Sprinting as a clinical tool for the prevention of exercise-mediated hypoglycaemia in Type 1 Diabetes Mellitus

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Bachelor of Science (Honours)

This Thesis is presented for the degree of Doctor of Philosophy at the University of Western Australia

School of Sport Science, Exercise and Health

2015
Statement of Candidate Contribution

The work involved in designing and conducting the studies described in this thesis has been carried out primarily by Vanessa Bussau (the candidate). The thesis outline and experimental design of the studies was developed and planned by the candidate in consultation with Professor Paul A. Fournier (the candidate’s primary supervisor) and Professor Timothy W. Jones (co-supervisor). All participant recruitment and management was carried out entirely by the candidate, along with the actual organisation, implementation and performance of the experiments. In addition, the candidate was responsible for all data analysis and original drafting of the thesis and peer-reviewed publications. Professor Paul Fournier (and Dr Luis Ferreira) have provided feedback for further drafts and completion of the thesis and manuscripts.

Signed:

Vanessa Bussau             Paul Fournier
(Candidate)                (Supervisor)


Abstract

Despite the numerous physiological and psychological health benefits of a physically active lifestyle for individuals with type 1 diabetes, the risk of hypoglycaemia increases both during and after exercise. It is important to note, however, that not all types of exercise result in an elevated risk of hypoglycaemia. For instance, prolonged high-intensity aerobic exercise in these individuals result in an increase in glycaemia during and after exercise. This raises the intriguing possibility that this type of exercise might be beneficial if adopted to counter a fall in glycaemia in complication-free individuals with type 1 diabetes, thus helping to prevent or delay hypoglycaemia if no carbohydrate is readily available. Unfortunately, this type of exercise modality to prevent hypoglycaemia is unlikely to be well tolerated by most individuals with type 1 diabetes due to the impractical duration of this type of exercise. This raises the primary aim at the core of this thesis to determine whether a much shorter bout of exercise lasting only 10 sec and performed at maximal intensity could be adopted to prevent glycaemia from falling. For this reason, the primary goal of this thesis was to determine whether a 10-sec maximal sprint effort performed after (Chapter 3) or before (Chapter 4) moderate intensity exercise provides a possible means other than carbohydrate intake to prevent glycaemia from falling when exercise is performed under hyperinsulinaemic conditions by complication-free individuals with type 1 diabetes. Also, given that for this type of study, it is common practice to subject participants to a graded exercise test to set exercise intensity relative to $\hat{V}O_{2peak}$, a secondary objective of this thesis was to determine whether the risk of hypoglycaemia is increased early during recovery from this type of exercise protocol (Chapter 2). Finally, since the counterregulatory response to sprinting has not been examined in hyperinsulinaemic individuals with type 1 diabetes, thus making it difficult to compare the findings of Chapters 3 and 4 with the literature, our last aim was to examine the counterregulatory responses to sprinting in type 1 diabetic individuals under hyperinsulinaemic conditions (Chapter 5).

The first study of this thesis (Chapter 2) examines whether the risk of hypoglycaemia increases in response to graded exercise testing in individuals with type 1 diabetes. Eight non-diabetic male participants and seven complication-free type 1 diabetic male individuals in good glycaemic control were recruited. On the morning of testing, the diabetic participants followed their normal insulin regimen, and both groups ate their usual breakfast. Then, participants were subjected to graded exercise testing
approximately four hours later. We found that this type of exercise result in a rapid post-exercise increase in blood glucose levels (> 2 mM), which remain elevated for the first two hours of recovery. On clinical grounds, these findings suggest for the first time that the early post-exercise risks of hypoglycaemia associated with graded exercise testing are minimal when performed under near basal plasma insulin levels, with no carbohydrate administration required soon before or after testing to prevent hypoglycaemia.

The primary goal of our next study (Chapter 3) was to determine whether a short 10-sec maximal sprint effort is preferable to only resting as a means to counter a further fall in glycaemia during recovery from moderate intensity exercise in hyperinsulinaemic individuals with type 1 diabetes. To meet our objective, seven healthy complication-free male participants with type 1 diabetes injected their normal insulin dose and ate their usual breakfast. Then, when their postprandial glycaemia fell to ~11 mM they pedalled at 40% \( \dot{V}O_{2\text{peak}} \) for 20 min on a cycle ergometer followed immediately by either a maximal 10-sec sprint or a rest. Our results show that, during exercise, blood glucose levels fell rapidly. However, sprinting immediately after exercise opposes a further fall in blood glucose levels for at least 120 min while glycaemia decreases significantly (p < 0.05) by ~ 3.5mM when no sprint was performed. We also found that sprinting is likely to counter the exercise-mediated decrease in blood glucose levels through an increase in catecholamine, lactate, and growth hormone levels. Interestingly, these glucoregulatory benefits of sprinting are remarkable considering the sprint trial was performed when insulin levels were elevated, a time when exercise is not usually recommended. On the basis of these findings, one might tentatively recommend that in order to minimise the risk of early hypoglycaemia post-moderate intensity exercise, it is preferable for complication-free young individuals with type 1 diabetes to engage in a 10-sec maximal sprint effort before resting than to only rest during recovery, particularly if a source of dietary carbohydrate is not readily available.

Given the glycaemia stabilising effect of sprinting performed after moderate-intensity exercise (Chapter 3), the study described in Chapter 4 examines whether performing a short sprint effort immediately prior to moderate-intensity exercise may offer a novel way of preventing glycaemia from falling both during and after moderate-intensity exercise. To this end, seven complication-free type 1 diabetic males injected their normal morning insulin dose and ate their usual breakfast. When post-meal glycaemia fell to ~11 mM, they were asked to perform a 10 sec all-out sprint (sprint trial) or to rest (control trial)
immediately before cycling at 40% of peak rate of oxygen consumption for 20 min. We found, against expectations, that sprinting for 10 sec immediately before moderate-intensity exercise performed under hyperinsulinaemic conditions does not affect the rapid decline in glycaemia during exercise. However, sprinting rather than resting before moderate-intensity exercise did prevent glycaemia from falling for at least the first 45 min of recovery in individuals with type 1 diabetes. This suggests that including a short sprint as part of the warm-up routine of individuals with type 1 diabetes before they engage in sustained moderate-intensity exercise might provide another means of temporarily stabilising glycaemia during early recovery.

