The range for the analytical cell – electrode 1 was 100 µA and the analytical cell – electrode 2 was 20 nA. The filter was set to 5 seconds.

DHBA, a chemical that behaves similar to plasma catecholamines with regards to alumina extraction and electrochemical detection, was used as an internal standard. To quantify catecholamines, a 5 point calibration curve with a working range of 0 pg/mL to 1000 pg/mL for noradrenaline and adrenaline was used. The lower limit of detection was 20 pg/ml for noradrenaline and adrenaline. The Chromeleon 7 software was used to integrate the area under the curves and quantify unknowns based on the calibration curve. A bilevel quality control (Bio-Rad Lyphochek® Endocrine Control) was used at the start of each run to verify extraction efficiency and the calibration curve. Results were accepted if QC results were within ± 2 standard deviations of the target values.

Glucagon levels in EDTA-treated plasma collected with aprotinin were measured by radioimmunoassay using the Linco glucagon radioimmunoassay kit (St. Charles, MO). The analytical sensitivity of this assay is 18.5pg/ml with working ranges of 18.5 to 400pg/ml. The intra-assay CV was 4 to 6.6% and inter-assay CV was 7.3 to 13.5%. Serum was assayed for growth hormone levels using a non-competitive enzyme immunoassay with a chemiluminescent substrate (Siemens Immulite 2000XPi; Siemens Medical Solutions, Pleasanton, CA, USA). The lower limit of detection was <0.3mu/L. The reproducibility data with the mean/coefficients of variation (CV%) obtained from the quality control materials for this assay were 9.3/4.7; 26.7/4.4 and 43.5/4.4%.

Cortisol levels were assayed from venous serum by competitive chemiluminescent immunoassay (Abbott). The lower limit of detection was <15 nmol/L. The reproducibility data with the mean/coefficients of variation (CV%) obtained from the quality control materials for this assay were 48/8.7; 90/5.2; 430/2.9 and 43645/3.1%. Finally, C-peptide levels were determined by solid-phase competitive chemiluminescent enzyme immunoassay on an Immulite 2000 Analyser using the Immulite C-Peptide Assay kit (Diagnostic Products).

Chapter 4

Effect of exercise intensity on glucose requirements to maintain stable glycaemia during exercise in type 1 diabetes

Foreword

This chapter is about the first study undertaken in this thesis, where the first two thesis aims are addressed. These two aims were to investigate:

1. The relationship between exercise intensity and the rate of exogenous glucose infusion required to maintain stable blood glucose levels during and early after exercise when plasma insulin is near basal levels.
2. To elucidate the glucoregulatory mechanisms relating exercise intensity and glucose infusion rate.

The studies I completed to address these questions were published in *Journal of Clinical Endocrinology and Metabolism* which is included as chapter 4:

Shetty VB, Fournier PA, Davey RJ, Retterath AJ, Paramalingam N, Roby HC, Cooper MN, Davis EA, Jones TW. Effects of exercise intensity on glucose requirements to maintain euglycaemia during exercise in type 1 diabetes. *Journal of Clinical Endocrinology and Metabolism*. 2016;101(3): 972-980.

4.1 Abstract

Objective: No recommendations exist to inform the amount of carbohydrates required to maintain stable blood glucose levels during and after exercise of different intensities in individuals with type 1 diabetes (T1D). This is in part because the relationship between exercise intensity and the exogenous carbohydrate requirements to maintain stable euglycaemia in individuals with T1D remains to be determined. It was predicted that an inverted U relationship exists between exercise intensity and the amount of glucose required to maintain stable euglycaemia during exercise at basal insulinaemia. Our objective was to investigate this relationship and elucidate the underlying gluco-regulatory mechanisms.

Design and Participants: We subjected nine individuals with T1D (mean \pm SD; age of 21.5 ± 4.0 y, duration of disease 11.4 ± 6.4 y, glycated haemoglobin of $7.9 \pm 0.8\%$ [60 mmol/mol], body mass index of 25.4 ± 5.5 kg/m², $\dot{V}O_2$ peak of 34.8 ± 5.1 ml kg⁻¹min⁻¹ and lactate threshold of $59.9 \pm 5.9\%$ of $\dot{V}O_2$ peak) to a euglycaemic clamp, whereby euglycaemia was maintained by infusing insulin at a basal rate with concomitant infusion of [6,6-²H₂]glucose for determining glucose kinetics. Glucose was infused to maintain euglycaemia during and for 2 hours after exercise of different intensities (35, 50, 65, and 80% $\dot{V}O_2$ peak).

Results: The mean glucose infusion rate (GIR) to maintain stable euglycaemia during exercise increased with exercise intensity up to 50 (4.0 ± 1.6 g/h, $p < 0.05$) and 65% $\dot{V}O_2$ peak (4.1 ± 1.7 g/h), but no glucose was required at 80% $\dot{V}O_2$ peak. Glucose Ra and Rd increased with exercise intensity, and reached, together with plasma catecholamines, higher levels at 80% $\dot{V}O_2$ peak.

Conclusion: Our findings support the predicted inverted U relationship between exercise intensity and the exogenous glucose requirements to maintain stable euglycaemia when insulin is at a basal level. However, the relationship between intravenous and oral glucose requirement needs to be investigated to translate these GIR data to clinical practice.

4.2 Introduction

Regular exercise has numerous health benefits for people with type 1 diabetes (T1D) (Seeger et al. 2011). However, the increased risk of hypoglycaemia during exercise (Tansey et al. 2006) and recovery in these individuals (McMahon et al. 2007; Davey et al. 2013a), and the resulting fear of hypoglycaemia are important barriers to exercise (Brazeau et al. 2008), accounting for their reluctance to participate in sports, their low levels of physical activity (Valerio et al. 2007), and their below average fitness levels (Komatsu et al. 2005).

In T1D, one strategy to decrease the risk of exercise-mediated hypoglycaemia is to consume extra carbohydrates (CHO) before, during, and after exercise. Although there are many guidelines about the amount of CHO that should be ingested to prevent hypoglycaemia during and after exercise (Robertson et al. 2014; Chiang et al. 2014; Riddell et al. 2017; Colberg et al. 2016; Adolfsson et al. 2018; Craig et al. 2011), more evidence is needed to inform recommendations for varying exercise intensities. In particular, the relationship between exercise intensity and the exogenous glucose requirements to maintain stable euglycaemia has not been investigated, thus raising the question as to the insulin levels under which this relationship should be investigated. Given that the glycaemia lowering effect of exercise increases with plasma insulin levels (Francescato et al. 2004), it is generally recommended to exercise at a time of day when plasma insulin levels are at near basal levels (Robertson et al. 2014).

During moderate intensity exercise, the fall in glycaemia is usually related to the duration and intensity of exercise (Kemmer and Berger 1983; Rabasa-Lhoret et al. 2001). Although the effect of low to moderate intensity exercise on the exogenous CHO requirements to maintain stable euglycaemia under basal insulinaemic conditions has never been examined, this is not the case for aerobic exercise performed at a high intensity. Indeed, this type of exercise performed under basal insulinaemic conditions has been shown to increase transiently plasma glucose levels during and after the cessation of exercise (Mitchell et al. 1988; Marliss and Vranic 2002; Purdon et al. 1993; Sigal et al. 1999), thus

implying that no glucose ingestion is required to prevent blood glucose levels from falling when intense exercise is performed under these conditions. Based on these observations, the objective of this study was to test the hypothesis that there is an inverted U relationship between exercise intensity and the amount of exogenous CHO required to maintain stable euglycaemia under basal insulinaemic conditions, with no glucose ingestion required when exercise is intense, and to elucidate the glucoregulatory mechanisms underlying this relationship.

4.3 Participants and methods

4.3.1 Participants

Nine individuals with T1D (6F, 3M), aged 14.6-25.0 y at the start of the study (2 adolescents, 7 adults), who had c-peptide <0.05 nmol/L, with a mean \pm SD age of 21.5 ± 4.0 y, duration of disease of 11.4 ± 6.4 y, glycated haemoglobin of $7.9 \pm 0.8\%$ (60 mmol/mol), body mass index of 25.4 ± 5.5 kg/m², $\dot{V}O_2$ peak of 34.8 ± 5.1 ml kg⁻¹min⁻¹ and a lactate threshold of $59.9 \pm 5.9\%$ of $\dot{V}O_2$ peak were enrolled in this study. None of the participants were pre-pubertal. All participants were recreationally active (4.8 ± 2.3 h/week) and free from diabetes complications. The participants were not taking any medications other than insulin. Three females were on oral contraceptive pills. Five participants were on a subcutaneous insulin infusion pump, and four on multiple daily insulin injections. Each participant attended an initial familiarisation session followed by four testing sessions. The protocol was approved by the Princess Margaret Hospital for Children Human Research Ethics Committee, and informed consent obtained from the participants and their parents if aged below 18 y.

4.3.2 Familiarisation session

During the familiarisation session, anthropometric measurements were taken, and participants completed a peak rate of oxygen consumption ($\dot{V}O_2$ peak) test on a cycle ergometer (Lode Corival Ergometer) connected to a computer fitted with a Cyclemax software (UWA, Australia) to assess their aerobic fitness (Chapter3; 3.1.2). Initial workload

was set at 25 watts and was subsequently increased by 25 watts every 3 min until exhaustion, with blood sampled at each step for lactate assay. Breath by breath analysis was conducted using a Vmax spectra analysis system (V max Spectra, SensorMedics Corporation, USA). $\dot{V}O_2$ peak was the highest rate of oxygen consumption reached in the incremental test using the 30-second epochs, and workloads corresponding to 35, 50, 65 and 80% of $\dot{V}O_2$ peak were calculated. These exercise intensities were chosen on the grounds that they correspond to light, moderate, and vigorous (low and upper) intensities (Norton et al 2010).

4.3.3 Testing sessions

After the familiarisation session, all participants completed up to 40 min of exercise at 4 different exercise intensities on 4 separate days, each administered following a randomised, counterbalanced study design, and separated by at least 1 week. Female participants not on oral contraceptive pills were investigated during the follicular phase of their menstrual cycles (day 8 ± 3), with 4 weeks between testing sessions. Testing was rescheduled if the participants experienced hypoglycaemia 48 hours prior to study, and they were required to abstain from caffeine, alcohol, injecting insulin to the legs and any physical activity other than light walking 24 hours before each study since both antecedent hypoglycaemia (Galassetti et al. 2003) and antecedent exercise (Galassetti et al. 2001a) affect the glucoregulatory responses to subsequent exercise. For these reasons, participants were fitted with a real time continuous glucose monitoring system (CGMS) for two days before testing. In addition, participants were required to keep a food diary for 24 hours prior to their first study, and then match their food intake the day before their subsequent three studies.

On the morning of testing, each participant arrived in the laboratory at 8:00am after an overnight-fast. Participants on multiple daily injections (MDI) insulin regimes were instructed to decrease their Glargine dose by 50% the night before the study and skip their morning bolus of insulin. For those on insulin pumps, the pump was disconnected on arrival. A cannula was inserted in the dorsum of one hand, and this hand was placed in a

Hotbox (Omega CN370, Sydney, Australia) at approximately 60°C for the sampling of arterialised venous blood. Another cannula was inserted in the contralateral antecubital fossa for the infusion of glucose and insulin.

4.3.3.1 Determination of basal insulin requirements

The initial insulin infusion rate was set at the participant's basal rate estimated either by the participant's lowest basal insulin infusion rate if on the insulin infusion pump or the dose of the long-acting insulin if on the multiple-dose insulin regimen. For each participant, insulin (Humalog, Eli Lilly Australia Pty Ltd, Australia) at a concentration of 0.1 unit of insulin per ml infusate was infused using an Alaris Asena[®] GH syringe pump (Alaris Medical Systems, United Kingdom). While insulin was infused, glucose levels were maintained between 5 and 6mmol/L by titrating the variable glucose infusion rate based on 5 to 15-minute glucose assays performed using a YSI glucose analyser (YSI, Yellow Springs, Ohio). Once blood glucose level was stable, insulin infusion rate was decreased progressively in a stepwise fashion until insulin infusion rate reached a level where no exogenous glucose was required to maintain a stable glycaemia between 5 to 6 mmol/L (euinsulinaemic-euglycaemic clamp; Chapter 3, 3.1.1). Insulin infusion rate was considered to have reached a basal level when the target range of blood glucose levels did not change for a period of 30 min while no glucose was infused at a constant insulin infusion rate. Once this insulin infusion rate was achieved, this rate remained constant for the duration of the experiment. This experimental approach has been used previously in our laboratory (McMahon et al. 2007) and by others (Marliss and Vranic 2002; Galassetti et al. 2003).

In order to determine the rate of glucose appearance (Ra) and disappearance (Rd), the non-radioactive stable isotope [6,6-²H]glucose was infused. To this end, a blood sample was drawn for the determination of background enrichment of [6,6-²H₂]glucose, and once basal insulin rate was achieved, a priming bolus dose of 3.3 mg.kg⁻¹ of [6,6-²H₂]glucose was administered. This was followed by the constant infusion of [6,6-²H₂]glucose at the rate of 2.4 mg.kg⁻¹.h⁻¹ until the end of the experiment. Blood sampling to determine

steady [6,6-²H₂]glucose enrichment was conducted at 15 to 30-minute intervals from 15 min prior to reaching isotopic equilibrium until the end of the testing session.

4.3.3.2 Exercise phase

Once isotopic equilibrium (approximately 150 min after the start of the [6,6-²H₂]glucose infusion) and stable euglycaemia with no exogenous glucose infusion were achieved for at least 45 min, blood samples were collected for baseline measurements of glucoregulatory hormones, metabolites, and [6,6-²H₂]glucose enrichment. Expired air was collected using an indirect calorimetry system (V Max Spectra; Sensor Medics Corp, Yorba Linda, California) for at least 10 min to determine baseline rates of O₂ consumption and CO₂ production. Then, at approximately 12:30 pm, each participant was randomised and subjected on separate days to the following treatments for 40 min or until fatigue on the same cycle ergometer as that used for $\dot{V}O_2$ peak testing: exercise at (a) 35% (b) 50% (c) 65%, and (d) 80% $\dot{V}O_2$ peak (fit individuals can sustain 80% $\dot{V}O_2$ peak intensity for at least 30 min; (Warren et al. 2009), with heart rate monitored continuously at each exercise intensity. These exercise intensities correspond to light, moderate, vigorous (low), and vigorous (high) intensity, respectively (Haskell et al. 2007).

During exercise, the rate of constant [6,6-²H₂]glucose tracer infusion was increased as described by others depending on the intensity of exercise to avoid marked changes in isotopic enrichments (Romijn et al. 1992, 2000; Manzon et al. 1998; Van Loon et al. 2001; Horton et al. 2005; Jenni et al. 2008; Sigal et al. 1999) and to assure the validity of glucose turnover calculations (Fisher et al. 1996). The rate of tracer infusion remained the same for exercise at 35% $\dot{V}O_2$ peak and was doubled for 50 and 65% $\dot{V}O_2$ peak. For exercise at 80% $\dot{V}O_2$ peak, the rate was doubled at the start of exercise and increased 3.5-fold 5 min into exercise, and until the end of exercise. The rate of tracer infusion was similarly decreased in a stepwise manner after exercise, and returned to the pre-exercise rate and maintained at this rate until 2 hours post-exercise. During the recovery period, blood sampling to determine steady enrichment was conducted at 30 to 45-minute intervals.

Insulin infusion rate remained unchanged and glycaemia was maintained between 5.0 and 6.0 mmol/L by measuring blood glucose levels every 5 min and by adjusting glucose infusion rate (GIR) of a 20% (w/v) dextrose solution accordingly. Before, during and hourly after exercise, the rates of O₂ consumption ($\dot{V}O_2$), CO₂ production ($\dot{V}CO_2$) and respiratory exchange ratio (RER) were measured using a mask linked to an indirect calorimetry system (V Max Spectra, SensorMedic, Viasys Australia) to calculate the rate of CHO oxidised. Throughout the 2-hour period post-exercise, the participants rested in a seated position.

4.3.3.3 Measurement of glucoregulatory hormones and [6,6-²H₂]glucose enrichment

Arterialised venous blood samples were taken for the analyses of blood glucose and lactate levels as well as the levels of insulin and glucoregulatory hormones (epinephrine, norepinephrine, glucagon GHs, and cortisol) as described in chapter 3 (3.2.2). Briefly, the methods employed for analysis were: HPLC for epinephrine and norepinephrine levels, RIA for glucagon, non-competitive immunoassay for insulin, GH and cortisol levels.

The [6,6-²H₂]glucose enrichment of the arterialised blood samples was determined by gas chromatography-mass spectrometry (Bio analytical Mass Spectrometry Facility, The University of NSW, Sydney, Australia). The readings obtained were corrected for background enrichment of naturally occurring [6,6-²H₂]glucose, and the rates of endogenous glucose appearance (Ra) and disappearance (Rd) were calculated from the changes in glucose enrichment using the single compartment, non-steady-state model of Steele (Wolfe 2005), as modified by Finegood et al (1983).

4.3.4 Calculations

The glucose requirements to maintain euglycaemia during exercise and recovery was obtained from the GIR during exercise and recovery, respectively. Glucose Ra shown in Fig 4.1 is the endogenous glucose Ra calculated by subtracting GIR from total rate of glucose appearance. Exercise-mediated CHO oxidation rates were calculated from the $\dot{V}O_2$ and $\dot{V}CO_2$ measurements (Frayn 1983) using the non-protein respiratory exchange ratio

determined using the equation $4.585 \dot{V}CO_2 - 3.226 \dot{V}O_2$ (Peronnet and Massicotte 1991). Fat oxidation rates and energy expenditure during exercise were calculated using appropriate non-protein respiratory quotient stoichiometric equations (Peronnet and Massicotte 1991; Frayn 1983).

4.3.5 Statistical analyses

Data were analysed using one-way (treatment) or two-way repeated-measures ANOVA (treatment and time) and Fisher's least significant difference test for posteriori analysis using SPSS software (version 20.0; SPSS, Chicago, IL, USA). Statistical significance was accepted at $p < 0.05$. Unless otherwise stated, all results are expressed as mean \pm SEM. This was an exploratory study, as while we had a hypothesis as to the relationship between intensity and glucose requirements, we had no preliminary data. A pragmatic target sample size of 10 was selected based largely on logistical elements of the study - cost of sessions, access to eligible participants, and time and burden on the participants.