Unfortunately, one difficulty with comparing the counterregulatory responses described in Chapters 3 and 4 with the literature is the lack of information on the effect of short-duration sprinting *per se* on the responses of counterregulatory hormones in hyperinsulinaemic diabetic individuals. For this reason, the purpose of the study described in Chapter 5 was to investigate the effect of a single 10-sec sprint on the levels of the counterregulatory hormones in type 1 diabetic individuals under hyperinsulinaemic conditions designed to approach those reported in Studies 3 and 4. In this study, we found that performing a 10-sec maximal sprint resulted in patterns of change in plasma catecholamines, growth hormone, cortisol and glucagon levels comparable to those observed when a sprint is performed immediately after a bout of moderate-intensity exercise in individuals with type 1 diabetes (Study 2) and also comparable to those observed in response to a sprint performed after an overnight fast (Fahey et al., 2012). What remains to be established in future studies is the extent to which the changes in the levels of these counterregulatory hormones contribute to the glucoregulatory benefits of sprinting.

In conclusion, although a number of issues must be addressed before recommending the adoption of short-duration sprinting as a safe and reliable tool for the short-term management of blood glucose levels in individuals with type 1 diabetes, this thesis shows that sprinting has the potential to help individuals with type 1 diabetes to exercise more safely and take advantage of the many physiological and psychological health benefits of a physically active lifestyle.
Acknowledgements

In writing my acknowledgements, I am overwhelmed with a sense of gratitude and appreciation to all the people who have made this thesis possible. I have a huge smile as I reminisce about the amazing people I have met and worked with along the way. Words can not express how thankful I am for help and support of so many amazing people. During the course of my PhD it seems like I have experienced almost every major life event and the some of the absolute best and worst of times. Throughout the journey I have had the support the best family anyone could wish for together with wonderful colleagues who have become lifelong friends. I look forward to thanking you in person but please realise how extremely grateful I am for your help, support and friendship during the course of my PhD. In particular, I would like to sincerely thank the following incredible people for their contribution towards my thesis:

My study participants - Thank you for your invaluable contribution. Recruitment was such a challenge for this thesis so an extra huge thank you for your time and effort. It was great to get to know you all and I wish you all the very best always. I hope you can safely enjoy a more active life as a result of the research in this field.

Professor Paul Fournier (and Angeline) - for your passion, professionalism, infinite knowledge and expertise, commitment, work ethic, endless enthusiasm and support (not only with my PhD but with my career and life in general).

Dr Luis Ferreira (and Daniela) - for your research knowledge and expertise, balanced advice, encouragement (together with your friendship and sharing my love of the Eagles, sport and the important things in life).

Dr Kym Guelfi - for always being there throughout the journey with practical help, advice, knowledge, friendship and huge morale support, you are an amazing friend and researcher.

Alex D’Vauz (nee Baptista) - for your amazing friendship, encouragement, help, sense of fun and for always bringing a smile to my face.
Dr Ray Davey - from ‘Little Aths’ in Margaret River as kids to PhD buddies in the same research team - thanks for your friendship, encouragement, advice and football banter.

Scott, Lian, Jen, Rob D, Hans (& Emma), Rob M, James (& Bree), Tim (& Sarah), Les, Pete, Elisa, Tom, Jonas, Si, Nat, Stephen, Sani, Brad & the ‘Postgrad team’ - to each and every one of you for your friendship, help, advice, camaraderie, fun times and great memories (from corridor cricket & Friday tennis to our Annual Cocktail Party and so many celebrations). Hans - it still seems surreal that you are not with us but your memory will remind us to always cherish each day and we will never forget your love for your family, friends and your passion for Exercise Physiology and Muscle Metabolism.

UWA Type 1 Diabetes Team – Paul, Luis, Kym, Alex, Tim, Avril, Katherine, Chee, Harris & the PMH Team - Prof. Tim Jones, A/Prof. Liz Davis, Niru, Leanne, Michael, Sarah, Vanessa. Thank you to a fantastic team of passionate researchers. It has been a long journey but I have learnt so much from ‘being there at the start’ and seeing our research team and field grow.

Our Nurses – Alisha, Nurse Bettye, Niru and Christy - thanks for your professionalism, friendship and ability to make ‘testing days’ more enjoyable for all.

SSEH Team – Bec, Kerry, Prof. ‘Daws’, Karen, Danny, Brenda, Inga, Pat, Margaret, Robin, Don & the Technical Staff for your help and support. Heads of Schools (Prof. Brian Blanksby, Prof. Bruce Elliot, Prof. Tim Ackland) – for your support and exciting career, research and teaching opportunities. To Prof. Tim Ackland, Sharon Gam and Jo Francis for your huge help in this final phase.

Past Teachers, Lecturers and Mentors – from tiny Karridale Primary to Margaret River High and UWA - to everyone who helped nurture my love of learning and science.

Inspiring Researchers, specialists, diabetes educators, endocrinologists and the incredible people with type 1 diabetes I was privileged to meet. Hearing your thoughts and discussing ideas at various conferences around the world was a huge privilege and highlight of my PhD journey.

Mum, Dad, Daniel, Natalie - throughout life, school, ‘Undergrad’, Honours, PhD and now life with my boys and business. Thanks for your unwavering love and support always. Mum and Dad, thank you for always encouraging me to work hard and to pursue my dreams. Thank you for all the opportunities you gave me throughout my education and for encouraging me to follow my dreams and create a career in a field that I love. I feel so incredibly grateful every time I see a patient and think how much I love helping people and promoting exercise and health. Mum, Dad, Daniel (& Lucy) and Nat (& Mike) – thank you for all your never-ending support along the journey – from school, study, career and life. I love you all so unbelievably much and will never forget your endless love and support always. Your contribution to this thesis is immense and will always be cherished.

Dad - I miss you so much and wish you were here in person to firstly meet Marcus & my ‘boys’ and to see this PhD completed. I remember how proud you were when my 1st paper was accepted for publication in your final days with us and hope you can enjoy a celebration from ‘above’ with us.

To my extended family and friends, thank you to each and every one of you. In particular, thank you to Ann, Noelle, Carrina for inspiring me in your own way. To Stu and Kay, Rayma (& Geoff) and George for welcoming me into your family and for all your help and support with our boys.

Finally, to Marcus, Owen, Max (and Lexie). Thanks for making me so unbelievably happy and bringing so much joy and happiness into my life. I love you all more than words can say. Marcus – thank you for your immense support. I will be eternally grateful for the huge effort and sacrifices you and the boys have made so I could finish my PhD.
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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>AIHW</td>
<td>Australian Institute of Health and Welfare</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>CON</td>
<td>Control</td>
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<tr>
<td>CGMS</td>
<td>Continuous glucose monitoring system</td>
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<tr>
<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
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<td>Direcnet</td>
<td>Diabetes Research in Children Network</td>
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<tr>
<td>FFA</td>
<td>Free fatty acids</td>
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<td>GIR</td>
<td>Glucose infusion rate</td>
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<td>Growth hormone</td>
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<td>Glucose transport protein</td>
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<td>HbA1c</td>
<td>Glycosylated haemoglobin</td>
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<td>IHE</td>
<td>Intermittent high-intensity exercise</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
</tr>
<tr>
<td>J</td>
<td>Joule</td>
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<tr>
<td>JDRF</td>
<td>Juvenile Diabetes Research Foundation</td>
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<tr>
<td>LOPEH</td>
<td>Late onset post-exercise hypoglycaemia</td>
</tr>
<tr>
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<td>Minute</td>
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<tr>
<td>mM</td>
<td>Millimoles per litre</td>
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</tr>
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<td>Radioimmunoassay</td>
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<td>Standard error of the mean</td>
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<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
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<tr>
<td>T1DM</td>
<td>Type 1 diabetes mellitus</td>
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<tr>
<td>µL</td>
<td>Microlitres</td>
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<tr>
<td>$\dot{V}O_2$</td>
<td>Rate of oxygen consumption</td>
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<tr>
<td>$\dot{V}O_{2\text{peak}}$</td>
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Publications Arising from this Thesis

Peer-Reviewed Publications


Conference Proceedings


Chapter 1

Introduction and Review of the Literature
1.1 Introduction and Type 1 Diabetes Mellitus

Type 1 diabetes mellitus, formerly known as insulin-dependent or juvenile-onset diabetes mellitus, is an endocrine disease that occurs commonly in childhood and adolescence, but can be recognised and become symptomatic at any age (Dinneen and Rizza, 2001). Type 1 diabetes affects millions of people worldwide including approximately 140 000 Australians (IDF, 2009). The International Diabetes Federation found Australia to be one of the top ten countries with the highest incidence of type 1 diabetes in children (Soltesz et al., 2009), with over 8,000 new cases between 2000 and 2008, an average of two new cases every day (AIHW, 2010). In individuals over 15 years, 9000 new cases of type 1 diabetes were diagnosed in Australia between 2000-2006 or three new cases per day (AIHW, 2008).

Type 1 diabetes is characterised by the absence of insulin secretion due to the autoimmune destruction of the beta cells of the Islets of Langerhans of the pancreas (Rizza et al., 2001). The small proportion of individuals that appear not to have an autoimmune basis for their beta cell destruction have a sub-type of diabetes referred to as idiopathic type 1 diabetes (Dinneen and Rizza, 2001). As a consequence of the destruction of the beta cells, the body loses its capacity to produce insulin. Given that insulin promotes the storage of carbohydrates and fat, inhibits ketone body production, and stimulates a decrease in blood glucose levels by both inhibiting hepatic glucose production and stimulating peripheral glucose uptake, it is not surprising that the absence of insulin leads to an increase in blood glucose (hyperglycaemia) and ketone body levels (Lernmark, 2001). This results in the many symptoms typical of untreated type 1 diabetes including glucose and ketone body loss in the urine (glucosuria and ketonuria), excessive urine production (polyuria), extreme thirst and consumption of large quantities of water (polydipsia), excessive consumption of food (polyphagia), and rapid weight loss (Eisenbarth et al., 2008). Other symptoms may include nausea, vomiting, blurred vision, confusion, shortness of breath and extreme fatigue (Eisenbarth et al., 2008). Severe hyperglycaemia and elevated ketone body levels increase markedly the risk of septicaemia and ketoacidosis, respectively. This explains why, without treatment, death generally occurs within 1-2 years of the onset of type 1 diabetes (Campagne and Lampman, 1994).

The discovery of insulin in 1921 and the initiation of treatments based on regular insulin injections have extended considerably the life expectancy of individuals with type 1
diabetes. Currently, insulin is delivered by injection or constant subcutaneous infusion via a pump (Sherr et al., 2009; Bergenstal et al., 2010). A number of types of insulin have been developed, each with different absorption rates, onset, time to peak action and duration of action. The dosage of insulin is often adjusted in response to self-monitored blood glucose levels, nutritional intake and physical activity (Eisenbarth et al., 2008).

1.2 Treatment of Type 1 diabetes Mellitus and Associated Hypoglycaemia

The main challenge in the treatment of type 1 diabetes is to maintain blood glucose levels close to a normal physiological range as this reduces the risk of developing a number of severe diabetic complications including microvascular and macrovascular diseases, nephropathy (kidney disease), retinopathy (damage to the retina of the eye) and neuropathy (disease of the nervous system) (Brownlee et al., 2008; Eisenbarth et al., 2008). This link between low risk of developing such complications and the importance of maintaining blood glucose levels as close to normal as possible were demonstrated by The Diabetes Control and Complications Trial in 1993 (Diabetes Control and Complications Trial Research Group, 1993) and more recent studies (Nathan et al., 2009). There is also evidence that, irrespective of glycaemic control, severe glycaemic excursions also contribute to the development of these complications (Soupal et al., 2014).

Unfortunately, the treatment of type 1 diabetes to maintain blood glucose levels within the narrow physiological range found in non-diabetic individuals increases considerably the risk of hypoglycaemic episodes (Cryer, 2010a). This is because of the absence of feedback mechanisms between blood glucose levels and insulin secretion normally found in non-diabetic individuals (Galassetti and Riddell, 2013). If too much insulin is administered, the state of relative hyperinsulinaemia that ensues decreases hepatic blood glucose production and increases glucose uptake, thus causing glucose utilisation to exceed glucose production rate and, as a result, blood glucose levels decrease below normal levels, a condition referred to as hypoglycaemia (Gerich, 2001).

In general, when blood glucose levels fall below 3 mM, warning symptoms alert an individual to the presence of hypoglycaemia (McAulay et al., 2001). These include “neuroglycopenic” symptoms due to insufficient glucose reaching the brain (Cryer, 1999) and may include a loss in concentration, fatigue, weakness, confusion, difficulty in
thinking and speaking (Hepburn et al., 1991; Towler et al., 1993). Hypoglycaemia-induced stimulation of the sympatho-adrenal system also triggers neurogenic (autonomic) symptoms. These include “adrenergic” symptoms including tremor, heart palpitations and anxiety as well as “cholinergic” symptoms such as sweating, hunger and paraesthesia (Towler et al., 1993). Perception of these neurogenic symptoms is essential in one’s awareness of hypoglycaemia and self-recognition that blood glucose levels are low (Towler et al., 1993). Unfortunately, some individuals, diagnosed as “hypoglycaemia unaware” experience minimal symptoms when their blood glucose levels fall to dangerously low levels, with a late response to warning symptoms increasing their possibility of experiencing severe hypoglycaemic episodes (Gold et al., 1994; McAuley et al., 2001; Cryer et al., 2009; Candace et al., 2013).

The increased risk of hypoglycaemia associated with insulin-based therapy is a major concern because severe hypoglycaemia can lead to central nervous system (CNS) damage, and in extreme cases coma and even death (Ben-Ami et al., 1999; Cryer, 2007). This is because a continuous supply of glucose is essential for normal cerebral function, since the brain uses glucose as its main source of fuel under normal conditions (Gerich, 2000). Moreover, the brain stores little carbohydrate as glycogen (Herzog et al., 2010). Given the importance of supporting the glucose requirements of the brain, it comes as no surprise that most of the glucose synthesised in the body is utilised by this organ.