4.4 Results

4.4.1 Participant's response to exercise

All nine participants completed the 40-min of exercise at 35 and 50% $\dot{V}O_2$ peak. Seven completed the 40-min exercise at 65% $\dot{V}O_2$ peak; with two participants stopping exercise at 30 min and 15 min, respectively, because they could not sustain exercising at this intensity. For the exercise trial at 80% $\dot{V}O_2$ peak, three participants completed the 40-min exercise bout, with the remaining six participants stopping exercising at 20, 10, 12, 10, 24 and 8.5 min, respectively. Relative to lactate threshold, the exercise intensities of 35, 50, 65 and 80% $\dot{V}O_2$ peak corresponded to intensities (mean \pm SD) of 61 ± 6 , 87 ± 8 , 113 ± 10 and 139 ± 13 % lactate threshold. The inability of some of our participants to complete the 40-min exercise trial at 65 and 80% $\dot{V}O_2$ peak is most likely due to the exercise intensity being well above their lactate threshold and thus difficult to sustain. Due to the differences in duration of the exercise performed at 65 and 80% $\dot{V}O_2$ peak, data recorded only during

baseline, mid-exercise and end of exercise were used in analysis of glucose kinetics and comparison of glucoregulatory hormones.

4.4.2 Euglycaemic clamp and glucose infusion rates to maintain euglycaemia

During all the euglycaemic clamps, plasma glucose levels were maintained between 5.0 and 6.0 mmol/L, with no difference between trials except during exercise at 80% $\dot{V}O_2$ peak, where glucose levels increased by 0.5 mmol/L ($p < 0.05$, Fig 4.1A). Plasma insulin levels were similar at all exercise intensities (120 ± 13 , 118 ± 22 , 126 ± 19 , and 100 ± 19 pmol/L at the commencement of the exercise performed at 35, 50, 65 and 80% $\dot{V}O_2$ peak, respectively). Plasma insulin levels remained stable during exercise and recovery, with no difference between trials ($p > 0.05$, Fig 4.2A).

The individual GIR to maintain euglycaemia during exercise varied from 0 to 15 g/h across all exercise intensities (Table 4.1), except for exercise at 80% $\dot{V}O_2$ peak, where none of the participants required any glucose. Two participants did not require any glucose under all four exercise intensities (Table 4.1). The mean GIR to maintain euglycaemia during exercise increased with increasing exercise intensity from 2.0 ± 0.9 g/h at 35% $\dot{V}O_2$ peak to 4.0 ± 1.6 g/h and 4.1 ± 1.7 g/h at 50 and 65% $\dot{V}O_2$ peak, respectively, which in turn were higher ($p < 0.05$) than at 80% $\dot{V}O_2$ peak where no glucose was required (Fig 4.3). The highest mean GIR of 4.1 g/h required at exercise intensity of 65% $\dot{V}O_2$ peak was equivalent to a GIR relative to body mass of 1 mg/kg/min. The individual GIR to maintain euglycaemia during the 2-hour post-exercise period varied from 0 to 10.4 g/h, and all participants required glucose to maintain euglycaemia during the 2-hour recovery from exercise at 65% $\dot{V}O_2$ peak (Table 4.2). There was no difference in the mean GIR between participants on pump and insulin injections ($p > 0.05$), as well as between the first- and second-hour post-exercise across all intensities ($p > 0.05$). There was also no difference in the mean GIR to maintain euglycaemia during the 2-hour recovery following exercise performed at 35, 50 and 80% $\dot{V}O_2$ peak ($p > 0.05$); however, the mean GIR during the first hour post-exercise at 65% $\dot{V}O_2$ peak (3.5 ± 1.3 g/h) was significantly higher ($p < 0.05$) than at 80% $\dot{V}O_2$ peak (1.5 ± 1.1 g/h; Table 4.2).

Table 4.1 Glucose infusion rate (g/h) for individual participants during exercise performed at four different intensities

	Glucose infusion rate (g/h) at different exercise intensities			
	35 % $\dot{V}O_{2peak}$	50 % $\dot{V}O_{2peak}$	65 % $\dot{V}O_{2peak}$	80 % $\dot{V}O_{2peak}$
Participant 1	0	6	15	0
Participant 2	4.5	9.4	0	0
Participant 3	0	0	7.2	0
Participant 4	0	0	0	0
Participant 5	0	0	0	0
Participant 6	0.7	3	0	0
Participant 7	2.5	0.5	3.3	0
Participant 8	3.2	4.6	5.6	0
Participant 9	7.1	12.2	5.5	0
Mean \pm SEM	2.0 \pm 0.9*	4.0 \pm 1.6*[†]	4.1 \pm 1.7*	0

*P < 0.05 vs 80% $\dot{V}O_{2peak}$. [†]P < 0.05 vs 35% $\dot{V}O_{2peak}$.

Table 4.2 Glucose infusion rate (g/h) for individual participants during the 2-hour post-exercise period after four different exercise intensities

Glucose infusion rate (g/h) during the first and second hour after exercise of different intensities								
	35% $\dot{V}O_{2peak}$		50% $\dot{V}O_{2peak}$		65% $\dot{V}O_{2peak}$		80% $\dot{V}O_{2peak}$	
	1st h	2nd h						
Participant 1	0	0	1.4	0.3	0	7.3	0	0
Participant 2	0.3	0	3.0	4.2	0.8	0	0	0
Participant 3	0	4.5	0	0	9.0	3.6	10.4	5.2
Participant 4	0	0	0	0	1.3	0.2	0	2.2
Participant 5	2.9	4.5	0	1.8	0	4.5	0	0
Participant 6	1.2	5.0	5.0	5.2	7.8	6.6	0	0
Participant 7	5.1	6.0	2.5	3.7	0.2	0	0.3	0
Participant 8	2.0	3.4	4.6	6.4	8.1	2.0	2.8	2.0
Participant 9	7.0	4.5	8.2	7.6	4.3	8.0	0	4.7
Mean \pm SEM	2.1\pm0.8	3.1\pm0.8	2.7\pm1.0	3.2\pm1.0	3.5* \pm1.3	3.6\pm1.1	1.5\pm1.1	1.6\pm0.7

*P < 0.05 vs 1st h, 80% $\dot{V}O_{2peak}$.

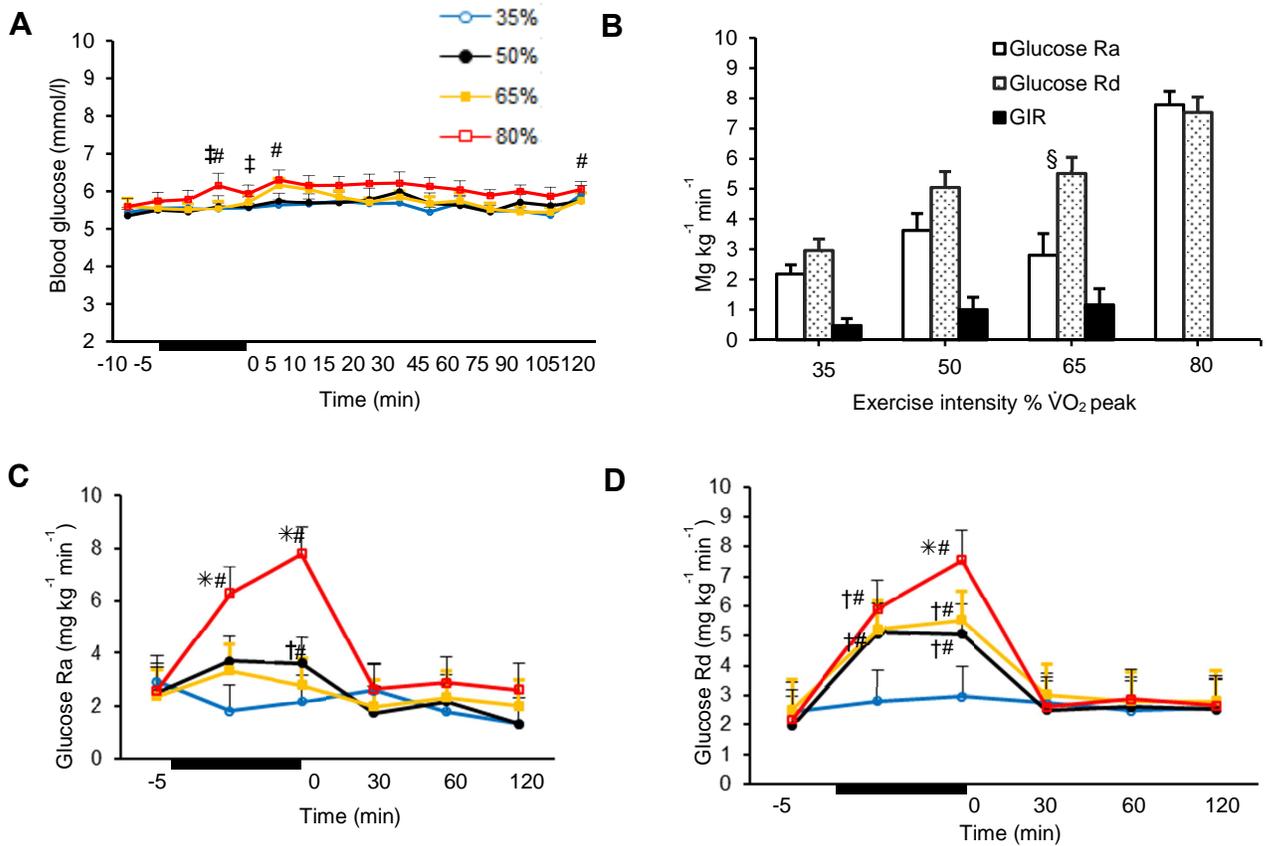


Figure 4.1. Effect of exercise intensities at 35% (blue open circles), 50% (black circles), 65% (yellow squares) and 80% $\dot{V}O_2$ peak (red open squares) on blood glucose levels, glucose Ra, glucose Rd and glucose infusion rate (GIR). (A) Blood glucose levels during exercise and for 2 hours post-exercise. (B) Effect of exercise intensity on glucose Ra, glucose Rd at the end of exercise, and GIR during exercise. (C) Effect of exercise on glucose Ra. (D) Effect of exercise on glucose Rd. All data are mean \pm SEM (n=9). Horizontal bar, exercise. #P<0.05 vs baseline. *P<0.05 vs 35, 50 & 65% $\dot{V}O_2$ peak. †P<0.05 vs 35 & 50% $\dot{V}O_2$ peak, ‡P<0.05 vs 35% $\dot{V}O_2$ peak, §P<0.05 glucose Rd vs Ra 65% $\dot{V}O_2$ peak.

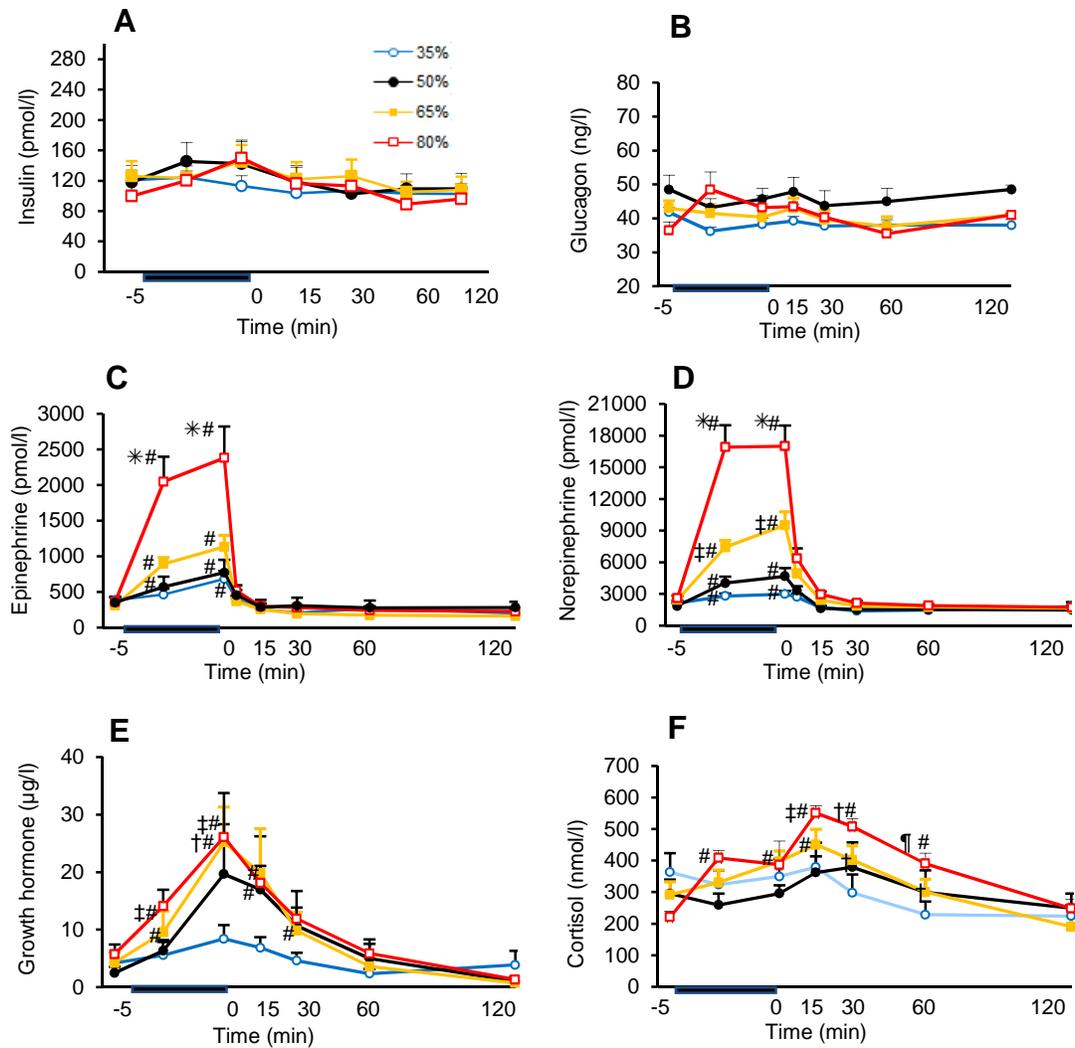


Figure 4.2. Hormonal response to exercise at intensities of 35% (blue open circles), 50% (black circles), 65% (yellow squares) and 80% $\dot{V}O_2$ peak (red open squares). (A) Plasma insulin levels. (B) Plasma glucagon levels. (C) Plasma epinephrine levels. (D) Plasma norepinephrine levels. (E) Serum growth hormone levels. (F) Serum cortisol levels. All data are mean \pm SEM ($n=9$). Horizontal bar, exercise. # $P < 0.05$ vs baseline. * $P < 0.05$ vs 35, 50 & 65% $\dot{V}O_2$ peak. ‡ $P < 0.05$ vs 35 & 50% $\dot{V}O_2$ peak. † $P < 0.05$ vs 35% $\dot{V}O_2$ peak.

4.4.3 Tracer kinetics, rates of endogenous glucose appearance (Ra) and disappearance (Rd)

The glucose enrichment was stable for 15 minutes prior to exercise. There was no significant difference in enrichment 15 and 5 min prior to exercise for all 4 exercise intensities ($p > 0.05$), with these enrichments 15 and 5 min prior to exercise being 0.0448 ± 0.0008 and 0.0448 ± 0.0007 at 35% $\dot{V}O_2$ peak; 0.0446 ± 0.0006 and 0.0449 ± 0.0006 at 50% $\dot{V}O_2$ peak; 0.0452 ± 0.0005 and 0.0457 ± 0.0005 at 65% $\dot{V}O_2$ peak; and 0.0454 ± 0.0011 and 0.0457 ± 0.0012 at 80% $\dot{V}O_2$ peak respectively. Basal glucose Ra and Rd prior to exercise were equivalent in all four trials. In response to exercise, glucose Ra did not change significantly during exercise at 35, 50 and 65% $\dot{V}O_2$ peak, except at the end of exercise at 50% $\dot{V}O_2$ peak where glucose Ra was significantly higher than basal (Fig 4.1C). Glucose Rd increased with increasing exercise intensity, rapidly declining to baseline within 30 min post-exercise (Fig 4.1D). The glucose Rd was higher than glucose Ra at the lower 3 intensities, but the difference was significant only at 65% $\dot{V}O_2$ peak (Fig 4.1B). During exercise at 80% $\dot{V}O_2$ peak, there was a significant increase in both glucose Ra and Rd (Fig 4.1C & 4.1D), but glucose Rd was similar to glucose Ra ($p > 0.05$; Fig 4.1B).

4.4.4 Cardiorespiratory and metabolic variables

Prior to exercise, there were no differences in heart rate, rate of oxygen consumption ($\dot{V}O_2$), rate of carbon-dioxide production ($\dot{V}CO_2$), CHO and fat oxidation rate, energy production and lactate levels between the four exercise trials ($p > 0.05$). In response to exercise, all these parameters except fat oxidation rate increased progressively with intensity, with maximal level attained at 80% $\dot{V}O_2$ peak (Table 4.3). The lactate levels of 5.19 ± 0.39 mmol/l at the end of 80% exercise intensity seems low for such high intensity. This is due to early fatigue and the short duration of exercise.

Table 4.3 Comparison of the effect of exercise intensity on cardio-respiratory and metabolic variables at rest and end of exercise

		Exercise Intensity (% $\dot{V}O_2$ peak)			
		35%	50%	65%	80%
Heart rate (beats/min)	Rest	89±6	98±5	95±2	80±3
	Exercise	119±4 ^{#*}	149±6 ^{#,†}	160±6 [#]	174±5 [#]
$\dot{V}O_2$ (L/min)	Rest	0.32±0.03	0.30±0.04	0.31±0.03	0.32±0.03
	Exercise	0.90±0.08 ^{#,*}	1.30±0.15 ^{#,‡}	1.80±0.19 ^{#,†}	2.10±0.20 [#]
$\dot{V}CO_2$ (L/min)	Rest	0.26±0.03	0.25±0.03	0.25±0.03	0.26±0.03
	Exercise	0.80±0.07 ^{#,*}	1.20±0.13 ^{#,‡}	1.70±0.19 ^{#,†}	2.20±0.23 [#]
RER	Rest	0.80±0.02	0.79±0.02	0.80±0.02	0.80±0.02
	Exercise	0.86±0.01 ^{#,*}	0.90±0.01 ^{#,‡}	0.95±0.02 ^{#,†}	1.04±0.02 [#]
Fat oxidation (g/min)	Rest	0.11±0.02	0.20±0.02	0.10±0.02	0.11±0.02
	Exercise	0.20±0.03 ^{#,†}	0.21±0.05 [#]	0.14±0.07 [†]	0 [#]
CHO oxidation (g/min)	Rest	0.15±0.03	0.11±0.02	0.14±0.02	0.14±0.03
	Exercise	0.69±0.09 ^{#,*}	1.25±0.16 [#]	2.02±0.29 [#]	3.29±0.45 [#]
Energy cost (KJ/min)	Rest	6.52±0.58	6.31±0.75	6.27±0.66	6.48±0.71
	Exercise	18.41±1.75 ^{#,*}	27.44±3.14 ^{#,‡}	36.53±3.97 ^{#,†}	44.3±4.31 [#]
Lactate (mmol/l)	Rest	0.54±0.07	0.47±0.04	0.52±0.03	0.47±0.03
	Exercise	0.82±0.12 ^{#,*}	1.54±0.34 ^{#,‡}	3.17±0.52 ^{#,†}	5.19±0.39 [#]

All data are mean ± SEM. [#]P < 0.05 vs Rest. ^{*}P < 0.05 vs 50, 65 & 80% $\dot{V}O_2$ peak. [†]P < 0.05 vs 65 & 80% $\dot{V}O_2$ peak. [‡]P < 0.05 vs 80% $\dot{V}O_2$ peak.