Unfortunately, hypoglycaemia is a common problem in the everyday life of most individuals with type 1 diabetes. In fact, individuals with type 1 diabetes experience on average two episodes of symptomatic hypoglycaemia per week, which equates to thousands of hypoglycaemic episodes during their lifetime (Alsahli and Gerich, 2008). It is important to remember that these rates are likely to underestimate the true number of hypoglycaemic events due to many missed asymptomatic and symptomatic episodes together with incorrect reporting (Cryer et al., 2009). In contrast, rates of severe episodes of hypoglycaemia that require extended intervention to treat are more reliable, with individuals with type 1 diabetes likely to suffer one such event per year often involving coma or seizures (Cryer, 2008a, 2010b). Even more alarming is the fact that people with type 1 diabetes die from hypoglycaemia (Laing et al., 1999). For these reasons, it is understandable that hypoglycaemia is the primary and most feared complication of insulin therapy (Amiel, 2009). It is not surprising, therefore, that such a therapy-induced hypoglycaemia, or iatrogenic hypoglycaemia, is considered as a major limiting factor in
the glycaemic management of type 1 diabetes as it causes recurrent morbidity in most individuals with type 1 diabetes (Cryer, 2005; 2010b). For this reason, any strategy aimed at reducing the risk of hypoglycaemia is likely to be well received by individuals with type 1 diabetes.

1.3 Counterregulatory Response to Hypoglycaemia in Non-Diabetic Individuals

Fortunately, the body possesses a range of mechanisms to counter hypoglycaemia. In order to best appreciate how this is achieved in individuals with type 1 diabetes, it is important in the first instance to examine how hypoglycaemia is countered in non-diabetic individuals. Firstly, 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 detect glucose changes or hypoglycaemia (McCrimmon, 2009; Watts and Donovan, 2010). In healthy non-diabetic individuals, 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 to restore euglycaemia (Cryer, 1993, 2008b). The magnitude of such counterregulatory responses is determined in part by the depth and duration of hypoglycaemia as well as by other factors including age, gender, rate of decline in blood glucose before the onset of hypoglycaemia, antecedent exercise (Galassetti et al., 2001a), and antecedent hypoglycaemia (Davis et al., 1991, 2000b).

1.3.1 Insulin

The first line of defence against hypoglycaemia is suppression of insulin secretion (Bolli, 1999) when blood glucose falls to a level of approximately 4.5 mM (Cryer et al., 2003). The resultant decrease in circulating insulin levels stimulates an increase in hepatic glucose production (Cherrington et al., 1998) and a decrease in glucose utilisation rate (Cryer, 2001), which as a result counter declining glycaemia. However, if blood glucose levels continue to fall to between 3.6 to 3.9 mM, the body responds by increasing the secretion of counterregulatory hormones (Cryer et al., 2003). These hormones act to restore glucose levels within a normal, safe physiological range by increasing glucose production and inhibiting peripheral glucose uptake. It is important to note that the contribution of each counterregulatory hormone is different, with some hormones being more important than others as discussed below (Rizza et al., 1979b; Schwartz et al., 1987; Mitrakou et al., 1991; Fanelli et al., 1994).
1.3.2 Glucagon

One of the key counterregulatory hormones is glucagon. Glucagon is secreted by the alpha cells of the Islets of Langerhans of the pancreas (Quesada et al., 2008) when blood glucose levels are low, with this hormone having an immediate effect on glucose kinetics (Alsahli and Gerich, 2008). The secretion of glucagon by the alpha cells of the pancreas is mainly influenced by blood glucose levels, with an increased secretion rate when blood glucose levels are low (Jiang and Zhang, 2003; Porcellati et al., 2003). Other factors that stimulate glucagon secretion are catecholamines (Gerich et al., 1973a), amino acids (Rocha et al., 1972; Schmid et al., 1989) and short-term exposure to fatty acids (Hong et al., 2005). In contrast, high levels of insulin and somatostatin inhibit glucagon secretion (Ito et al., 1995; Hauge-Evans et al., 2009). Acting independently of these factors, autonomic neural activation of the islet is thought to influence glucagon response to hypoglycaemia (Havel et al., 1993).

Glucagon stimulates an increase in glucose levels firstly by activating hepatic glycogenolysis and gluconeogenesis (Lecavalier et al., 1989; Roden et al., 1996; Camacho et al., 2005). Even small changes in glucagon levels can increase glucose production (Myers et al., 1991). Glucagon may also prevent blood glucose levels from falling by inhibiting glycogen synthesis in the liver (Jiang and Zhang, 2003). Glucagon is considered the most important counterregulatory hormone as demonstrated by studies where recovery from hypoglycaemia has been shown to be impaired by a deficiency in glucagon (Rizza et al., 1979b; Boyle et al., 1989). In particular, the importance of glucagon is best shown by the observation that when glucagon response to hypoglycaemia is prevented, this results in a blunted compensatory increase in endogenous glucose production despite an increase in epinephrine secretion (De Feo et al., 1991b). In contrast, glucagon does not affect renal glucose production or utilisation (Stumvoll et al., 1998a).

It is important to note that it is the level of portal glucagon relative to that of insulin as expressed by the portal venous glucagon to insulin ratio that plays a central role in the control of glycaemia (Quesada et al., 2008) as these hormones acutely increase or decrease glucose production and utilisation to maintain blood glucose levels within a narrow physiological range (Cryer, 2008b). A number of studies have highlighted the importance of the portal venous glucagon-to insulin ratio in the regulation of hepatic glucose production (Ferrannini et al., 1982; Lins et al., 1983; Steiner et al., 1990). When
this ratio is high, blood glucose levels increase as a result of decreased glucose utilisation and increased glucose production (Boyle et al., 1989). The opposite occurs when this ratio is low (Boyle et al., 1989).

1.3.3 Catecholamines

Catecholamines, including epinephrine and norepinephrine, are also thought to play a major role in counterregulation in non-diabetic individuals. Epinephrine is released by the chromaffin cells of the adrenal medulla, while norepinephrine is released by both the adrenal medulla and the sympathetic nerve endings (Deschenes et al., 1991; Zouhal et al., 2008). Hypoglycaemia, in its early stage, induces an elevation in sympathetic activity in non-diabetic individuals, which results in an increase in catecholamine levels (Sotsky et al., 1989; Bolli, 1999; Davis et al., 2000b). Although this increase in epinephrine and norepinephrine levels is well established, there has been a long standing controversy regarding the relative importance of catecholamines versus glucagon in counterregulation (Cryer, 1981; Bolli, 1999). An important role for catecholamines in the stimulation of glucose production during prolonged hypoglycaemia is highlighted by the observation that pharmacological blockage of catecholamine action results in severe hypoglycaemia despite increases in other counterregulatory hormones including glucagon (De Feo et al., 1991a). In contrast, others have concluded that when glucagon response to hypoglycaemia is normal, catecholamines play only a minor role (Rizza et al., 1979b; Cryer et al., 2003). However, when glucagon response is impaired or absent, catecholamines, in particular epinephrine, play a critical role in counterregulation (Rizza et al., 1979b; Boyle et al., 1989; Cryer et al., 2003).

Catecholamines increase glycaemia by acting on multiple organs (Meyer et al., 1999; Alsahli and Gerich, 2008). An increase in epinephrine and norepinephrine levels results in an elevation in blood glucose levels due to an increase in hepatic (Sacca et al., 1980) and renal (Stumvoll et al., 1998b) glucose production together with a fall in insulin-mediated stimulation of glucose utilisation (Rizza et al., 1979a; Nonogaki, 2000; Coker & Kjaer, 2005). Catecholamines increase the rate of hepatic glucose production by stimulating an increase in both glycogenolysis and gluconeogenesis (Barth et al., 2007). This increase in hepatic glucose production is initially due to the stimulation of glycogenolysis, while hepatic gluconeogenesis later becomes the predominant contributor to sustained hepatic glucose production (Sacca et al., 1983). Catecholamines also decrease the rate of glucose utilisation with both epinephrine (Rizza et al., 1979a;
Deibert and DeFronzo, 1980; Lager et al., 1986) and norepinephrine (Lembo et al., 1994) inhibiting insulin-mediated stimulation of glucose uptake by skeletal muscles. As discussed later, however, there are conditions where catecholamines and other adrenoceptor agonists have been reported to increase muscle glucose uptake (Abe et al., 1993; Nonogaki, 2000; Ngala et al., 2013).

Catecholamines also indirectly increase blood glucose levels. High levels of catecholamines suppress endogenous insulin release (Clutter et al., 1980; Sherwin et al., 1980; Sacca et al., 1983) and stimulate the supply of gluconeogenic substrates (Sacca et al., 1983; Stumvoll et al., 1998b) including lactate from resting muscles (Sacca et al., 1983). Catecholamines also stimulate glucagon (Gerich et al., 1973a) and growth hormone release (Blackard and Heidingsfelder, 1968). Finally, catecholamines stimulate lipolysis (Clutter et al., 1980) and therefore increase the levels of plasma free fatty acids (Sherwin et al., 1980; Sacca et al., 1983), decreasing carbohydrate utilisation and increasing hepatic glucose production.

1.3.4 Growth hormone
Hypoglycaemia also results in the activation and release of GH, a group of counterregulatory hormones produced by the pituitary gland and which also plays some role in the defence against hypoglycaemia. Indeed, growth hormone is a heterogeneous class of protein hormones consisting of a series of related isoforms (Baumann, 2009). When the hypothalamus senses hypoglycaemia, growth hormone releasing hormone and somatostatin are thought to be released together with other growth hormone-releasing factors resulting in the pulsatile secretion of growth hormone from the anterior pituitary gland (Reiter and Rosenfeld, 2008).

Growth hormone is not considered important in opposing acute hypoglycaemia, but it may play a role during prolonged hypoglycaemia. This is based on the observation that the pharmacological blockage of growth hormone release or action during prolonged hypoglycaemia impairs the increase in glucose production, thus resulting in more severe hypoglycaemia (De Feo et al., 1989b; Boyle and Cryer, 1991). This effect of growth hormone on glucose turnover takes several hours to take place (De Feo et al., 1989b; Boyle and Cryer, 1991). Growth hormone has also an indirect effect on counterregulation by stimulating lipolysis. The resulting increase in glycerol and free fatty acids levels provide gluconeogenic substrates and fuels, respectively, with the capacity to spare
glucose by inhibiting glucose utilisation (De Feo et al., 1989b). Prolonged growth hormone exposure has been shown to have insulin-antagonistic effects (Fowelin et al., 1991) whereas in contrast short duration growth hormone infusion does not invoke insulin resistance (Djurhuus et al., 2004).

It is noteworthy that some studies have shown that growth hormone has an acute effect on glucose metabolism. Indeed, local growth hormone exposure results in a rapid decrease in forearm glucose uptake (Zierler and Rabinowitz, 1963; Rabinowitz et al., 1965; Gibney et al., 2007). Furthermore, the administration of a physiological growth hormone pulse in non-diabetic individuals has been reported to result in a rapid fall in muscle glucose uptake (Møller et al., 1990, 1992b, 2003) and a 1-2 hour delayed increase in lipolysis, circulating free fatty acid levels, and fat oxidation rates (Møller et al., 1990, 1992b; Gravholt et al., 1999; Møller et al., 2003; Djurhuus et al., 2004), which altogether could contribute further to lowering glucose utilisation rates (Møller et al., 1992b) and stimulating hepatic glucose production.

1.3.5 Cortisol
Another counterregulatory hormone implicated in prevention of hypoglycaemia is cortisol. When the hypothalamus senses hypoglycaemia, this results in the release of corticotrophin-releasing factor in the pituitary portal vessels which in turn stimulates the secretion of adrenocorticotropic (ACTH) hormone by the anterior pituitary gland. ACTH then activates the secretion of cortisol by the cortex of the adrenal glands (Macdonald and King, 2007), which acts indirectly in the acute counterregulatory response by further stimulating catecholamine secretion by an intra-adrenal effect.

Like GH, cortisol is not considered important in the acute response to hypoglycaemia (Heller, 2011). Despite this, cortisol plays an important role in long term counterregulation when hypoglycaemia is prolonged (De Feo et al., 1989a; Boyle and Cryer, 1991). Under these conditions, cortisol increases glucose production and inhibit glucose utilisation after approximately 3 hours (De Feo et al., 1989a; Boyle and Cryer, 1991). Prolonged infusion of cortisol increases glycaemia and reduces the required glucose infusion rate to maintain euglycaemia by increasing glucose production and decreasing glucose uptake by the peripheral tissues, partly by inducing hepatic and peripheral insulin resistance (Rizza et al., 1982; Rooney et al., 1993).
During prolonged hypoglycaemia, cortisol has also an indirect effect on blood glucose levels by stimulating systemic and regional lipolysis (Djurhuus et al., 2002, 2004). If growth hormone is present, the lipolytic effect of both hormones is additive (Djurhuus et al., 2004). This increase in lipolysis, in turn, has independent insulin-resistant or glucose sparing effects (De Feo et al., 1989b; Corral et al., 1998).