4.4.5 Hormonal responses

The levels of all hormones prior to exercise were similar between trials (Fig 4.2). Epinephrine and norepinephrine levels increased during all exercise intensities, with the highest levels attained in response to exercise at 80% $\dot{V}O_2$ peak ($P < 0.05$). At the end of exercise, catecholamines decreased rapidly to basal levels within 30 min post-exercise (Fig 4.2C & 4.2D). Glucagon levels did not change significantly in response to all exercise intensities (Fig 4.2B). Growth hormone levels increased in response to exercise, reaching peak levels at the end of exercise, and declining to baseline values within 60 min after exercise (Fig 4.2E). Cortisol levels also increased in response to exercise, but with peak levels reached 15 min post-exercise, returning to pre-exercise levels within 120 min after exercise (Fig 4.2F).

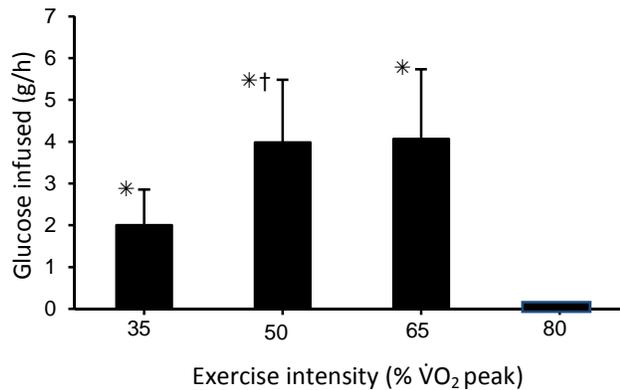


Figure 4.3. Rate of glucose infusion during exercise performed at intensities of 35, 50, 65 and 80% $\dot{V}O_2$ peak. All data are mean \pm SEM. * $P < 0.05$ vs 80% $\dot{V}O_2$ peak, $^\dagger P < 0.05$ vs 35% $\dot{V}O_2$ peak.

4.5 Discussion

The primary aim of this study was to investigate the relationship between exercise intensity and the exogenous glucose requirements to maintain stable euglycaemia under basal insulinaemic conditions. We found an inverted U relationship between exogenous glucose requirement and exercise intensity (Fig 4.3), with mean GIR to maintain euglycaemia increasing to an average peak of 4.1 g/h at 65% $\dot{V}O_2$ peak, and with no exogenous glucose required to maintain euglycaemia during exercise at an intensity of 80% $\dot{V}O_2$ peak. This rise in glucose requirements with exercise of low to moderate intensity is due to a disproportionate increase in glucose Rd relative to glucose Ra (Fig 4.1B). In contrast, at an exercise intensity of 80% $\dot{V}O_2$ peak, glucose Rd is similar to glucose Ra (Fig 4.1B), explaining why no exogenous glucose is required to maintain euglycaemia at this exercise intensity.

Although the inverted U relationship found here between exercise intensity and the exogenous glucose requirements to maintain euglycaemia has never been reported before, it was expected on the basis of current literature. Indeed, aerobic exercise at an intensity above 80% $\dot{V}O_2$ peak and under basal insulinaemic conditions has been shown to result in a transient increase in plasma glucose levels (Mitchell et al. 1988; Purdon et al. 1993; Sigal et al. 1999; Marliss and Vranic 2002), implying that no exogenous CHO is required to prevent blood levels glucose from falling. This observation together with earlier findings from our laboratory (McMahon et al. 2007; Davey et al. 2013a) that exercise of moderate intensity performed under near basal insulinaemic conditions results in a significant increase in exogenous glucose requirements to maintain euglycaemia is consistent with the inverted U relationship described here (Fig 4.3).

The inverted U relationship between GIR and exercise intensity can be explained by the pattern of change in glucose Ra and Rd. During low to moderate intensity exercise, our results show that glucose Rd is greater than glucose Ra (Fig 4.1B), thus explaining the increase in exogenous glucose required to maintain euglycaemia at these exercise

intensities. As shown by us (Table 4.3) and others, this rise in glucose Rd is associated with a marked increase in glucose oxidation rate with exercise intensity (van Loon et al. 2001). In contrast, the absence of any exogenous glucose requirements during exercise at 80% $\dot{V}O_2$ peak is explained on the basis that the exercise-mediated increase in glucose Rd was accompanied by a similar rise in glucose Ra (Fig 4.1B). Although a higher exercise intensity was not tested, previous studies performed under basal insulinaemic conditions have shown that exercise at intensities above 80% $\dot{V}O_2$ peak causes a disproportionate increase in glucose Ra relative to glucose Rd, resulting in a rise in plasma glucose level (Purdon et al. 1993; Sigal et al. 1999; Marliss and Vranic 2002).

The significant increase in glucose Ra and the disproportionate rise in catecholamines levels in response to exercise at 80% $\dot{V}O_2$ peak are consistent with the rise in glucose Ra at this intensity being most probably mediated by an increase in catecholamines levels. In support of this view, catecholamines have been shown to be the primary mediator of the rise in glucose Ra that occurs in response to high intensity aerobic exercise (Sigal et al. 1999; Purdon et al. 1993; Marliss and Vranic 2002); however, not all studies support this view (Coker and Kjaer 2005). In contrast, the significant exercise-mediated increase in growth hormone levels is unlikely to play an important role in mediating the rise in endogenous glucose Ra and Rd. This is because the inhibition of the increase in GH levels during intense aerobic exercise has been shown not to affect both glucose Ra and Rd and the exercise-mediated rise in blood glucose level (Sigal et al. 1996). The increase in cortisol level is also unlikely to have contributed to the increase in glucose Ra reported here because the rise in cortisol levels occurred after rather than during exercise, a finding consistent with those of others (Adolfsson et al. 2012; Purdon et al. 1993).

The absence of rise in glucose Ra during exercise at 35% $\dot{V}O_2$ peak and the trend for a minimal increase in glucose Ra during exercise at 50 and 65% $\dot{V}O_2$ peak are findings comparable to those of others in non-diabetic individuals (Romijn et al. 2000; van Loon et al. 2001; Petersen et al. 2004). This lack of a significant increase in glucose Ra despite a small increase in plasma catecholamines levels together with the absence of any change in

plasma insulin and glucagon levels suggests that glucose Ra at these exercise intensities is controlled by the insulin to glucagon ratio rather than by changes in catecholamines levels. Others have also found only small increases in catecholamines levels and marginal (Tansey et al. 2006; Galassetti et al. 2006b; Adolfsson et al. 2012) or unaltered change in glucagon levels in response to exercise in individuals with T1D (Petersen et al. 2004). Our results support the work of others who have shown that the insulin to glucagon ratio, as opposed to changes in catecholamines levels, is the primary mediator of the rise in glucose Ra during moderate intensity exercise performed under euglycaemic condition in non-diabetic individuals (Wasserman et al. 1989b).

Our findings show that, irrespective of exercise intensity, the average GIR required to maintain euglycaemia during exercise performed under basal insulinaemic conditions did not exceed 15 g/h, with some participants not requiring any intravenous glucose to maintain stable blood glucose level during exercise. Since the relationship between oral CHO intake and GIR is unknown, and considering the effect exercise may have on insulin absorption rate from injection site (Mallad et al. 2015), future studies are required to relate our GIR findings to translatable oral intake equivalent. It is also important to note that since the amount of CHO required to prevent hypoglycaemia during exercise increases with plasma insulin concentration (Francescato et al. 2004), higher hourly CHO intake (1.0-1.5g/kg per hour) is recommended for the prevention of hypoglycaemia when plasma insulin levels are elevated (Riddell et al. 1999).

As expected, our results also show that, in general, CHO must be administered post-exercise to maintain stable glycaemia and prevent hypoglycaemia during early recovery from exercise performed under basal insulinaemic conditions. These findings are consistent with those of others who have reported that even when exercise is performed under low basal insulin levels, blood glucose levels decrease after exercise (Nathan et al. 1985). This decrease in blood glucose levels immediately after exercise is likely mediated by a lasting effect of contraction-stimulated glucose uptake early during recovery (Maarbjerg et al. 2011) and by an increase in insulin sensitivity beyond the first few hours

after exercise (McMahon et al. 2007; Richter et al. 1982 1989), enhancing further the stimulatory effect of insulin on peripheral glucose utilisation rate (Goodyear and Kahn 1998).

One of our study limitations was that we did not compare matched-duration time periods for the participants that did not complete the exercise protocol, since it would have been difficult to apply and interpret. Instead we used data from mid-exercise and end-exercise, since counter regulatory hormone response and glucose kinetics are different during and immediately after exhaustion.

4.6 Conclusion

In conclusion, this study shows that the relationship between the glucose requirements to maintain stable euglycaemia and exercise intensity is not linear but follows an inverted “U” relationship, with no exogenous glucose required at high intensity exercise. Intravenous glucose requirements to maintain euglycaemia during exercise performed under basal insulinaemic conditions vary markedly between individuals and depend on exercise intensity. On clinical grounds, our finding supports the view that the risks of hypoglycaemia during and early after exercise are not high when exercise is performed while plasma insulin levels are at close to basal levels (Nathan et al. 1985; Robertson et al. 2014), particularly when exercise intensity is elevated (Marliss and Vranic 2002; Mitchell et al. 1988; Purdon et al. 1993; Sigal et al. 1999). It remains to be determined how intravenous glucose requirements and exercise intensity relate when exercise is performed under hyperinsulinaemic conditions as well as in response to other exercise modalities, and other subpopulations of individuals with T1D. In addition, the relationship between intravenous and oral glucose requirement needs to be investigated to translate our findings to clinical practice.

Chapter 5

Effect of exercise intensity on exogenous glucose requirements to maintain stable glycaemia at high insulin levels in type 1 diabetes

Foreword

In our first study we found that an inverted U relationship exist between exercise intensity and the amount of glucose that must be ingested to maintain stable glycaemia under near basal insulinaemic conditions. In order to investigate whether this inverted U relationship is maintained even under high insulin conditions, we conducted the second study which is described in this chapter.

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Effect of exercise intensity on exogenous glucose requirements to maintain stable glycaemia at high insulin levels in type 1 diabetes.

5.1 Abstract

Objective: Recently, we showed that in individuals with type 1 diabetes (T1D), there is an inverted U relationship between exercise intensity and the exogenous glucose requirements to maintain stable blood glucose levels under basal plasma insulin levels, with no exogenous glucose being required during high intensity exercise. Our aim was to test the hypothesis that this inverted U relationship also holds under hyperinsulinaemic conditions, but with extra glucose being required at all exercise intensities.

Design and participants: Nine young adults with T1D (mean \pm SD age, 22.6 ± 4.7 y; duration of disease, 12.9 ± 5.1 y; glycated haemoglobin, 61 ± 14 mmol/mol [$7.7 \pm 0.9\%$]; body mass index, 24.0 ± 3.3 kg/m²; $\dot{V}O_2$ peak, 36.6 ± 8.0 ml kg⁻¹min⁻¹; lactate threshold, $59.5 \pm 2.8\%$ of $\dot{V}O_2$ peak) were subjected to a hyperinsulinaemic-euglycaemic clamp (5-6 mmol/l), and exercised for 40 min at 4 different intensities (35, 50, 65 and 80% $\dot{V}O_2$ peak) on separate days following a randomised counterbalanced study design.

Results: The glucose infusion rate (GIR; \pm SEM) to maintain euglycaemia was 4.4 ± 0.4 mg kg⁻¹min⁻¹ prior to exercise, and increased significantly by 1.8 ± 0.4 , 3.0 ± 0.4 , 4.2 ± 0.7 , and 3.5 ± 0.7 mg kg⁻¹min⁻¹ during exercise at 35, 50, 65, and 80% $\dot{V}O_2$ peak, respectively, with no significant differences between the two highest exercise intensities ($p=0.145$). During the 2-h recovery period from exercise at 35, 50, 65, and 80% $\dot{V}O_2$ peak, GIR exceeded pre-exercise GIR by 1.2 ± 0.2 , 1.7 ± 0.2 , 3.1 ± 0.4 , and 3.1 ± 0.5 mg kg⁻¹min⁻¹, respectively ($p<0.05$), with no difference between 65 and 80% $\dot{V}O_2$ peak.

Conclusion: The relationship between exercise intensity and the exogenous glucose requirements to maintain stable euglycaemia under hyperinsulinaemic conditions follows a near hyperbolic rather than an inverted U relationship during and after exercise. Further research is required to translate our findings to clinical practice.

5.2 Introduction

The prevention of both hypoglycaemia and glycaemic excursions during and after exercise is an ongoing clinical challenge in the management of type 1 diabetes (T1D). Although insulin dose reduction before exercise provides an effective means to reduce significantly such a risk of hypoglycaemia (DirecNet Study 2006; Dube et al. 2005; Grimm et al. 2004; Mauvais-Jarvis et al. 2003; Rabasa-Lhoret et al. 2001; Riddell et al. 1999; Sonnenberg et al. 1990; Schiffrin and Parikh 1985; Moser et al. 2015), this approach is suitable only for planned exercise when insulin dose adjustments can be made in advance. Hence a number of guidelines have advocated the use of carbohydrates (CHO) intake as a management tool to prevent exercise-mediated hypoglycaemia both for unplanned and planned exercise (Adolfsson et al. 2018; Colberg et al. 2016; Riddell et al. 2017; Craig et al. 2011). One limitation with these guidelines is that they are concerned mainly with hypoglycaemia prevention rather than the reduction of glycaemic excursions, and none of these guidelines addresses evidence-based relationship between exercise intensity and the amount of dietary CHO required to prevent hypoglycaemia, and the effect plasma insulin level has on this relationship (Adolfsson et al. 2018; Colberg et al. 2016; Riddell et al. 2017; Craig et al. 2011).

Since the rate of blood glucose utilisation and rate of fall in glycaemia increase with exercise of low to moderate intensity (Kemmer 1992; Rabasa-Lhoret et al. 2001), one would predict that the CHO intake required to maintain stable blood glucose levels also increases with exercise intensity. However, recently we have shown that the relationship between exercise intensity performed under basal plasma insulin level and the amount of exogenous glucose required to maintain stable glycaemia is not linear, but follows an inverted U relationship (Shetty et al. 2016), with no exogenous glucose being required for exercise intensity of 80% $\dot{V}O_2$ peak (Shetty et al. 2016). These findings raise the issue of whether this relationship is affected by plasma insulin levels. This is an important issue to address given that it is not uncommon for individuals with T1D to exercise under hyperinsulinaemic conditions, particularly shortly after an insulin bolus.

The amount of administered CHO required to prevent hypoglycaemia during moderate intensity exercise increases with insulin concentrations (Chokkalingam et al. 2007a; Francescato et al. 2004) because elevated plasma insulin levels inhibit hepatic glucose production and enhance peripheral glucose uptake (Camacho et al. 2005; Chokkalingam et al. 2007a; Zinman et al. 1977). Hence, high plasma insulin levels would be expected to increase the exogenous glucose requirements associated with exercise of light or moderate intensity. On the other hand, since mild hyperinsulinaemia is without any marked effect on the increase in the rate of hepatic glucose production during intense aerobic exercise (Sigal et al. 1996), little extra glucose administration may be required during high intensity exercise performed under hyperinsulinaemic conditions, thus suggesting that an inverted U relationship may also hold even under these conditions (Fig 5.1, plot B). In order to determine whether this is the case, the primary objective of our study was to test the hypothesis that when plasma insulin levels are at the high end of the therapeutic range, there is an inverted U relationship between exercise intensity and the exogenous glucose requirements to maintain stable glycaemia, but with extra glucose being required at all exercise intensities.

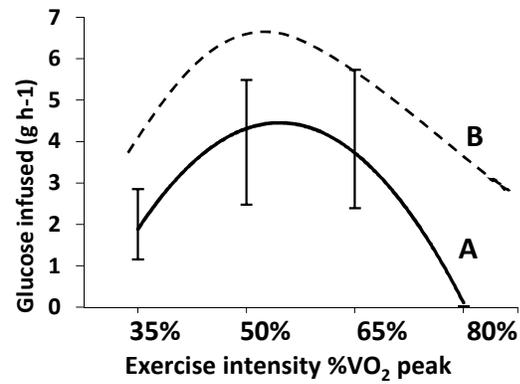


Figure 5.1. Relationship between exercise intensity and exogenous glucose requirements to maintain stable glycaemia under basal insulin conditions (solid line A, mean \pm SEM, $n=9$; Shetty et al 2016), and the predicted relationship between these variables (hatched lines) in response to hyperinsulinaemic conditions (B).

5.3 Participants and methods

5.3.1 Participants

Nine recreationally active (5.4 ± 3.7 h/week) young individuals aged between 16-30 years with well-controlled, complication-free T1D (one, multiple daily injections; eight, continuous subcutaneous insulin infusion) were enrolled for this study (Table 5.1). Participants were eligible if they had undetectable plasma C-peptide levels (<0.05 nmol/L), stable insulin regimen for at least 6 months prior to the study, and were not taking any prescribed medication other than insulin. Two women were on oral contraceptive pills. The protocol was approved by the Princess Margaret Hospital for Children Human Research Ethics Committee, and informed consent obtained from the participants and their parents if aged below 18 y.