1.3.6 Other factors
In recent years, the potential role that the cytokine interleukin-6 (IL-6) plays in the regulation of blood glucose levels and hepatic glucose production has received some attention (Glund and Krook, 2008; Hoene and Weigert, 2008; Pedersen, 2009). IL-6 is a pro-inflammatory cytokine that is involved in mediating many inflammatory processes, brain function, fatigue and immune response (Glund and Krook, 2008). The relatively recent discovery that skeletal muscle can produce and release cytokines such as IL-6 is at the origin of the term, myokine. This class of proteins expressed and released by skeletal muscle is associated with endocrine and/or paracrine effects (Pedersen, 2009). In fact, IL-6 was the first discovered muscle contraction-induced “exercise factor” or myokine (Pedersen, 2009).

Hypoglycaemia is associated with an increase in plasma IL-6 levels (Dotson et al., 2008). IL-6 infusion in vivo increases glucose uptake potentially due to increased translocation of GLUT4 from intracellular compartments to the plasma membrane (Carey et al., 2006). Indeed, in healthy men subjected to a hyperinsulinaemic, euglycaemic clamp, recombinant human IL-6 infusion increases the glucose infusion rate without affecting the total suppression of endogenous glucose production (Carey et al., 2006), thus suggesting an increase in glucose uptake. Although these effects of IL-6 would not be expected to prevent hypoglycaemia, IL-6 might be indirectly beneficial given that exogenous IL-6 administration has been shown to increase cortisol and glucagon levels together with a rise in blood glucose levels, thus probably helping with opposing hypoglycaemia (Dotson et al., 2008; Glund & Krook, 2008).

Other than IL-6, the levels of circulating lactate, glycerol and amino acids may also play some role in counterregulation. These metabolites act as substrates for gluconeogenesis to increase glucose production and modulate the secretion of hormones from the pancreas (Roden and Bernroider, 2003). They may also act as fuels for peripheral tissues to spare glucose or inhibit insulin-mediated glucose uptake. Indeed, both oral (Rossetti et al.,
2008) and intravenous (Porcellati et al., 2007) amino acids enhance the response of glucagon to hypoglycaemia (Porcellati et al., 2007). Since lactate is a significant fuel source, gluconeogenic precursor (Gerich, 1988), and may have a role in increasing insulin resistance (Harmer et al., 2008), it has the potential to play a role in both increasing glucose production and decreasing glucose utilisation. Some have proposed that increased muscle lactate utilisation together with decreased muscle glucose uptake make major contributions to glucose counterregulation in response to hypoglycaemia humans (Meyer et al., 2005).

There is evidence that changes in circulating blood glucose concentrations have the capacity to affect hepatic glucose production and thus contribute to the counterregulatory response to hypoglycaemia. Such a non-hormonal autoregulatory mechanism accounts for approximately 25% of the rise in net hepatic glucose production during hypoglycaemia (Connolly et al., 1992). On the other hand, hyperglycaemia exerts a direct inhibitory effect on endogenous glucose production via a glycogen phosphorylase-mediated inhibition of glycogenolysis (Tonelli et al., 2005; Yki-Järvinen, 1993). In addition, animal models have shown that net hepatic gluconeogenesis is reduced under hyperglycaemic conditions when glycogen levels are depleted (Tonelli et al., 2005). The mechanisms underlying the aforementioned autoregulation are only partly understood (Moore et al., 1998; Tonelli et al., 2005).

1.4 Counterregulatory Response to Hypoglycaemia in Individuals with Type 1 Diabetes

In individuals with type 1 diabetes, many of the counterregulatory responses described above are either absent or impaired, increasing the risk of severe and potentially life-threatening episodes of hypoglycaemia (Galassetti and Riddell, 2013). Firstly, when blood glucose levels begin to fall, the levels of insulin do not decrease, which is the initial response to a decrease in glycaemia in non-diabetic individuals (Briscoe et al., 2007). This is because the level of circulating insulin in insulin-treated individuals with type 1 diabetes is determined primarily by the rate of passive absorption from the site of insulin injection together with the pharmokinetics of the particular type of insulin administered (Cryer et al., 2003), with no feedback existing between blood glucose levels and insulin release.
Early after the onset of type 1 diabetes, patients generally have normal insulin-independent counter-regulatory responses to hypoglycaemia. However, this defence mechanism against hypoglycaemia deteriorates thereafter (Richter and Galbo, 1986; Gerich, 1988). Reduced glucagon response to a fall in glycaemia is the first and possibly most important counterregulatory response to be impaired (Gerich et al., 1973b) despite normal glucagon secretion in response to other stimuli such as exercise (Cryer et al., 1989; Shilo et al., 1990). It is well established that this deficient glucagon response to hypoglycaemia is strongly correlated to duration of diabetes (Bolli et al., 1983); however the mechanism(s) involved is still unclear. Recent studies have provided evidence of beta cell regulation of alpha cell glucagon secretion (Cooperberg and Cryer, 2009), with an increase in insulin level signalling a decrease in glucagon secretion in response to hypoglycaemia (Banarer et al., 2002; Cooperberg and Cryer, 2010). Therefore, current evidence suggests defective glucagon response may be due to beta cell failure. Furthermore, there may also be a central nervous system component to the impaired or absent glucagon response since insulin's inhibitory effect on glucagon release is partly mediated by the ventromedial hypothalamus under both normoglycaemic and hypoglycaemic conditions (Paranjape et al., 2010).

As a result of their attenuated or lack of glucagon response to hypoglycaemia (Gerich et al., 1973b; Bolli et al., 1983; Hirsch and Shamoon, 1987; Meyer et al., 1998), individuals with type 1 diabetes become more dependent on catecholamines (Marker et al., 1991), in particular epinephrine (Bolli et al., 1982; Cryer et al., 1989), together with other counterregulatory hormones to overcome hypoglycaemia (De Feo et al., 1983). Unfortunately, individuals with type 1 diabetes have an attenuated epinephrine (Bolli et al., 1983; Amiel et al., 1988; Dagogo-Jack et al., 1993; Meyer et al., 1998) and norepinephrine (Meyer et al., 1998) response to hypoglycaemia compared to non-diabetic individuals. This is exacerbated by the fact that the rate of fall of glycaemia can affect epinephrine response to hypoglycaemia, with a rapid rate of fall resulting in a smaller epinephrine response compared with a slower rate of fall. In diabetic individuals with autonomic neuropathy, catecholamine response to hypoglycaemia is further impaired (Bolli et al., 1983; Bottini et al., 1997; Meyer et al., 1998). In summary, the three key normal responses that play an important role in the defence against hypoglycaemia when glycaemia is falling (decreased insulin levels, increased glucagon and increased epinephrine levels) are often deficient in individuals with type 1 diabetes (Cryer, 2003).
This is highly problematic for those individuals with both glucagon and epinephrine
deficiency as they are at an increased risk of severe hypoglycaemia (White et al., 1983).