5.3.2 Familiarisation session

Each participant attended a familiarisation session followed by four testing sessions. During the familiarisation session, anthropometric measurements were taken and participants completed a peak rate of oxygen consumption ($\dot{V}O_2$ peak) test on a cycle ergometer (Lode Corival Ergometer) connected to a computer fitted with Cyclemax software (UWA, Australia) to assess their aerobic fitness and lactate threshold. Workloads corresponding to 35, 50, 65 and 80% of $\dot{V}O_2$ peak were calculated as described in Chapter 3 (3.1.2).

Table 5.1. Baseline characteristics of study participants

Characteristic	n = 9
Age (years)	22.6 ± 4.7
Gender: male/female, n	6/3
Weight (kg)	76.0 ± 11.5
Height (m)	1.77 ± 0.06
BMI (kg/m ²)	24.0 ± 3.3
VO ₂ peak (ml [kg body weight] ⁻¹ min ⁻¹)	36.6 ± 8.0
Lactate threshold (%VO ₂ peak)	59.5 ± 2.8
Diabetes duration (years)	12.9 ± 5.1
HbA1c (%)	7.7 ± 0.9
HbA1c (mmol/mol)	61 ± 14

Data are expressed as mean ± SD.

5.3.3 Testing session

Prior to testing, all participants were required to abstain from caffeine, alcohol, injecting insulin to the legs, and any physical activity other than light walking for 24 hours before testing since both antecedent hypoglycaemia (Galassetti et al. 2003) and antecedent exercise (Galassetti et al. 2001a) affect the glucoregulatory responses to subsequent exercise. For these reasons, participants were fitted with a real time continuous glucose monitoring system for two days before testing. In addition, participants were required to keep a food diary for 24 hours prior to the first testing session, and then match their food intake the day before their subsequent three testing sessions. Testing was rescheduled for any participant experiencing hypoglycaemia 48 hours prior to testing, and female participants not on oral contraceptive pills were investigated during the follicular phase of their menstrual cycles (day 8 ± 3), with 4 weeks between testing sessions.

On the morning of testing, each participant arrived in the laboratory at 8:00 am after an overnight-fast. Participants on multiple daily injections (MDI) insulin regimes were instructed to decrease their Glargine dose by 50% the night before the study and skip their morning bolus of insulin. For those on insulin pumps, the pump was disconnected on arrival. A cannula was inserted in the dorsum of one hand, and this hand was placed in a Hotbox (Omega CN370, Sydney, Australia) at approximately 60°C for the sampling of arterialed venous blood. Another cannula was inserted in the contralateral antecubital fossa for the infusion of glucose and insulin.

Participants were then subjected to a hyperinsulinaemic-euglycaemic clamp (DeFronzo et al. 1979) as described in Chapter 3 (3.1.2). Exogenous short-acting insulin (Humalog, Eli Lilly Australia Pty Ltd, Australia), at a concentration of 1 unit of insulin per ml infusate, was infused using an Alaris Asena[®] GH syringe pump (Alaris Medical Systems, United Kingdom) at a continuous rate of 30 mU/m²/min after receiving a priming dose of the same insulin during the initial 10 min in a logarithmically decreasing manner so as to raise plasma insulin acutely to attain target plasma insulin levels of 350-550 pmol/L, which

reflect levels within the physiological range observed after the ingestion of a large CHO-rich meal (270-600 pmol/L) (Lee and Woleve 1998; Chokkalingam et al. 2007a).

During the clamp procedure, blood glucose levels were monitored every 5 to 10 min, and the samples for measuring insulin levels were collected at regular intervals. The rate of infusion of a 20% (w/v) dextrose solution was adjusted during the clamp as per a published algorithm (DeFronzo et al. 1979) to maintain blood glucose levels between 5-6 mmol/l throughout the clamp. Since steady state blood glucose level is normally attained two hours after the start of the clamp (DeFronzo et al. 1979), exercise was initiated at least two hours after starting the clamp, and only when stable blood glucose level was maintained between 5-6 mmol/l for at least 30 min. Glucose was infused during exercise and recovery period to stabilise blood glucose to the same levels as those maintained prior to exercise.

Once stable euglycaemia was achieved for at least 30 min, blood samples were collected for baseline measurements of glucoregulatory hormones. Expired air was collected using an indirect calorimetry system (V Max Spectra; Sensor Medics Corp, Yorba Linda, California) for at least 10 min for the determination of baseline rates of O₂ consumption ($\dot{V}O_2$) and CO₂ production ($\dot{V}CO_2$). Then, at approximately 11:30 am, each participant was randomised and asked to cycle for 40 min on the same cycle ergometer as that used for $\dot{V}O_2$ peak testing. The cycling sessions were performed at an intensity of (a) 35% (b) 50% (c) 65%, and (d) 80% $\dot{V}O_2$ peak (fit individuals can sustain 80% $\dot{V}O_2$ peak intensity for at least 30 min; (Warren et al. 2009), and were administered following a randomised, counterbalanced study design, and separated by at least one week. These exercise intensities correspond to light, moderate, vigorous (low), and vigorous (high) intensity, respectively (Haskell et al. 2007). Heart rate was monitored continuously at each exercise intensity, and insulin infusion rate remained unchanged. During and after exercise, expired air was collected for the determination of the rates of O₂ consumption and CO₂ production. Throughout the 2-hour period post-exercise, the participants rested in a seated position.

5.3.4 Measurement of glucoregulatory hormones

Arterialised venous blood samples were sampled for the analyses of blood glucose and lactate levels as well as the levels of the following glucoregulatory hormones: epinephrine, norepinephrine, glucagon, growth hormones, and cortisol. Determination of serum free insulin levels was performed using a one-step non-competitive chemiluminescent immunoassay after insulin extraction with polyethylene glycol (Coat-a-Count Insulin Kit; Diagnostic Products). Glucagon levels from EDTA-treated plasma collected with trasylol (Bayer Pharmaceuticals) were measured using a Linco Glucagon RIA Kit (Linco Research), whereas growth hormone and cortisol levels were determined from venous serum by chemiluminescent immunoassays (Immulite Growth Hormone and Cortisol Assay Kits; Diagnostic Products). The levels of epinephrine and norepinephrine in heparinised plasma treated with sodium metabisulphite were measured via reverse-phase high performance liquid chromatography using a Waters Novapak C18 reverse phase column and a model 5200A Coulochem detector (ESA Biosciences).

5.3.5 Calculations

The exogenous glucose requirements to maintain stable euglycaemia during exercise and recovery were obtained from the glucose infusion rate (GIR) during exercise and recovery, respectively. Total GIR was the actual rate of glucose infused to maintain stable blood glucose levels during exercise and recovery. Baseline GIR was the GIR required to maintain stable blood glucose levels prior to the start of exercise. Extra GIR required to maintain euglycaemia during exercise and recovery was calculated by deducting basal GIR from total GIR. Exercise-mediated CHO oxidation rates were calculated from the $\dot{V}O_2$ and $\dot{V}CO_2$ measurements (Frayn 1983) using the non-protein respiratory exchange ratio and the equation $4.585 \dot{V}CO_2 - 3.226 \dot{V}O_2$ (Peronnet and Massicotte 1991). Fat oxidation rates and energy expenditure during exercise were calculated using appropriate non-protein respiratory quotient stoichiometric equations (Peronnet and Massicotte 1991; Frayn 1983).

5.3.6 Statistical analyses

Data were analysed using one-way (treatment) or two-way repeated-measures ANOVA (treatment and time) and Fisher's least significant difference test for posteriori analysis using SPSS software (version 20.0; SPSS Inc). Statistical significance was accepted at $p < 0.05$. Unless otherwise stated, all results are expressed as mean \pm SEM. Based on previous work from our laboratory using this experimental approach (McMahon et al. 2007; Shetty et al. 2016), a group size of 8 was calculated to provide enough statistical power ($1 - \beta = 0.8$) to identify clinically significant differences in the primary outcome measures.

5.4 Results

5.4.1 Participant's response to exercise

All nine participants completed the 40 min of exercise at 35, 50 and 65% $\dot{V}O_2$ peak. For the exercise trial at 80% $\dot{V}O_2$ peak, only two participants completed the 40-min exercise bout. Two participants ceased exercise at 25 min, two at 20 min, one at 15 min, and two at 10 min. Relative to lactate threshold, the exercise intensities of 35, 50, 65 and 80% $\dot{V}O_2$ peak corresponded to intensities (mean \pm SD) of 55 ± 13 , 79 ± 19 , 103 ± 24 and $126 \pm 30\%$ lactate threshold. The inability of most of our participants to complete the 40-min exercise trial at 80% $\dot{V}O_2$ peak is most likely due to the exercise intensity being well above lactate threshold and thus difficult to sustain. Due to the differences in the duration of the exercise performed at 80% $\dot{V}O_2$ peak, data recorded only during baseline, mid-exercise and end of exercise were used for the comparison of glucoregulatory hormones as described previously (Shetty et al. 2016).

5.4.2 Hyperinsulinaemic-euglycemic clamp and glucose infusion rates to maintain euglycaemia

During all the hyperinsulinaemic-euglycaemic clamps, plasma glucose levels were maintained between 5.0 and 6.0 mmol/L, with no difference between trials ($p > 0.05$, Fig

5.2). Serum free insulin levels prior to the start of exercise were similar at all exercise intensities (Fig 5.5A).

The baseline GIR were similar at all exercise intensities before the commencement of exercise (5.4 ± 1.4 , 4.4 ± 0.8 , 4.3 ± 1.2 , and 3.5 ± 0.8 $\text{mg kg}^{-1}\text{min}^{-1}$ prior to 35, 50, 65 and 80% $\dot{V}O_2$ peak, respectively). The mean extra GIR to maintain euglycaemia for the total duration of exercise at 35% $\dot{V}O_2$ peak (1.8 ± 0.4 $\text{mg kg}^{-1}\text{min}^{-1}$) was lower than at the other intensities ($p < 0.05$ vs 50, 65 & 80% $\dot{V}O_2$ peak), and the mean extra GIR to maintain euglycaemia at 50% $\dot{V}O_2$ peak (3.0 ± 0.4 $\text{mg kg}^{-1}\text{min}^{-1}$) was lower than at 65% (4.2 ± 0.7 $\text{mg kg}^{-1}\text{min}^{-1}$; $p < 0.05$ vs 65% $\dot{V}O_2$ peak). The mean extra GIR at 80% $\dot{V}O_2$ peak (3.5 ± 0.7 $\text{mg kg}^{-1}\text{min}^{-1}$) was similar to that at 50% ($p = 0.257$) and 65% $\dot{V}O_2$ peak ($p = 0.145$). The mean extra GIR for the first 10 min of exercise was similar for all four exercise intensities (Fig 5.3A). The total amount of extra glucose infused during the 40 min of exercise was 6.8 ± 1.2 , 10.7 ± 1.8 and 11.7 ± 2.3 g at 35, 50, and 65% $\dot{V}O_2$ peak, respectively.

The mean extra GIRs to maintain euglycaemia during the 2-h recovery period increased with exercise intensity, reaching 1.2 ± 0.2 , 1.7 ± 0.2 , 3.1 ± 0.4 , and 3.1 ± 0.5 $\text{mg kg}^{-1}\text{min}^{-1}$ at 35, 50, 65, and 80% $\dot{V}O_2$ peak ($p < 0.05$), respectively, but did not differ between 65% and 80% $\dot{V}O_2$ peak (Fig 5.3B).

The temporal pattern of rise in GIR during exercise was comparable for the first three lowest exercise intensities where all participants completed the 40-min exercise session (Fig 5.4). Exercise at 80% $\dot{V}O_2$ peak was excluded from this comparison since the duration of exercise was not the same for all participants at this intensity. A rise in GIR was detected at 10 min of exercise for all the three exercise intensities of 35, 50 and 65% $\dot{V}O_2$ peak, with these rises persisting throughout the remainder of exercise and recovery (Fig 5.4).

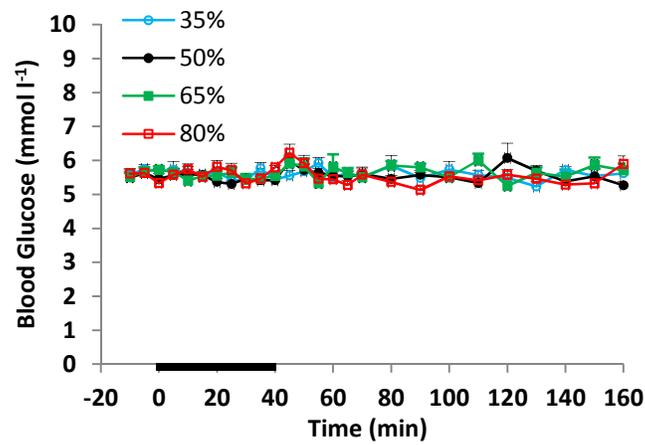


Figure 5.2. Effect of exercise intensities at 35, 50, 65 and 80% $\dot{V}O_2$ peak on blood glucose levels during exercise and for 2 hours post-exercise. All data are mean \pm SEM (n=9). Horizontal bar, exercise.

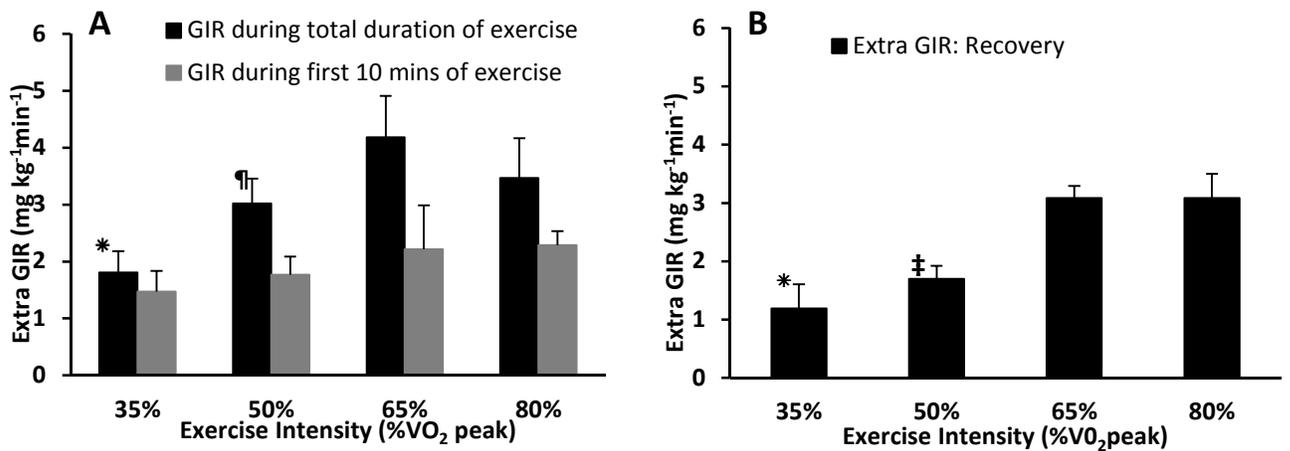


Figure 5.3. Effect of exercise intensities at 35, 50, 65 and 80% $\dot{V}O_2$ peak on extra glucose infusion rate (GIR) during (A) the total duration of exercise (black bar) and the first 10 min of exercise (grey bar) and (B) during the two hours of recovery post-exercise. All data are mean \pm SEM (n=9). * $P < 0.05$ vs 50, 65 & 80% $\dot{V}O_2$ peak. [¶] $P < 0.05$ vs 65% $\dot{V}O_2$ peak. [‡] $P < 0.05$ vs 65 and 80% $\dot{V}O_2$ peak.

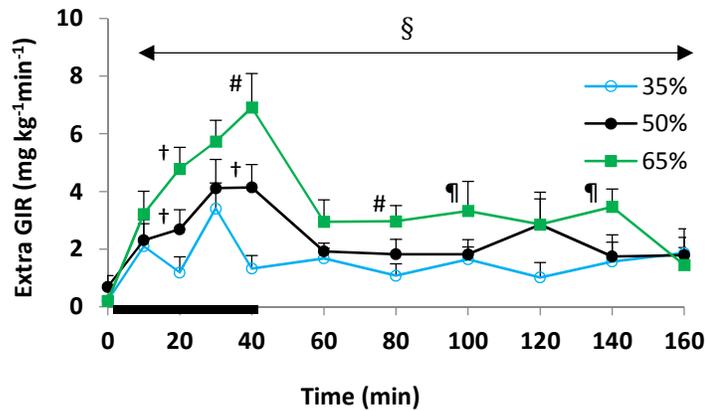


Figure 5.4. Effect of exercise intensities at 35% (blue open circles), 50% (black circles) and 65% $\dot{V}O_2$ peak (green squares) on extra glucose infusion rate (GIR) during exercise and 2 hours post-exercise. All data are mean \pm SEM (n=9). Horizontal bar, exercise. [§]P < 0.05 vs time 0 which is start of exercise; # p < 0.05 vs 35 and 50% $\dot{V}O_2$ peak; [†] p < 0.05 vs 35% $\dot{V}O_2$ peak; [¶] p < 0.05 vs 50% $\dot{V}O_2$ peak.

5.4.3 Cardiorespiratory and metabolic variables

Prior to exercise, there were no differences in heart rate, rate of oxygen consumption ($\dot{V}O_2$), rate of carbon-dioxide production ($\dot{V}CO_2$), CHO and fat oxidation rate, energy consumption, and lactate levels between the four exercise trials ($p>0.05$; Table 5.2). In response to exercise, all these variables, except fat oxidation rate, increased significantly with exercise intensity, with maximal level attained at 80% $\dot{V}O_2$ peak ($p<0.05$). Fat oxidation rates were maximal for exercise at 65% $\dot{V}O_2$ peak (Table 5.2). The lactate levels of 6.35 ± 0.94 mmol/l at the end of 80% intensity seem low for such high intensity. This is due to early fatigue and the short duration of exercise.

5.4.4 Hormonal responses

The plasma insulin levels were similar at all exercise intensities with the median \pm SD [IQR] at the commencement of the exercise being 165 ± 39 [40], 132 ± 41 [41], 130 ± 46 [71], and 124 ± 69 [78] pmol/L performed at 35, 50, 65 and 80% $\dot{V}O_2$ peak, respectively. The levels reflected physiological post-prandial levels. Serum free insulin levels remained stable during exercise and recovery, except for a transient rise in insulin levels during exercise, but with no significant difference between conditions at any time (Fig 5.5A). The rise in insulin levels during exercise was observed despite insulin infusion rates remaining constant. The median \pm SD [IQR] of plasma insulin levels at the end of exercise were 168 ± 29 [35], 238 ± 67 [88], 195 ± 56 [31], and 261 ± 91 [154] pmol/L performed at 35, 50, 65 and 80% $\dot{V}O_2$ peak, respectively. The levels of all other hormones prior to exercise were similar between trials (Fig 5.5). Epinephrine and Norepinephrine levels increased during the two higher exercise intensities, with the highest levels attained in response to exercise at 80% $\dot{V}O_2$ peak ($P < 0.05$) and with these levels decreasing rapidly to basal levels within 15 min post-exercise (Fig 5.5C,D). Glucagon levels did not change significantly in response to all exercise intensities, except at 80% $\dot{V}O_2$ peak where they increased significantly before returning to baseline values by 15 min post-exercise (Fig 5.5B). Growth hormone levels increased in response to exercise, with higher levels being attained in response to

higher exercise intensities, reaching peak levels at the end of exercise, and declining to baseline values by 60 min of recovery (Fig 5.5E). Cortisol levels did not change and remained stable during exercise and recovery (Fig 5.5F).

Table 5.2. Comparison of the effect of exercise intensity on cardiorespiratory and metabolic variables at rest and end of exercise

		Exercise Intensity (% $\dot{V}O_2$ peak)			
		35%	50%	65%	80%
Heart rate (beats/min)	Rest	89±6	84±4	82±4	86±4
	Exercise	120±5 ^{#,*}	142±4 ^{#,‡}	162±54 ^{#,†}	183±4 [#]
$\dot{V}O_2$ (L/min)	Rest	0.28±0.03	0.34±0.02	0.33±0.03	0.29±0.03
	Exercise	1.04±0.10 ^{#,*}	1.49±1.15 ^{#,‡}	1.94±0.21 ^{#,†}	2.32±0.22 [#]
$\dot{V}CO_2$ (L/min)	Rest	0.25±0.02	0.29±0.02	0.28±0.02	0.26±0.03
	Exercise	0.96±0.10 ^{#,*}	1.34±0.15 ^{#,‡}	1.85±0.21 ^{#,†}	2.40±0.31 [#]
RER	Rest	0.87±0.01	0.88±0.01	0.87±0.02	0.88±0.02
	Exercise	0.94±0.01 ^{#,‡}	0.93±0.01 ^{#,†}	0.95±0.02 ^{#,†}	1.05±0.05 [#]
CHO oxidation (g/min)	Rest	0.22±0.03	0.25±0.02	0.29±0.01	0.23±0.03
	Exercise	1.06±0.11 ^{#,*}	1.43±0.25 ^{#,‡}	2.55±0.61 [#]	3.78±0.76 [#]
Fat oxidation (g/min)	Rest	0.06±0.01	0.07±0.01	0.07±0.01	0.06±0.01
	Exercise	0.13±0.16 [#]	0.18±0.03 [#]	0.19±0.05 [†]	0.08±0.03
Energy cost (kJ/min)	Rest	1.60±0.63	1.66±0.13	1.59±0.14	1.43±0.16
	Exercise	21.42±2.10 ^{#,*}	30.79±3.30 ^{#,‡}	40.20±4.22 ^{#,†}	49.30±4.93 [#]
Lactate (mmol/l)	Rest	0.634±0.06	0.59±0.03	0.65±0.05	0.79±0.05
	Exercise	0.88±0.18 [*]	1.54±0.24 ^{#,‡}	2.61±0.43 ^{#,†}	6.35±0.94 [#]

All data are mean ± SEM. [#]P < 0.05 vs Rest. ^{*}P < 0.05 vs 50, 65 & 80% $\dot{V}O_2$ peak. [†]P < 0.05 vs 65 & 80% $\dot{V}O_2$ peak. [‡]P < 0.05 vs 80% $\dot{V}O_2$ peak.

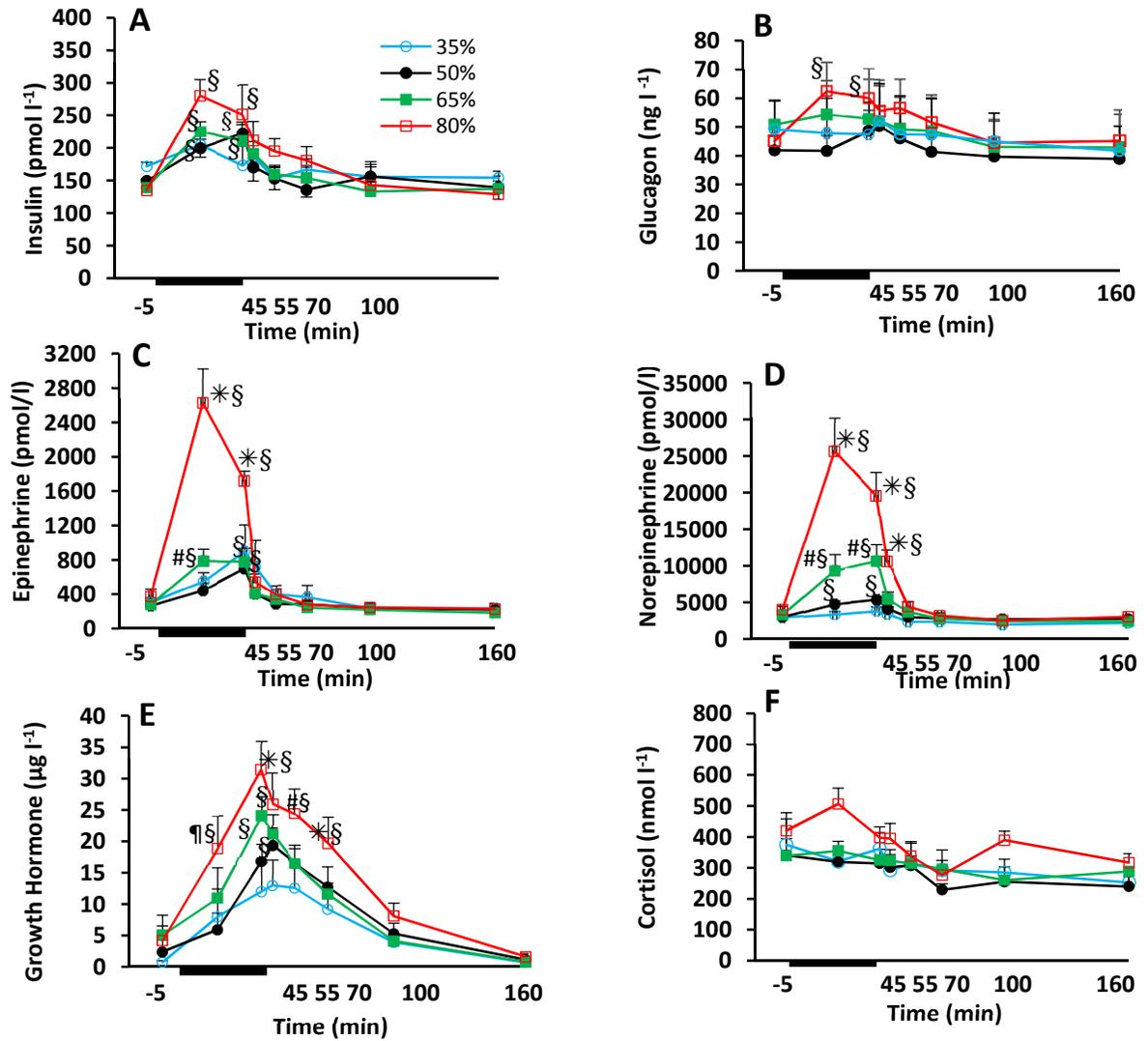


Figure 5.5. Hormonal response to exercise at intensities of 35, 50, 65 and 80% $\dot{V}O_2$ peak. **(A)** Plasma insulin levels. **(B)** Plasma glucagon levels. **(C)** Plasma epinephrine levels. **(D)** Plasma norepinephrine levels. **(E)** Serum growth hormone levels. **(F)** Serum cortisol levels. All data are mean \pm SEM (n=9). Horizontal bar, exercise. § P < 0.05 vs baseline (-5) * P < 0.05 vs 35, 50 and 65% $\dot{V}O_2$ peak; # P < 0.05 vs 35 and 50% $\dot{V}O_2$ peak; ¶ P < 0.05 vs 50% $\dot{V}O_2$ peak; † P < 0.05 vs 35% $\dot{V}O_2$ peak.

5.5 Discussion

Recently, we showed that there is an inverted U relationship between exercise intensity and the exogenous glucose requirements to maintain stable blood glucose levels under basal insulin levels, with no exogenous glucose being required to maintain stable glycaemia during high intensity exercise. Since this relationship has never been investigated under hyperinsulinaemic conditions, our aim was to determine whether this inverted U relationship also holds under such conditions. Here we show, against expectations, that the relationship between exercise intensity and the exogenous glucose requirements to maintain stable blood glucose levels under hyperinsulinaemic conditions follows a near hyperbolic rather than an inverted U relationship both during and after exercise.

The relationship between exercise intensity performed under hyperinsulinaemic conditions and the exogenous glucose requirements to maintain stable blood glucose levels does not follow the inverted U relationship previously observed under basal insulin levels (Chapter 4; Shetty et al., 2016). Our findings show that the extra glucose requirements to maintain euglycaemia increases with exercise intensity to reach a maximum at 65% $\dot{V}O_2$ peak, with similar requirements between 65 and 80% $\dot{V}O_2$ peak. Our findings that glucose must be administered to prevent blood glucose levels from falling during high intensity aerobic exercise (80% $\dot{V}O_2$ peak) performed under hyperinsulinaemic conditions are in marked contrast to what is observed under basal insulinaemic conditions where no glucose administration is required to maintain euglycaemia at an exercise intensity of 80% $\dot{V}O_2$ peak (Shetty et al. 2016).

Irrespective of exercise intensity, more exogenous glucose is required to maintain stable blood glucose during exercise performed under hyperinsulinaemic compared to basal insulin level (Chapter 4), with these requirements at 50% and 65% $\dot{V}O_2$ peak being 2.5 to 3 times higher under our hyperinsulinaemic conditions compared to published basal insulin levels (Shetty et al. 2016). Our finding that plasma insulin levels increase the amount of

exogenous glucose required to maintain stable glycaemia during exercise is supported by the work of others. Chokkalingam and colleagues (Chokkalingam et al. 2007a) found that almost 3 times more glucose had to be infused under hyperinsulinaemic conditions compared to low insulin condition to maintain stable euglycaemia during 45 min of moderate intensity exercise (60% $\dot{V}O_2$ peak) in people with T1D. Similarly, Francescato and colleagues showed that the total amount of CHO required to prevent hypoglycaemia during exercise increases linearly with insulin concentration (Francescato et al. 2004).

The expected additive effect of high plasma insulin levels and muscle contraction on the rate of glucose utilisation (Chokkalingam et al. 2007a) together with the inhibitory effect of hyperinsulinaemia on hepatic glucose production (Edgerton et al. 2001; Sindelar et al. 1998) may explain why more exogenous glucose is required to prevent blood glucose from falling when exercise is performed under hyperinsulinaemic compared to basal insulinaemic conditions. Moreover, the significant transient rise in insulin levels observed during exercise, also reported by others (Sigal et al. 1994b; Purdon et al. 1993; Sigal et al. 1996; Chokkalingam et al. 2007a; Mallad et al. 2015) and probably due to decreased insulin clearance (Sigal et al. 1996), may have contributed further to the increase in the exogenous glucose requirements observed in our study. The plateauing in these requirements when exercise is performed at 80% compared to 65% $\dot{V}O_2$ peak may be explained on the grounds that the high catecholamines levels at 80% $\dot{V}O_2$ peak may have overridden, at least in part, the hyperinsulinaemia-mediated inhibition of hepatic glucose production, thus allowing hepatic glucose production to support part of muscle glucose demands. This interpretation is consistent with catecholamines being potent activators of hepatic glucose production during high intensity aerobic exercise (Marliss and Vranic 2002; Purdon et al. 1993; Sigal et al. 1999), and the observation that mild hyperinsulinaemia does not have any marked effect on the increase in the rate of hepatic glucose production during intense exercise (Sigal et al. 1996). Moreover, it is possible that the elevated levels of plasma glucagon and GH reported here during high intensity exercise at 80% $\dot{V}O_2$ peak may have contributed to both the stimulation of hepatic glucose production and leveling in exogenous glucose requirements.

The temporal pattern of increase in GIR during exercise at mild and moderate intensities reveals the absence of a lag before the exogenous glucose requirements reach above resting levels, with significantly higher than basal GIR being detected as early as 10 min after the onset of exercise. These findings are in contrast to our results obtained under basal insulinaemic conditions (Chapter 6) where the exogenous glucose requirements to maintain stable glycaemia during exercise at 50% $\dot{V}O_2$ peak were found to rise only 20 min after the start of exercise (Shetty et al. 2018). On clinical grounds, the absence of any lag in the rise in the exogenous glucose requirements during low to moderate intensity exercise performed under hyperinsulinaemic conditions suggests that CHO supplementation is required even for short duration exercise performed when prevailing insulin levels are high.

During recovery from exercise performed under hyperinsulinaemic conditions, our results show that extra glucose must also be administered at rates higher than those prior to exercise to maintain stable glycaemia, with these requirements increasing with exercise intensity. Although, earlier studies have reported that more exogenous glucose is required to maintain stable glycaemia during recovery from exercise performed under basal insulinaemic conditions (Shetty et al. 2016; Nathan et al. 1985), one major difference is that no relationship was found between the glucose requirements to stabilise blood glucose level during recovery and prior exercise intensity under basal insulin levels. Our findings are thus consistent with those of others who have reported that blood glucose levels decrease after exercise performed under high insulin levels (Sonnenberg et al. 1990). Such increased glucose demands post-exercise probably result from the lasting stimulatory effects of muscle contraction on glucose uptake (Maarbjerg et al. 2011) and insulin sensitivity (McMahon et al. 2007; Richter et al. 1982, 1989) enhancing further the stimulatory effect of insulin on peripheral glucose utilisation rate (Goodyear and Kahn 1998).

Although the plasma insulin levels achieved in this study reflect those attained post-prandially 1 to 2 hours following subcutaneous insulin administration (Mallad et al. 2015;

Francescato et al. 2004), one of the main limitations with our study is that only one insulinaemic condition was tested. This is an important limitation considering that plasma insulin levels can vary markedly depending not only on insulin bolus dose, but also on the time elapsed post-insulin injection (Francescato et al. 2004; Mallad et al. 2015). Another limitation is the lack of isotopic work to elucidate the mechanisms underlying our findings (Shetty et al. 2016). Also, since this is a small study performed under ideal physiological conditions and limited to recreationally active, lean participants aged between 16-26 years, the generalisability of our findings to people not meeting those criteria is not known. Finally, the relationship between intravenous and oral glucose requirement needs to be investigated to translate our GIR data to clinical practice.

5.6 Conclusion

In conclusion, this study shows that under hyperinsulinaemic conditions, the relationship between the exogenous glucose requirements to maintain stable euglycaemia and exercise intensity follows a near hyperbolic rather than an inverted “U” relationship during and after exercise, with similar amount of exogenous glucose being required at moderate and high intensity exercise. On clinical grounds, our findings support the view that when exercise is performed under hyperinsulinaemic conditions, CHO supplementation is needed to prevent blood glucose levels from falling during and after exercise of all intensities. Since the relationship between oral CHO intake and GIR is unknown and considering the effect exercise may have on insulin absorption rate from injection site (Mallad et al. 2015), future studies are required to relate our GIR findings to translatable oral CHO intake equivalent in individuals with insulin administered subcutaneously.

Chapter 6

The time lag prior to the rise in glucose requirements to maintain stable glycaemia during moderate exercise in a fasted insulinaemic state is of short duration and unaffected by the level at which glycaemia is maintained in type 1 diabetes

Foreword

Having investigated the relationship between exercise intensity and the CHO requirements to maintain stable glycaemia and the glucoregulatory mechanisms underlying this relationship, we undertook to explore the time period during which hypoglycaemia risk is reduced following the onset of exercise performed under near basal insulinaemic conditions. Hence, we conducted the study described in this chapter investigating the temporal pattern of increase in glucose requirements with increasing duration of exercise under both eu- and hyperglycaemic conditions.

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This journal article has been reproduced in this chapter in its entirety.

6.1 Abstract

Objective: The exogenous glucose requirements to maintain stable blood glucose level during exercise performed under basal insulinaemic condition have been reported to be low early during exercise and elevated 1.5-2.0 hours later in individuals with type 1 diabetes (T1D), irrespective of the level at which blood glucose is maintained. Our aim was to determine whether this low hypoglycaemia risk period is affected by the level at which glycaemia is maintained under basal insulinaemic conditions.

Participants: Eight participants with T1D (mean \pm SD; age 21.5 ± 4.0 years) underwent either a euglycaemic (5-6 mmol/L) or hyperglycaemic clamp (9-10 mmol/L) on separate days, and were infused with insulin at basal rates and [6,6-²H]glucose while cycling for 40 min at 50% $\dot{V}O_2$ peak, with both treatments administered following a randomised counterbalanced study design. The main outcome measures were glucose infusion rates (GIR) to maintain stable glycaemia, glucoregulatory hormones levels, and rates of glucose appearance (Ra) and disappearance (Rd).

Results: During the first 20 min of exercise, GIR did not increase significantly irrespective of the level at which glycaemia was maintained, but increased acutely between 20-25 min under both conditions. Maintaining higher glycaemia resulted in higher GIR during, but not early post-exercise. With the exception of epinephrine, the glucoregulatory hormone levels and glucose Ra and Rd were similar between conditions.

Conclusion: Irrespective of the levels at which glycaemia is maintained, there is a 20-minute low exogenous glucose demand period during which the exogenous glucose requirements to maintain stable glycaemia do not increase during moderate exercise performed at basal insulin level.

6.2 Introduction

Exercise provides numerous health benefits for individuals with T1D and for this reason is recommended as an important part of their therapy (Robertson et al. 2014). However, the risks of hypoglycaemia generally increase during and after exercise (McMahon et al. 2007). One approach to decrease such a risk is to consume extra carbohydrates (CHO) (Robertson et al. 2014; Chiang et al. 2014; Craig et al. 2011; Riddell and Perkins 2006) and to exercise while plasma insulin is at or near basal levels since exercising under such conditions is associated with low exogenous glucose requirements to maintain euglycaemia (Shetty et al. 2016) and little changes in blood glucose levels (Nathan et al 1985; Martin et al. 1982) as opposed to exercising under hyperinsulinaemic conditions. In this respect, we have shown that there is an inverted “U” relationship between exercise intensity and the exogenous glucose requirements to maintain stable euglycaemia under basal insulinaemic conditions (Shetty et al. 2016).