The aforementioned impaired counterregulatory responses to hypoglycaemia also
implicate the glycaemic threshold for the release of counterregulatory hormones, which
is often different in type 1 diabetic individuals compared to non-diabetic individuals
(Cryer et al., 2003). The glycaemic threshold for individuals with poorly controlled type
1 diabetes is higher (Amiel et al., 1988; Boyle et al., 1988) than in non-diabetic
individuals. In contrast, the plasma glucose level threshold is generally lower in tightly
controlled type 1 diabetes (Amiel et al., 1988). The degree of attenuation, absence or
lowering of the glycaemic threshold for these counterregulatory responses appears to be
associated with a longer duration of type 1 diabetes (Bolli et al., 1983). Strict glycaemic
control and intensive insulin therapy (Amiel et al., 1987; 1998) are associated with
impaired counterregulation, thus increasing the risk of severe hypoglycaemia (DCCT,
1997).

One key factor that can alter the counterregulatory response to hypoglycaemia in
individuals with type 1 diabetes is a recent previous episode of hypoglycaemia. Recent
antecedent hypoglycaemia reduces the glycaemic thresholds for the activation of
counterregulatory responses to subsequent hypoglycaemia (Amiel et al., 1988). This is
because recurrent hypoglycaemia interferes with glucose sensors and neural networks that
detect hypoglycaemia (McCrimmon, 2009). Antecedent hypoglycaemia also blunt the
magnitude of the response of counterregulatory hormones to subsequent hypoglycaemia
and increases the risk of future episodes of hypoglycaemia (Davis and Shamoon, 1991;
Heller and Cryer, 1991; Widom and Simonson, 1992; Dagogo-Jack et al., 1993; Hvidberg
et al., 1996; Davis et al., 1997, 2000b, 2000c; Shum et al., 2001; Cryer et al., 2003). Even
mild episodes of hypoglycaemia can attenuate this counterregulatory response; however
the depth of hypoglycaemia is an important determinant of the impaired
counterregulatory response to a subsequent hypoglycaemic episode with a dose-response
effect of antecedent hypoglycaemia (Davis et al., 1997). For instance, mild
hypoglycaemia of ~3.9 mM can reduce epinephrine, muscle sympathetic nervous system
and glucagon responses to hypoglycaemia by ~30% the next day (Davis et al., 1997).
Lower glycaemia of ~3.3 mM also reduces the response of these gluco-regulatory
hormones together with blunting norepinephrine, growth hormone, endogenous glucose
production, pancreatic polypeptide and lipolytic responses to hypoglycaemia (Davis et
Interestingly, an even lower antecedent blood glucose level of ~2.9 mM elicits a similar impaired counterregulation to ~3.3 mM (Davis et al., 1997). Together with this dose-response effect of antecedent hypoglycaemia on subsequent counterregulatory response to hypoglycaemia, the duration and frequency of hypoglycaemic episodes can also influence this counterregulatory response. Indeed, there seems to be a hierarchical effect of the duration of antecedent hypoglycaemia on the reduction of the counterregulatory responses to subsequent hypoglycaemic episodes (Davis et al., 2000b).

Recurring episodes of hypoglycaemia are also associated with marked attenuation of the warning symptoms of hypoglycaemia (Bolli, 2003). This leads to a situation where individuals with type 1 diabetes often suffer from a reduced ability or failure to recognise hypoglycaemia, a condition referred to as hypoglycaemia unawareness (Cryer et al., 2003; Alsahli and Gerich, 2008). As a result, these patients are unable to detect and therefore correct hypoglycaemia (Bolli, 2003), increasing markedly their risk of severe hypoglycaemia (Gold et al., 1994).

Antecedent exercise can also impair one’s capacity to counter a subsequent episode of hypoglycaemia in individuals with type 1 diabetes as exercise impairs the counterregulatory response to subsequent hypoglycaemia (Galassetti et al., 2001a; Sandoval et al., 2004, 2006). Although exercise and hypoglycaemia can blunt counterregulation to a similar level (Sandoval et al., 2006), the increase in insulin sensitivity that occurs as a result of exercise can elevate the risk for hypoglycaemia during subsequent hypoglycaemic episodes (Briscoe et al., 2007, Galassetti and Riddell, 2013).

Finally, one important factor that can aggravate the risk of hypoglycaemia in individuals with type 1 diabetes is physical activity. Participation in regular physical activity provides numerous health benefits for individuals with type 1 diabetes, including weight control, improvement of muscle strength, lowering atherosclerosis risk factors, and overall improvement in cardiovascular function (Chimen et al., 2012). Unfortunately, exercise for these individuals increases the risk of hypoglycaemia during and for several hours after exercise (MacDonald, 1987; Tsalikian et al., 2005; McMahon et al., 2007). For this reason, many individuals with type 1 diabetes are often reluctant to be physically active and thus miss out on the many physical and psychological benefits of an active lifestyle (Ludvigsson et al., 1980). In order to fully appreciate how exercise increases the
risk of hypoglycaemia, we must examine how blood glucose levels are regulated during exercise in healthy individuals. Here we will focus on sustained aerobic exercise of moderate intensity, aerobic/anaerobic exercise of high intensity, intermittent high intensity exercise, and maximal sprint effort, but not on other forms of exercise such as resistance exercise.

1.5 Gluoregulatory Responses to Moderate Intensity Exercise in Non-Diabetic Individuals

During moderate intensity exercise in healthy non-diabetic individuals, stable blood glucose levels are maintained by feedback mechanisms that allow the increase in glucose utilisation by exercising muscles to be precisely matched by an equal increase in glucose production rate (Richter and Galbo, 1986; Marliss and Vrankic, 2002). What is still a source of some debate is the mechanisms that coordinate liver and muscle responses to exercise and the role of insulin, glucagon, catecholamines together with other counterregulatory hormones and metabolites in this precise matching of glucose kinetics.

At the start of moderate intensity exercise, it is well accepted that the contraction of skeletal muscle induces a rapid increase in glucose uptake (Wahren and Ekberg, 2007). At rest, only 15-20% of peripheral glucose utilisation is attributed to skeletal muscle, but during moderate intensity exercise at 55-60% \( \dot{V}O_{2max} \) skeletal muscle accounts for up to 80-85% of whole body glucose disposal (Hargreaves and Spriet, 2006). This marked increase in muscle glucose uptake during exercise (Camacho et al., 2005; Hargreaves and Spriet, 2006) is explained by an exercise-mediated stimulation of the translocation of a non-insulin responsive pool of the glucose transporter GLUT4 (Douen et al., 1989; Coderre et al., 1995), together with increased blood flow, capillary recruitment, glucose extraction and improved glucose delivery to skeletal muscle.