The duration of exercise and the extent to which blood glucose is maintained above euglycaemic levels are some of the factors that increase the amount of exogenous glucose required to maintain stable glycaemia during exercise performed under near basal insulinaemic conditions. Indeed, Jenni and colleagues (2008) reported a 2.6-fold increase in total glucose requirements to maintain hyperglycaemia (11 mmol/L) compared to euglycaemia (5 mmol/L) during two hours of moderate intensity exercise performed under low plasma insulin levels in individuals with T1D. In addition, they showed that, irrespective of the levels at which glycaemia is maintained, the exogenous glucose requirements to maintain stable glycaemia are low early during moderate intensity exercise, but elevated 1.5-2 hours later (Jenni et al. 2008). This suggests that following the onset of exercise, there is a time period during which hypoglycaemia risk is reduced when exercise is performed under near basal insulinaemic conditions, thus making exercise safer. Since the temporal pattern of increase in glucose requirements was not described in the study of Jenni and colleagues (2008) the primary aim of our study was to determine whether the low hypoglycaemia risk period following the start of moderate intensity

exercise performed under basal insulinaemic conditions is affected by the level at which glycaemia is maintained in individuals with T1D.

6.3 Methods

6.3.1 Participants

Eight recreationally active (6.1 ± 4.1 h/week) young individuals aged between 16-26 years with well-controlled, complication-free T1D (three participants on multiple daily injections; five participants on continuous subcutaneous insulin infusion) were enrolled for this study (Table 6.1). Participants were eligible if they had undetectable plasma C-peptide levels (<0.05 nmol/L), stable insulin regimen for at least 6 months prior to the study, and were not taking any prescribed medication other than insulin. Three women were on oral contraceptive pills. The protocol was approved by our hospital Ethics Committee, and informed consent obtained from the participants and their parents if aged below 18 y.

6.3.2 Familiarisation session

Each participant attended a familiarisation session followed by two testing sessions. During the familiarisation session, anthropometric measurements were taken, and participants completed a maximal rate of oxygen consumption ($\dot{V}O_2$ peak) and lactate threshold tests on a cycle ergometer (Corival Ergometer; Lode) to assess their aerobic fitness, with both tests performed as described previously (Chapter 3; Shetty et al 2016).

Table 6.1 Baseline characteristics of study participants

Characteristics	n = 8
Age (years)	21.5 ± 4.0
Gender (male/female n)	4/4
Weight (kg)	79.6 ± 19.4
Height (m)	1.75 ± 0.09
BMI (kg m ² ⁻¹)	25.7 ± 5.8
$\dot{V}O_2$ peak (ml kg ⁻¹ body weight ⁻¹ min ⁻¹)	34.5 ± 10.9
Lactate threshold (% $\dot{V}O_2$ peak)	59.5 ± 2.8
Diabetes duration (years)	12.4 ± 6.4
HbA1c (mmol mol ⁻¹)	60 ± 8
HbA1c (%)	8.0 ± 0.7

Data are expressed as mean ± SD

6.3.3 Testing sessions

During the 48-hour period before each testing session, participants were required to abstain from caffeine, alcohol, injecting insulin to the legs, and any physical activity other than light walking. Participants were fitted with a real time continuous glucose monitoring (CGM) system for two days before testing. Testing was rescheduled if the participants experienced hypoglycaemia 48 hours before testing. These precautions were taken because both antecedent hypoglycaemia (Galassetti et al. 2003) and antecedent exercise (Galassetti et al. 2001) affect the glucoregulatory responses to subsequent exercise. Participants kept a food diary for 24 hours prior to their first testing session, and were asked to match their food intake the day before their subsequent testing session.

All participants underwent on separate occasions a euglycaemic (5-6 mmol/L) or hyperglycaemic (9-10 mmol/L) clamp performed at basal insulin levels, with each clamp separated by at least 1 week, and administered following a repeated measure counterbalanced study design. The participants were blinded to the glycaemic level. All women were investigated during the follicular phase of their menstrual cycles (day 8 ± 3), with 4 weeks between testing sessions.

On the morning of testing, each participant arrived in the laboratory at 8:00 am after an overnight-fast. Participants on multiple daily injections insulin regimens were instructed to decrease their Glargine dose by 50% the night before the study and skip their morning bolus of insulin. For those on insulin pumps, the pump was disconnected on arrival. A cannula was inserted in the dorsum of one hand and this hand was placed in a Hotbox (Omega CN370, Sydney, Australia) for the sampling of arterialed venous blood. Another cannula was inserted in the contralateral antecubital fossa for the infusion of glucose and insulin.

As describe previously (Chapter 3, 3.1.1; Shetty et al. 2016), the insulin infusion rate was adjusted to a level where no exogenous glucose was required to maintain a stable glycaemia of 5-6 mmol/L. Then, a blood sample was drawn for the determination of

background enrichment of [6,6-²H₂]glucose, and a priming bolus dose of 3.3 mg.kg⁻¹ of [6,6-²H₂]glucose was administered as described previously (Chapter 3, 3.2; Shetty et al. 2016). This was followed by the constant infusion of 2.4 mg.kg⁻¹.h⁻¹ of [6,6-²H₂]glucose for the remainder of the experiment. Blood glucose levels were determined every 5 to 15 min to ensure maintenance of euglycaemia at 5-6 mmol/L. Participants were then randomised and subjected on separate days to either a euglycaemic or hyperglycaemic clamp. On the day of the euglycaemic clamp, blood glucose levels were maintained between 5-6 mmol/L. On the hyperglycaemic clamp day, the variable infusion of glucose was spiked with [6,6-²H]glucose (2.48 mg/mL) to minimise changes in isotopic enrichment (Finegood et al. 1987) and was infused at a higher rate to maintain blood glucose between 9-10 mmol/L.

Once isotopic equilibrium (approximately 150 min after the start of the [6,6-²H₂]glucose infusion) and stable euglycaemia with no glucose infusion were achieved for at least 45 min on the euglycaemic day and stable hyperglycaemia with a constant glucose infusion rate (GIR) was achieved for at least 45 min on the hyperglycaemic day, blood samples were collected for baseline measurements of glucoregulatory hormones, metabolites, and [6,6-²H₂]glucose enrichment. Expired air was collected using an indirect calorimetry system (V Max Spectra; Sensor Medics Corp, Yorba Linda, California) for at least 10 min for the determination of baseline rates of O₂ consumption ($\dot{V}O_2$) and CO₂ production ($\dot{V}CO_2$). Between 12:00 and 12:30 pm, each participant was subjected to 40 min of exercise at an intensity of 50% $\dot{V}O_2$ peak, with heart rate monitored continuously during and after exercise. During exercise, the rate of constant [6,6-²H₂]glucose tracer infusion was doubled to avoid marked changes in isotopic enrichments (Romijn et al. 1992, 2000; Manzon et al. 1998; Van Loon et al. 2001; Horton et al. 2005; Jenni et al. 2008; Sigal et al. 1999) and to ensure the validity of glucose turnover calculations (Fisher et al. 1996). The rate of tracer infusion was decreased after exercise to the pre-exercise rate, and maintained at this rate until 2 hours post-exercise while participants remained seated. Insulin infusion rate remained unchanged and glycaemia was maintained on target for both the euglycaemic and hyperglycaemic conditions by infusing glucose. During and after

exercise, expired air was collected for the determination of $\dot{V}O_2$ and $\dot{V}CO_2$. Urine samples were collected during the study day for glucose determination.

6.3.4 Measurement of glucoregulatory hormones and [6,6-²H₂]glucose enrichment

Arterialised venous blood samples were analysed for blood glucose and lactate levels as well as for the assays of insulin and glucoregulatory hormones levels: epinephrine, norepinephrine, glucagon, growth hormones, and cortisol, as we described previously (Chapter 3, 3.3.2). The [6,6-²H₂]glucose enrichment was determined by gas chromatography-mass spectrometry (Bio analytical Mass Spectrometry Facility, The University of NSW, Sydney, Australia) as described previously (Chapter 3, 3.3.1). The readings obtained were corrected for background enrichment of naturally occurring [6,6-²H₂]glucose, and the rates of endogenous glucose appearance (Ra) and disappearance (Rd) were calculated from the changes in glucose enrichment using the single compartment, non-steady-state model of Steele (Wolfe 2005), as modified by Finegood and Bergman (Finegood and Bergman 1983) and as we described previously (Guelfi et al. 2007b).

6.3.5 Calculations and statistical analyses

Our primary outcome measure, the exogenous glucose requirements to maintain stable glycaemia during exercise and recovery, was calculated from the GIR data and expressed relative to body mass. Glucose Ra was calculated by subtracting GIR from the total rate of glucose appearance. Exercise-mediated CHO oxidation rates were calculated from the $\dot{V}O_2$ and $\dot{V}CO_2$ measurements (Frayn 1983) using the non-protein respiratory exchange ratio and the equation $4.585 \dot{V}CO_2 - 3.226 \dot{V}O_2$ (Peronnet and Massicotte 1991). Fat oxidation rates and energy expenditure during exercise were calculated using appropriate non-protein respiratory quotient stoichiometric equations that assume negligible protein oxidation (Frayn 1983; Peronnet and Massicotte 1991).

Based on previous work from our laboratory using this experimental approach (McMahon et al. 2007), a group size of 8 was calculated to provide enough statistical power ($1 - \beta = 0.8$) to identify clinically significant differences in the primary outcome measures. Data

were analysed using one-way (treatment) or two-way repeated-measures ANOVA (treatment and time) and Fisher's least significant difference test for posteriori analysis using SPSS software (version 20.0; SPSS, Chicago, IL, USA). Statistical significance was accepted at $p < 0.05$. Unless otherwise stated, all results are expressed as mean \pm SEM.

6.4 Results

6.4.1 Glucose clamp and GIRs to maintain stable glycaemia

During the euglycaemic and hyperglycaemic clamps, plasma glucose levels were maintained between 5-6 mmol/L and 9-10 mmol/L, respectively, and there was a significant difference between conditions (Fig 6.1a). Plasma insulin reached levels of 113.5 ± 16.8 and 106.0 ± 13.7 pmol/L ($p=0.35$) prior to exercise performed under euglycaemic and hyperglycaemic conditions, respectively.

The GIR to maintain stable glycaemia was higher at rest and during exercise in the hyperglycaemic as opposed to the euglycaemic condition (Fig 6.1b). The total amount of glucose infused during exercise was higher for hyperglycaemia than for euglycaemia (1425 ± 258 and 587 ± 120 mg.kg⁻¹, $p < 0.05$). The GIR during exercise increased from 1.5 ± 0.5 to 5.3 ± 0.7 mg.kg⁻¹min⁻¹ for hyperglycaemia ($p=0.03$) and from 0 to 2.0 ± 0.7 mg.kg⁻¹min⁻¹ for euglycaemia ($p=0.01$). This increase in GIR during exercise did not differ between conditions ($p=0.087$). At the start of exercise, GIR did not change irrespective of the level at which glycaemia was maintained, but rose suddenly after 20 min for the hyperglycaemic and euglycaemic conditions, with the GIR at this time point increasing from 1.5 ± 0.5 to 3.3 ± 1.2 mg.kg⁻¹min⁻¹ ($p=0.03$) and from 0 to 2.1 ± 0.7 mg.kg⁻¹min⁻¹ ($p=0.03$), respectively. During the 2-h recovery period, there was no significant difference ($p=0.128$) in the GIR required to maintain stable glycaemia between conditions (Fig 6.1b).

6.4.2 Rates of endogenous glucose appearance (Ra) and disappearance (Rd)

Basal glucose Ra did not differ between conditions and did not increase in response to exercise for the eu- and hyperglycaemic conditions (Fig 6.1c). Glucose Rd prior to exercise

did not differ between conditions, and increased in response to exercise to reach end-of-exercise levels of 4.2 ± 1.0 and $6.2 \pm 1.6 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for the eu- and hyperglycaemic conditions, respectively (Fig 6.1d). There was no significant difference in the glucose Rd response to exercise between conditions ($p=0.19$). During recovery, glucose Ra remained unchanged whereas glucose Rd returned to baseline values within 30 min, and reached levels that did not differ between conditions (Figs 6.1c, 6.1d).

6.4.3 Cardiorespiratory and metabolic variables

Prior to exercise, there were no differences in heart rate, rate of oxygen consumption ($\dot{V}O_2$), rate of carbon dioxide production ($\dot{V}CO_2$), CHO and fat oxidation rates, respiratory exchange ratio (RER), energy consumption, and lactate levels between conditions ($p>0.05$, Table 6.2). In response to exercise, all these dependent variables, except heart rate, increased to a similar extent between conditions (Table 6.2). The whole-body CHO oxidation rate increased rapidly in the first 10 min of exercise, and then remained stable throughout the exercise period, with no difference between conditions (Fig 6.2). At the end of exercise, CHO oxidation rates were 17.1 ± 2.0 and $14.8 \pm 1.9 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for eu- and hyperglycaemic trials, respectively, and were higher than the respective glucose Rd (Fig 6.1d).

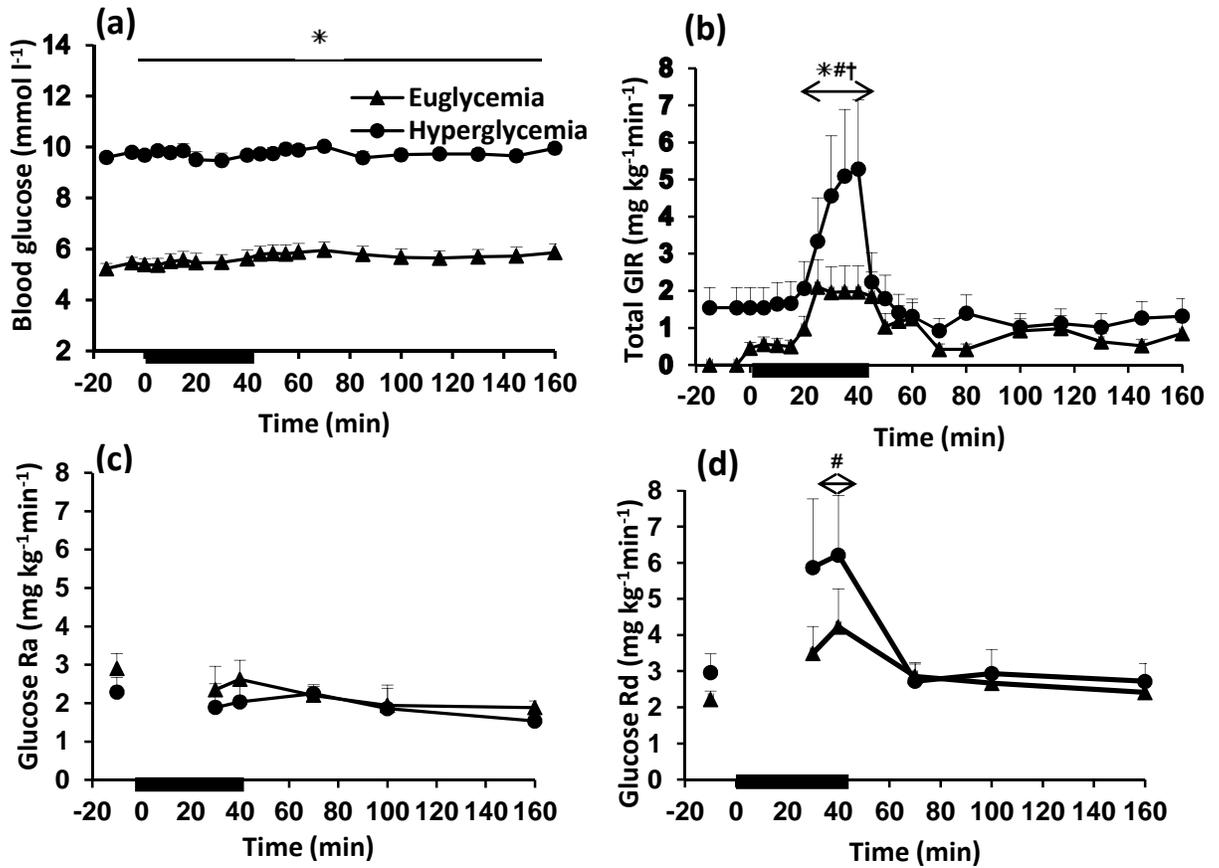


Figure 6.1. Effect of exercise combined with euglycaemic (black triangles) and hyperglycaemic (black circles) euinsulinaemic clamps on (a) Blood glucose level, (b) GIR, (c) Glucose Ra, and (d) Glucose Rd. All data are expressed as mean \pm SEM (n=8). Horizontal bar, exercise at 50% $\dot{V}O_2$ peak. #, P<0.05 vs baseline. †, P<0.05 vs first 20 mins of exercise. *, P<0.05 Euglycaemia vs Hyperglycaemia at all-time points. GIR. glucose infusion rate. Ra, rate of glucose appearance. Rd, rate of glucose disappearance.

Table 6.2 Effect of euglycaemic and hyperglycaemic euinsulinaemic clamps on cardio-respiratory and metabolic profiles at rest and end of exercise

	Euglycaemia	Hyperglycaemia
Heart rate (beats min ⁻¹)		
Rest	82±8	76±6
Exercise	146±7 ^a	140±7 ^{a, b}
$\dot{V}O_2$ (l min ⁻¹)		
Rest	0.31±0.02	0.34±0.02
Exercise	1.45±0.16 ^a	1.43±0.16 ^a
$\dot{V}CO_2$, (l min ⁻¹)		
Rest	0.25±0.02	0.28±0.02
Exercise	1.33±0.15 ^a	1.29±0.15 ^a
Respiratory exchange ratio		
Rest	0.79±0.02	0.80±0.01
Exercise	0.92±0.02 ^a	0.90±0.01 ^a
CHO oxidation rate (mg·kg ⁻¹ ·min ⁻¹)		
Rest	1.47±0.26	1.89±0.18
Exercise	17.07±2.04 ^a	14.86±1.91 ^a
Fat oxidation rate (mg·kg ⁻¹ ·min ⁻¹)		
Rest	1.46±0.18	1.47±0.06
Exercise	2.59±0.60 ^a	3.02±0.56 ^a
Energy cost (kJ min ⁻¹)		
Rest	6.23±0.33	6.90±0.46
Exercise	29.80±3.18 ^a	29.32±3.05 ^a
Lactate (mmol l ⁻¹)		
Rest	0.46±0.04	0.52±0.05
Exercise	1.59±0.39 ^a	1.16±0.21 ^a

All data are mean ± SEM (n=8). ^a P< 0.05 vs rest. ^b P< 0.05 vs euglycaemia

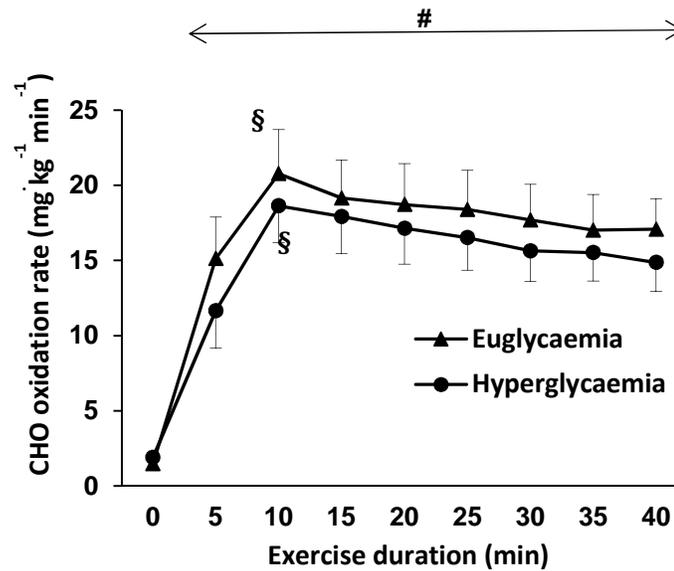


Figure 6.2. Effect of exercise combined with euglycaemic (black triangles) and hyperglycaemic (black circles) euinsulinaemic clamps on whole body CHO oxidation rate during exercise at 50% $\dot{V}O_2$ peak. All data are expressed as mean \pm SEM (n=8). [#], P<0.05 vs baseline (Time 0). [§], P<0.05 vs Time 5 min.

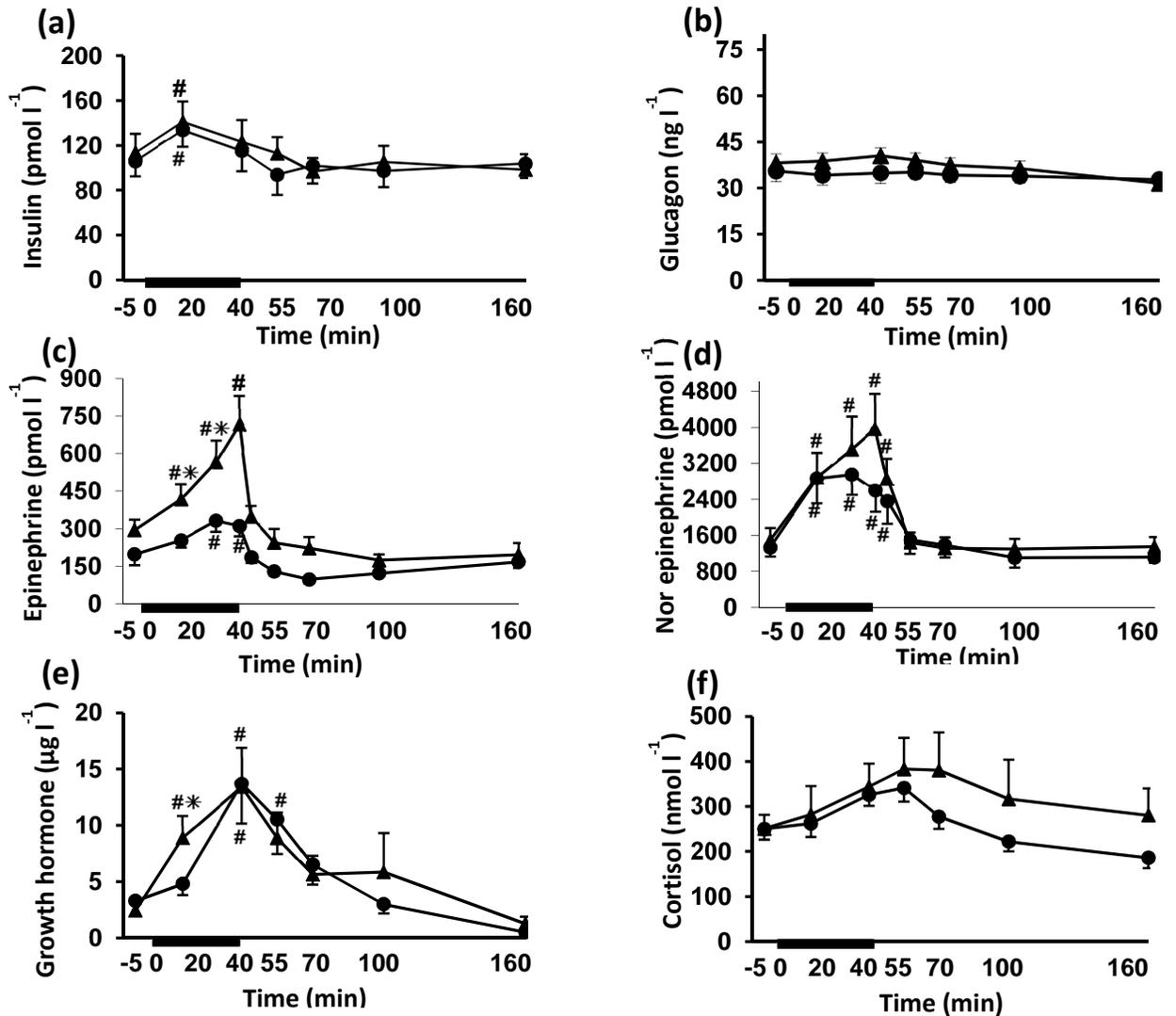


Figure 6.3. Effect of exercise combined with euglycaemic (black triangles) and hyperglycaemic (black circles) euinsulinaemic clamps on plasma levels of (a), Insulin (b), Glucagon (c), Epinephrine (d), Norepinephrine (e), Growth Hormone and (f), Cortisol. All data are expressed as mean \pm SEM (n=8). Horizontal bar, exercise at 50% $\dot{V}O_2$ peak.

#, P<0.05 vs baseline. *, P<.005 Euglycaemia vs Hyperglycaemia.

6.4.4 Hormonal responses

The levels of all hormones prior to exercise were similar between conditions (Fig 6.3). Plasma insulin levels remained stable during exercise and recovery, except for a small transient rise in insulin levels during mid-exercise, but with no difference between conditions at any time (Fig 6.3a). Epinephrine, norepinephrine and growth hormone levels increased during exercise in both conditions (Figs 6.3c, 6.3d, 6.3e), but with a greater rise in epinephrine levels in the euglycaemic state (Fig 6.3c). Epinephrine levels during exercise under euglycaemia were higher than under hyperglycaemia, with peak levels achieved at the end of exercise (Fig 6.3c). Catecholamine decreased rapidly to basal levels within 15 min of recovery (Figs 6.3c, 6.3d). Glucagon levels did not change in response to exercise (Fig 6.3b). The marginal increase in cortisol levels during exercise was similar between conditions (Fig 6.3f).

6.5 Discussion

The exogenous glucose requirements to maintain stable blood glucose level during moderate intensity exercise performed under basal insulinaemic condition have been reported to be low early during exercise and elevated 1.5-2.0 hours later in individuals with T1D (Jenni et al. 2008). Our aim was to determine whether the duration of the low exogenous glucose requirements period following the start of exercise is affected by the level at which glycaemia is maintained. This study shows that the exogenous glucose requirements to maintain stable glycaemia during exercise does not increase during the first 20 min of moderate intensity exercise performed under euglycaemic and hyperglycaemic conditions, with the duration of this low hypoglycaemia risk period being unaffected by the levels at which glycaemia is maintained. This study also shows that mild hyperglycaemia compared to euglycaemia increases the exogenous glucose requirements to maintain stable glycaemia before and during exercise, but not during the post-exercise period. These findings thus show that little or no extra CHO intake is required to maintain stable blood glucose level during exercise if exercise lasts for less than 20 min, but with more CHO being required if mild hyperglycaemia is maintained during exercise. These

findings are thus in agreement with those of others who have reported that short duration exercise performed at or near basal insulinaemic levels has little or no effect on blood glucose levels in individuals with T1D (Martin et al. 1982)

Our findings are consistent with those of Jenni and colleagues (2008) who reported that there is a lag before the exogenous glucose requirements to maintain stable glycaemia rise during exercise performed under near basal insulinaemic conditions, with more exogenous glucose being required to maintain stable hyperglycaemia compared to euglycaemia during exercise. Jenni and colleagues (2008) reported a 2.6-fold increase in the total amount of glucose administered to maintain hyperglycaemia compared to euglycaemia during two hours of moderate intensity exercise performed under low plasma insulin levels. This is similar to the 2.4-fold difference reported here between conditions, a finding that is not surprising given the comparable exercise intensity and average plasma insulin levels between studies (Jenni et al. 2008). The main difference between our results and those of Jenni and colleagues (2008) is that the temporal pattern of increase in exogenous glucose requirements was examined only in our study. The glucose requirements were shown to increase acutely at close to 20 min following the start of exercise, with the levels at which glycaemia is maintained having no effect on the duration of this low hypoglycaemia risk.

The absence of increase in GIR during the first 20 min of exercise performed under eu- and hyperglycaemic conditions despite elevated CHO oxidation rates and non-significant rise in glucose Rd suggests an increased reliance of skeletal muscles on the oxidation of their glycogen stores during that time as this can be achieved without increasing glucose Rd. This interpretation is further supported by the work of others who showed that muscle glycogen as opposed to blood glucose is the major CHO oxidised to support muscle energy demands early during moderate intensity exercise performed under basal insulinaemic conditions (Romijn et al. 1993). Of note, the rise in glucose Rd and GIR together with the absence of any increase in CHO oxidation rate and glucose Ra as exercise duration extended beyond 20 min suggests that muscle glycogen oxidation rate

decreases with time, as is generally the case during aerobic exercise (MacLaren et al. 1999). This lesser reliance on muscle glycogen for exercise lasting longer than 20 min would explain, at least in part, our findings of a marked rise in GIR at that time.

The patterns of change in GIR during exercise performed under both eu- and hyperglycaemic conditions are best explained by the corresponding patterns of change in glucose Rd rather than glucose Ra, since glucose Ra did not change significantly during exercise (Fig 6.1c). Such an absence of increase in glucose Ra in response to low-to-moderate intensity exercise corroborates earlier findings in individuals without diabetes (Hawley et al. 1994; Romijn et al. 1993; Carter et al. 2004) and in some (Shetty et al. 2016; Jenni et al. 2008) but not all studies involving individuals with T1D (Petersen et al. 2004). Glucose Rd tended to be higher during exercise in hyperglycaemia than in euglycaemia (6.2 ± 1.6 vs 4.2 ± 1.0 mg.kg⁻¹.min⁻¹), though it did not reach statistical significance. This corroborates the work of others who reported a similar trend for glucose Rd to be higher during exercise performed under hyperglycaemic compared to euglycaemic conditions in individuals with (Jenni et al. 2008) and without T1D (Coyle et al. 1991). The lower glucose Rd during exercise performed under euglycaemic compared to hyperglycaemic condition is probably due, at least in part, to a lower mass action effect of glucose on glucose transport, and the higher epinephrine levels in the eu- compared to the hyperglycaemic condition since epinephrine has been shown to inhibit muscle glucose utilisation rate during exercise (Watt and Hargreaves 2002).

The carbohydrate oxidation rates did not differ between treatments, a finding that corroborates those of Jenni and colleagues (2008) who showed that carbohydrate oxidation rates in individuals with T1D exercising at moderate intensity did not differ between euglycaemic and hyperglycaemic conditions during the first 60 mins of exercise; however a shift towards higher carbohydrate oxidation rates was reported during the second hour of exercise in hyperglycaemic participants (Jenni et al. 2008). Although similar findings have also been shown by others in individuals with (Stettler et al. 2006) and without T1D (Carter et al. 2004), it is important to stress that some studies have reported

increased rate of CHO oxidation with higher delivery of glucose into the systemic circulation in individuals without diabetes (MacLaren et al. 1999).

During recovery, the lack of significant difference in GIR between euglycaemic and hyperglycaemic conditions (Fig 6.1b) has never been reported before, and is best explained by the absence of post-exercise difference in glucose Rd and Ra between these conditions. In addition, the lower GIR during recovery compared to during exercise (Fig 6.1b) is also consistent with the rapid post-exercise fall in glucose Rd. Despite this fall, GIR throughout recovery still remains above pre-exercise level during the euglycaemic condition and similar to pre-exercise level in the hyperglycaemic condition. These results and earlier ones from our laboratory (Shetty et al. 2016) thus show that CHO should be ingested to maintain stable glycaemia during early recovery from exercise performed under basal insulinaemic conditions (Nathan et al. 1985).

6.6 Conclusion

In conclusion, this study makes the clinically important observation that irrespective of the levels at which glycaemia is maintained, little or no extra exogenous CHO is required under basal insulinaemic conditions to maintain stable glycaemia during moderate intensity exercise lasting less than 20 min compared to the rest state. This study also shows for the first time that after this time lag, the rise in exogenous CHO requirements during exercise is acute and independent of the levels at which glycaemia is maintained. Finally, our study reveals that increasing the level at which blood glucose is maintained during moderate intensity exercise performed under basal insulinaemic conditions does not affect the exogenous glucose requirements to maintain stable glycaemia early after exercise.

It is important to stress that since exercise in this study was performed in a basal insulinaemic state, its findings cannot be extrapolated to exercising under hyperinsulinaemic conditions as blood glucose levels decrease rapidly under these latter

conditions (Guelfi et al. 2005a). Also, since insulin was administered intravenously rather than subcutaneously, the generalisability of our findings may be further limited on the grounds that there may be circumstances (e.g. site of insulin injection, ambient temperature) where the pattern of insulin response to exercise differs from that reported here. Since this is a small study performed under ideal physiological conditions and limited to recreationally active, lean and young participants, the generalisability of our findings to people not sharing those criteria is not known. Finally, only one exercise intensity and hyperglycaemic condition were investigated here, thus implying that generalising our findings to other conditions should be made with caution. Therefore, more studies performed in a real-life setting are thus required before translating our findings to clinical practice.

Chapter 7

General discussion

7.1 General discussion

Intensive blood glucose management to achieve tight glycaemic control is integral to the treatment of T1D. The availability of a variety of insulin preparations, insulin pumps, and continuous glucose monitoring devices has contributed markedly to improving glycaemic control (DCCT Research Group 1993, 1994; Nathan et al. 2009; White et al. 2001; Mohsin et al. 2005; Rewers et al. 2014a; Rodbard 2017). However, physical activity is one factor that has proven to make the achievement of good glycaemic management highly challenging as exercise can increase markedly the risk of hypoglycaemia (Davis et al. 1997a; Admon et al. 2005; Wasserman 2002; Jensen and Richter 2012; Zinman et al. 1977; Berger et al. 1979; Tansey et al. 2006). This is of concern not only because of the many deleterious effects of hypoglycaemia, but also because the fear of exercise-mediated hypoglycaemia is a major barrier to the adoption of a physically active lifestyle by people with T1D (Brazeau et al. 2008). Although regular blood glucose monitoring, carbohydrate (CHO) supplementation, and insulin dose adjustment pre- and post-exercise are generally advocated as means to help manage blood glucose levels during and after exercise, only these first two strategies are suitable for unplanned exercise (Kemmer 1992; Dube et al. 2005; Riddell et al. 1999; Dube et al. 2006; Grimm et al. 2004; Adolfsson et al. 2018; Riddell et al. 2017).

One of the main clinical challenges with using CHO supplementation to manage blood glucose level is one of ingesting enough CHO before, during and after exercise to prevent hypoglycaemia without causing excessive hyperglycaemia. That this challenge is seldom met is indirectly supported by the observation that regular exercise has little or no effect on glycaemic control (Beraki et al. 2014; Aman et al. 2009) despite the marked glycaemia lowering effect of most types of exercise. It is the overconsumption of CHO in an effort to avoid hypoglycaemia that is likely responsible, at least in part, for the small impact regular exercise has on improving glycaemic control (Ebeling et al. 1995). Hence, optimising CHO intake to maintain near stable blood glucose levels during exercise is necessary if exercise is to contribute to optimal management of T1D.

As discussed at length in Chapter 1 (section 1.3), the glycaemic response to exercise is affected by many factors such as the intensity and duration of exercise as well as plasma insulin levels. Surprisingly, the relationship between exercise intensity and the amount of exogenous CHO required to maintain stable blood glucose levels during and after exercise, and the impact both plasma insulin levels and the levels at which blood glucose is maintained during exercise have on this relationship are important clinical issues that have not been thoroughly examined. This is important to address because the CHO intake required to maintain stable glycaemia during and after exercise is expected to vary markedly depending on exercise intensity and plasma insulin levels. Also, how these CHO requirements are affected over time by the level at which blood glucose is maintained is a question without a clear answer. Although many guidelines describe the amount of CHO that should be ingested to prevent hypoglycaemia during and after exercise (Robertson et al. 2014; Chiang et al. 2014; Craig et al. 2011; Adolfsson et al. 2018; Riddell et al. 2017; Colberg et al. 2016), the research that informs these guidelines focuses primarily on hypoglycaemia prevention, with little regard for achieving this goal without excessive hyperglycaemia.

Since the effects of exercise intensity and duration on the exogenous CHO requirements to maintain blood glucose at stable low or high levels under basal or hyperinsulinaemic conditions were poorly understood at the time this thesis was initiated, our primary aims were to address these issues. More specifically, the **first aim** of this thesis was to investigate the relationship between exercise intensity and the amount of exogenous glucose required to maintain stable glycaemia during and immediately after exercise performed under basal insulin conditions in young adults with T1D, and to elucidate some of the glucoregulatory mechanisms involved (Chapter 4). On the basis that CHO oxidation rates increase during low and moderate intensity exercise and that no CHO is required to prevent blood glucose levels from falling during and immediately after high intensity aerobic exercise when plasma insulin is at near basal level, we hypothesised the presence of an inverted U relationship rather than a linear one between exercise intensity and the

amount of exogenous glucose required to maintain stable glycaemia under near basal insulinaemic conditions.

The **second aim** was to explore how exercising under hyperinsulinaemic conditions affects this predicted inverted U relationship. We hypothesised that this relationship is maintained even under hyperinsulinaemic conditions (Chapter 5). Finally, given the evidence that under basal insulinaemic conditions the exogenous glucose requirements to maintain stable glycaemia are low early during moderate intensity exercise, but elevated 1.5-2.0 hours later irrespective of the levels at which glycaemia is maintained (Jenni et al. 2008), the **third aim** of this thesis was to determine whether the duration of the low exogenous glucose requirement period during moderate intensity exercise performed under basal insulinaemic conditions is affected by the level at which glycaemia is maintained (Chapter 6). This is an important question to address as a better understanding of the effect of exercise duration on the amount of exogenous glucose required to maintain stable glycaemia could lead to the identification of a time period during which hypoglycaemia risk is reduced when exercise is performed under near basal insulinaemic conditions, thus making exercise safer.

In order to estimate precisely the effects of exercise intensity and plasma insulin levels on the exogenous glucose requirements to maintain stable blood glucose levels during exercise, this thesis has adopted the use of euinsulinaemic euglycaemic clamps (Chapter 4 and 6), euinsulinaemic hyperglycaemic clamps (Chapter 6) or hyperinsulinaemic euglycaemic clamps (Chapter 5). These different types of clamps allow the precise quantification of the amount of exogenous glucose required to maintain stable glycaemia while exercising, and provide excellent experimental models to explore the glucoregulatory mechanisms involved.

As shown in this thesis, the investigation of these mechanisms can be achieved by combining the assays of key glucoregulatory hormones known to play important roles in glucose homeostasis with the infusion of the stable [6,6-²H] glucose isotope to determine

the rate of glucose appearance (Ra) and disappearance (Rd). Of note, we chose to maintain blood glucose levels between 5.0 and 6.0 mmol/L for the euglycaemic clamps not only because these are physiological levels, but also because of the evidence that initiating exercise with blood glucose levels near 5 mmol/l while under near basal insulinaemic conditions has a lesser effect on blood glucose levels during exercise (Nathan et al. 1985). In contrast, the glucose levels targeted for the hyperglycaemic clamp described in Chapter 6 were close to 9-10 mmol/L to avoid the loss of glucose in the urine that occurs at plasma level exceeding 10-11 mmol/L (Moe et al. 2008).

Using euinsulinaemic euglycaemic clamps, we achieved basal insulin levels between 100-126 pmol/L for the studies described in Chapters 4 and 6, with these levels reflecting those found in insulin-treated individuals under the effect of basal insulin (Nielsen et al. 1995; Gulan et al. 1987). In contrast, the pre-exercise free insulin levels achieved during the hyperinsulinaemic euglycaemic clamps used in Chapter 5 were between 170 and 200 pmol/L, with these insulin levels being within the broad range of plasma insulin levels achieved post-prandially in response to subcutaneous insulin injection (Francescato et al. 2004) and hyperinsulinaemic clamps (Chokkalingam et al. 2007a; Bonora et al. 1989). Of note, however, the clamped hyperinsulinaemic state achieved in Chapter 5 differed from that associated with a subcutaneous insulin injection/infusion bolus in that plasma insulin levels were kept at high and stable levels during the clamp, whereas plasma insulin levels increase transiently and change markedly following post-subcutaneous insulin bolus administration.

With the help of euinsulinaemic euglycaemic clamps, we undertook to examine the relationship between exercise intensity and the exogenous glucose requirements to maintain stable glycaemia during exercise performed under basal insulin conditions (condition defined as the insulinaemic state where no exogenous glucose is required to maintain stable glycaemia at rest). For this study, nine participants with T1D were subjected on 4 occasions to different exercise intensities (35, 50, 65, 80% $\dot{V}O_2$ peak) combined with the concomitant infusion of [6,6- 2H_2]glucose to measure both glucose Ra

and Rd. Our findings showed that the exogenous glucose requirements to maintain stable glycaemia followed an inverted U relationship with exercise intensity performed under basal insulin conditions, with no exogenous glucose being required during high intensity aerobic exercise.

Our findings that the amounts of CHO required to maintain stable glycaemia were relatively low at all exercise intensities are consistent with the findings of others that the risk of hypoglycaemia during and early after exercise is very low when exercise is performed while plasma insulin is at close to basal level (Nathan et al. 1985), particularly when exercise intensity is elevated (Mitchell et al. 1988; Purdon et al. 1993; Sigal et al. 1999; Marliss and Vranic 2002). On clinical grounds, these findings support the recommendation that the best time to exercise with the least risk of hypoglycaemia is when plasma insulin is at close to basal level.

As explained in Chapter 4, the pattern of change in glucose Ra and Rd can explain the inverted U relationship between exercise intensity and the amount of exogenous glucose required to maintain stable glycaemia. Glucose Rd being greater than glucose Ra during low to moderate intensity exercise, more exogenous glucose was required to maintain stable blood glucose levels at these exercise intensities (Chapter 4). The absence of increase in glucose Ra to match the rise in glucose Rd during exercise at low and moderate intensity exercise despite a small increase in plasma catecholamine levels together with the absence of change in plasma insulin and glucagon levels altogether are findings that support the contention that glucose Ra at these exercise intensities is controlled primarily by insulin to glucagon ratio rather than by changes in catecholamines levels. In contrast, the marked increase in catecholamines levels in response to intense aerobic exercise (80% $\dot{V}O_2$ peak) may explain why no exogenous glucose was required to maintain stable glycaemia at such a high exercise intensity since the accompanying marked rise in glucose Ra matched glucose Rd, a response showed by others to be mediated by increased catecholamine levels (Sigal et al. 1999; Purdon et al. 1993; Marliss and Vranic 2002).

One unexpected finding was the marked inter-individual variability in the extent to which exogenous glucose is required to maintain stable glycaemia during exercise of low to moderate intensity. For instance, although such requirements were on average 4 g/h, they ranged from 0 to 15 g/h across participants, with two participants necessitating no exogenous glucose to maintain stable blood glucose level, irrespective of exercise intensity. On clinical grounds, these findings imply that even under stable basal insulinaemic conditions, there is marked inter-individual variability in the amount of exogenous CHO required to maintain stable glycaemia.

In contrast to what is observed during exercise, our results also show that, irrespective of exercise intensity, CHO must be administered to maintain stable blood glucose level during early recovery from exercise performed under basal insulinaemic conditions. It is noteworthy that the exogenous CHO requirements to maintain stable glycaemia early during recovery were not significantly different from those during exercise of low to moderate intensity, and higher after rather than during intense exercise performed at 80% $\dot{V}O_2$ peak. These findings thus emphasise the need to monitor blood glucose levels not only during but also early after exercise irrespective of exercise intensity.

The findings in Chapter 4 raise the issue of whether the inverted U relationship between exercise intensity and the exogenous glucose requirements to maintain stable glycaemia also holds under hyperinsulinaemic conditions. We hypothesised that when plasma insulin levels are at the high end of the therapeutic range, there is still an inverted U relationship, but with extra glucose being required at all exercise intensities. We considered performing such a study to be clinically important as it is common for individuals with T1D to be physically active within 1-2 hours after the injection of their bolus of rapid acting insulin.

In order to test our hypothesis, nine participants with T1D were studied on 4 occasions at different exercise intensities. On each occasion, they cycled for 40 min while being subjected to a hyperinsulinaemic euglycaemic clamp designed to achieve plasma insulin

levels at the high end of the therapeutic range. As predicted, we found that more exogenous glucose was required to maintain stable glycaemia during the hyperinsulinaemic conditions compared to basal insulinaemic conditions when exercise is of low to moderate intensity. Against expectations, however, we found that the relationship between exercise intensity and the exogenous glucose requirement to maintain stable glycaemia under hyperinsulinaemic conditions followed a near hyperbolic rather than an inverted U relationship.

These results thus imply that CHO must be administered to prevent blood glucose levels from falling during high intensity aerobic exercise (80% $\dot{V}O_2$ peak) performed under hyperinsulinaemic conditions. This finding is in marked contrast to what we and others found for high intensity aerobic exercise performed under basal insulinaemic conditions where no exogenous CHO was required to maintain euglycaemia (Mitchell et al. 1988; Purdon et al. 1993; Sigal et al. 1999; Marliss and Vranic 2002; Shetty et al. 2016). On clinical grounds, our findings imply that high intensity aerobic exercise performed under hyperinsulinaemic conditions increases the risk of hypoglycaemia as opposed to the protective effect high intensity aerobic exercise has against hypoglycaemia risk when performed under basal insulinaemic conditions. These findings are clinically important as they support the work of others who showed that the amount of CHO required to prevent hypoglycaemia during exercise increases with plasma insulin concentration (Francescato et al. 2004).

Our results in Chapter 5 also show that extra CHO must be administered to maintain stable glycaemia during recovery from exercise performed under hyperinsulinaemic conditions. We also found for the first time that these CHO requirements post-exercise increase with the intensity of the prior bout of exercise. These results are thus not only consistent with those of others who have reported that blood glucose levels decrease after exercise performed under high insulin levels (Sonnenberg et al. 1990), but also entail on clinical grounds that the risk of hypoglycaemia is almost as high during early recovery

from exercise as during exercise, with CHO supplementation being required during and after exercise to prevent hypoglycaemia, irrespective of exercise intensity.

In addition to being affected by exercise intensity and plasma insulin levels, others have reported that the exogenous CHO requirements to maintain stable glycaemia during exercise increase with not only the levels at which blood glucose levels are maintained under basal insulin conditions, but also exercise duration, with these CHO requirements being low early during moderate intensity exercise and elevated 1.5-2.0 hours later irrespective of the levels at which glycaemia is maintained (Jenni et al. 2008). Since the issue as to whether the duration of this lag period is affected by the level at which glycaemia is maintained when exercise is performed under basal insulin conditions was not examined in the study of Jenni and colleagues (2008), the final study outlined in Chapter 6 was undertaken to determine whether the temporal pattern of increase in exogenous CHO requirements during 40 min of moderate intensity exercise is affected by the levels at which blood glucose is maintained.

For this study, eight participants with T1D underwent either a euglycaemic or hyperglycaemic clamp on separate days, and were infused with both insulin at a basal rate and [6,6-²H]glucose while cycling for 40 min at an intensity of 50% $\dot{V}O_2$ peak. We found that irrespective of the levels at which glycaemia is maintained, there was a 20-minute period of low demand in exogenous glucose to maintain stable glycaemia. Of note, however, these findings differ from those in Chapter 5 where there was no lag in the rise in the exogenous CHO requirements to maintain stable glycaemia when exercise was performed under hyperinsulinaemic conditions as these requirements increased significantly within 10 min following the start of exercise and remained elevated throughout exercise. The absence of an increase in GIR during the first 20 min of exercise performed under basal insulin occurred despite elevated CHO oxidation rates and no significant rise in glucose Rd, thus implying an increased reliance of skeletal muscles on the oxidation of their glycogen stores during that time. Beyond the first 20 min of exercise, the progressive increase in glucose Rd and absence of an increase in CHO oxidation rate

and glucose Ra suggest that muscle glycogen oxidation rate decreases with time, as is generally the case during aerobic exercise (MacLaren et al. 1999), thus explaining the sudden rise in glucose requirements after 20 min of exercise. Of note, such a 20-min lag may explain, at least in part, the low initial exogenous glucose requirements described in Chapter 4 when aerobic exercise is performed at 80% $\dot{V}O_2$ peak as the exercise duration at such an intensity was less than 20 min for most of the participants involved in that study.

Our findings in Chapter 6 also show that maintaining mild hyperglycaemia compared to euglycaemia before and during exercise increases the exogenous CHO requirements to maintain stable glycaemia, but not during the post-exercise period. These findings have clinical implications since they suggest that although little or no extra CHO intake is required to maintain stable blood glucose level during exercise if it lasts less than 20 min under basal insulin conditions, more CHO is required to maintain stable glycaemia if mild hyperglycaemia is maintained during exercise. On clinical grounds, our findings suggest that the risk of exercise-mediated hypoglycaemia is minimal for short duration exercise (<20 min) performed under basal insulin conditions.

7.2 Clinical implications, limitations with our findings and future research directions

Our main finding of an inverted U relationship between exercise intensity and the exogenous carbohydrate requirements to maintain stable glycaemia under basal but not hyperinsulinaemic condition may impact the advice provided by health care professionals to patients with T1D on how best to exercise safely to prevent hypoglycaemia while maintaining near stable blood glucose level. In particular, future recommendations based on our findings could advocate minimal CHO supplementation for mild to moderate exercise intensities and no CHO supplementation for high intensity aerobic exercise performed under basal insulinaemic conditions, but with some CHO intake being required during recovery, irrespective of exercise intensity. In contrast, increasing the amount of

CHO supplementation is required during and after exercise performed under hyperinsulinaemic conditions, irrespective of exercise intensities.

Just as importantly, our finding that the amount of CHO required to maintain euglycaemia during early recovery from exercise performed in a basal insulinaemic state is comparable or even higher than that during the exercise is an important one as it suggests that the risk of hypoglycaemia during early recovery is comparable or even higher than during exercise. For instance, under basal insulinaemic conditions, we found that glucose must be infused during recovery from high intensity aerobic exercise to maintain stable glycaemia, but not during exercise. Also, the increased hypoglycaemia risk associated with recovery was evident in the study described in Chapter 4 where two participants did not require any exogenous glucose to maintain euglycaemia during all intensities of exercise, but required glucose during the two hours of recovery post-exercise.

As reported in Chapters 4 and 5, the exogenous CHO requirements to maintain stable glycaemia during exercise varies between individuals and exercise intensities under both basal and high insulin conditions, thus implying that the quest for one-size-fits-all recommendations for CHO intake is unwarranted. These considerations highlight the importance of monitoring blood glucose levels before, during and at time intervals after exercise for the prevention of hypoglycaemia, irrespective of exercise intensity or prevailing insulin levels.

Finally, another important clinical issue addressed in this thesis is that of the effect of exercise duration on the level of CHO intake required to maintain stable glycaemia. The current recommendation that CHO intake may not be required prior to moderate intensity exercise if of a short duration (<30 min) is not evidence based (Sherr et al. 2018). Our findings provide such evidence by suggesting that extra CHO supplementation may not be needed for moderate intensity exercise lasting less than 20 min when performed under basal insulinaemic conditions. This information might be relevant to children with

T1D as it is not uncommon for them to play for a short duration during school recess and lunch breaks.

To summarise, here are some of the key findings of this thesis that may have the potential to inform the development of future guidelines for the management of blood glucose levels in physically active individuals with T1D:

For exercise performed under basal insulin conditions,

1. The finding of an inverted U relationship between exercise intensity and the exogenous CHO requirements to maintain stable glycaemia highlights the necessity of ingesting CHO in preparation for mild and moderate intensity exercise, with no supplementation required for high intensity aerobic exercise.
2. For some people who exercise under basal insulin conditions, there may be no need to ingest any CHO to prevent blood glucose levels from falling, irrespective of exercise intensities.
3. There is a lag of ~20 min before more exogenous CHO are required to maintain stable glycaemia during exercise when plasma insulin is at near basal level, thus making exercise lasting less than 20 min safer with a lesser risk of hypoglycaemia. CHO supplementation may thus not be required prior to short duration exercise performed under basal insulin conditions.
4. The CHO requirements to maintain stable glycaemia during exercise depend on the level at which glycaemia is maintained, with more CHO being required to maintain stable blood glucose at higher blood glucose levels.
5. Irrespective of exercise intensity, the amount of CHO required to maintain stable blood glucose levels during recovery is either comparable (35, 50, 65% $\dot{V}O_2$ peak) or higher (80% $\dot{V}O_2$ peak) than those during exercise. These findings suggest that hypoglycaemia risk is comparable early after exercise compared to during exercise, and CHO should be ingested during recovery, irrespective of exercise intensity.

6. There is marked inter-individual variability with respect to the CHO requirements to maintain stable glycaemia in response to exercise, even when exercise is performed under basal insulinaemic conditions and highly standardised laboratory conditions.

For exercise performed under high insulin conditions,

1. There is a near hyperbolic rather than an inverted U relationship between exercise intensity and the levels of exogenous CHO required to maintain stable glycaemia, with CHO supplementation being required to maintain stable glycaemia even during high intensity aerobic exercise.
2. The CHO requirements to maintain stable glycaemia are increased during early recovery from exercise performed under hyperinsulinaemic conditions. This implies that CHO intake for the prevention of hypoglycaemia should be advocated for the early recovery period.

The ultimate aim of this thesis was to provide some evidence-based information to inform and improve future exercise guidelines for individuals with T1D. Our findings reinforce the recommendation that the safest approach to decrease the risk of exercise-mediated hypoglycaemia is to exercise while plasma insulin is at or near basal levels, since exercising under such conditions is associated with minimal changes in blood glucose levels and low exogenous glucose requirements as opposed to exercising under hyperinsulinaemic conditions.

However, before recommending any glucose supplementation based on our findings, the oral glucose equivalent of our intravenous glucose infusion data should be established. Indeed, we adopted a clamp-based approach to investigate the intravenous glucose requirements to maintain stable glycaemia during exercise of different intensities, implying that our results cannot be fully translated to clinical practice until future research are performed to convert our intravenous data to corresponding oral intake. More research is thus warranted to find out the oral glucose equivalent of the intravenous glucose requirements to translate some of the findings of this thesis to

clinical practice. Also, the use of results obtained from clamp-based studies to inform oral intake of carbohydrate would have to take into consideration the fact that plasma insulin levels after an insulin bolus is not stable as opposed to the stable hyperinsulinaemic state generated in our studies. Finally, the findings arising from this thesis are based on data obtained from a very specific population of healthy complication-free young adults with T1D, and for this reason may not apply to the wider population of individuals with T1D. In addition, the findings this project was undertaken on individuals with T1D who were treated with either multiple dose insulin regimen or on standard insulin pump therapy. With the introduction of closed loop pump therapy for management of T1D, we need to investigate in future if the recommendations from this project are applicable for individuals on closed loop pump therapy. For this reason, it is of paramount importance to determine whether the results from the studies described here are applicable to all age groups and different free-living conditions. We hope that the findings of this thesis will help inform future guidelines and improve the way blood glucose level is managed in physically active individuals with T1D, thereby helping them exercise with confidence and with no or little fear of hypoglycaemia.

Chapter 8

References

8.1 References

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