Although there is a significant increase in the rate of glucose removal from the blood during moderate intensity exercise, non-diabetic individuals do not become hypoglycaemic. Instead, their blood glucose levels remain stable as a result of a matched increase in the rate of hepatic glucose production (Camacho et al., 2005). The activation of hepatic glycogenolysis and gluconeogenesis increases hepatic glucose production, with the relative contributions of each metabolic pathway changing with exercise duration.
(Trimmer et al., 2002; Wahren and Ekberg, 2007) and intensity (MacRae et al., 1995; Staehr et al., 2007) and dietary state (Staehr et al., 2007), with both prolonged fasting and exercise being favourable to hepatic gluconeogenesis (Trimmer et al., 2002; Staehr et al., 2007). At the start of exercise, hepatic glycogenolysis accounts for most of the increase in glucose produced by the liver. As time progresses, the rate of glycogenolysis declines with decreasing hepatic glycogen stores, and gluconeogenesis becomes increasingly more important (Richter & Galbo, 1986; Camacho et al., 2005; Hargreaves & Spriet, 2006; Wahren & Ekberg, 2007). If the duration of exercise is more than two hours, the depletion of hepatic glycogen stores together with an inadequate compensatory increase in gluconeogenesis can result in declining glycaemia and even hypoglycaemia (Trimmer et al., 2002; Camacho et al., 2005).

The increase in hepatic glucose production during exercise results to some extent from the combined fall in insulin level and rise in glucagon concentration (Marliss and Vranic, 2002; Camacho et al., 2005). A fall in insulin level is required for a full increase in hepatic glycogenolysis (Wasserman et al., 1989b), whereas elevation in glucagon level is necessary for both increased hepatic glycogenolysis and gluconeogenesis (Wasserman et al., 1989a). Although such a fall in insulin and increase in glucagon levels are often observed during moderate intensity exercise, some studies have reported that the levels of these hormones, in particular glucagon levels, do not change or only change slightly (Wasserman et al., 1993; Wasserman, 2009). It is important to note, however, that the observation that the levels of these hormones change little or not at all (Wasserman et al., 1993; Wasserman, 2009) informs us little about their importance as ultimately it is the glucagon/insulin ratio (Richter & Galbo, 1986) and the portal levels of these hormones that determine their effects on hepatic glucose production (Wasserman et al., 1989a). The small changes in peripheral glucagon levels during exercise are due to the hepatic extraction of glucagon released by the pancreas, leading to lesser rise in peripheral glucagon levels and the underestimation of the physiological importance of glucagon in blood glucose regulation (Wasserman et al., 1993). It has been shown that even a small increase in glucagon level can have a marked effect on hepatic glucose production as the potency of a given glucagon level is enhanced considerably during exercise compared to rest (Wasserman et al., 1989a; Wasserman, 2009).

One powerful approach to evaluate the relative contributions of insulin and glucagon in the activation of glucose production during exercise without the confounding effect of the
counterregulatory response to falling blood glucose level is to manipulate portal glucagon and insulin levels by infusing an inhibitor (e.g. somatostatin, octreotide) of their pancreatic release together with the infusion of insulin, glucagon and glucose to maintain euglycaemia, a technique known as pancreatic islet clamp. Using this technique, insulin and glucagon have been reported to account for ~55% and ~60% of the exercise-mediated increase in hepatic glucose production in dogs, respectively (Wasserman et al., 1989a, 1989b). Similar techniques in humans have shown both hormones play an important role in increasing hepatic glucose production during exercise since the absence of changes in insulin and glucagon concentrations has been shown by many to prevent glucose production rate from increasing (Wolfe et al., 1986; Hirsch et al., 1991; Kjaer et al., 1993a; Lavoie et al., 1997). However, others have reported that the increase in hepatic glucose production during moderate exercise is little or not affected when plasma insulin and glucagon levels are held constant in humans (Bjorkman et al., 1983; Coker et al., 2001). Therefore, despite playing some role, changes in glucagon and insulin do not totally explain the increase in hepatic glucose production that takes place during moderate intensity exercise in humans, thus indicating that other factors are involved (Hargreaves and Spriet, 2006).

Catecholamines have been proposed to play some role in the activation of hepatic glucose production during moderate intensity exercise. However, most studies have failed to show a significant role for the sympathoadrenergic system although the increase in epinephrine and norepinephrine levels has led some researchers to suggest the opposite. Indeed, there is a strong correlation in humans between the exercise-induced rise in hepatic glucose production and increases in catecholamines levels, with the amount of active muscle mass influencing the magnitude of the rise in catecholamines (Kjaer et al., 1991). Hypoxic exercise is another condition which results in a greater rise in both hepatic glucose production and catecholamines (Cooper et al., 1986). Adrenal medulla removal in rats decreases hepatic glycogenolysis (Richter et al., 1981) and hepatic glucose production (Sonne et al., 1985) during exercise. However, some studies in rats have shown no effect of epinephrine on hepatic glycogen breakdown during exercise (Hargreaves and Spriet, 2006).

The small contribution of plasma catecholamines in mediating the increase in glucose production during exercise is suggested by the studies which have shown that alpha- and beta-adrenergic blockades have little or no effect on the rise in hepatic glucose production
during moderate exercise in dogs (Coker et al., 1997) and humans (Simonson et al., 1984; Marker et al., 1991). Also, direct adrenergic stimulation at physiological dose has little effect on the rate of hepatic glucose production during exercise even in the absence of changes in glucagon and insulin levels (Coker et al., 2002). The small role played by circulating catecholamines is not this surprising when one considers that epinephrine levels in the portal circulation are much lower than in peripheral blood where measurements are normally performed. This is because the gut extracts a large proportion of blood epinephrine before it reaches portal circulation (Coker and Kjaer, 2005).

The role of hepatic nerves in the activation of hepatic glucose production has also been investigated, with most studies suggesting that stimulation of hepatic nerves plays a minimal role. For instance, hepatic denervation does not affect the rise in hepatic glucose production during exercise in rats (Richter et al., 1980; Sonne et al., 1985) or dogs (Wasserman et al., 1990). Similarly, blockade of sympathoadrenergic activity in healthy males during exercise by local anaesthesia of the celiac ganglion that usually innervate the liver and adrenal medulla, together with the infusion of glucagon and insulin hormones and physiological doses of epinephrine to mimic usual responses to exercise, has no effect on the increase in hepatic glucose production rate during exercise, indicating that sympathetic liver nerve activity is unlikely to be involved (Kjaer et al., 1993a). Likewise, patients with denervated liver transplants experience similar increases in hepatic glucose production during exercise to those of control individuals, indicating hepatic nerve activity is not an important glucoregulatory factor during exercise (Kjaer et al., 1995).

Other than the sympathoadrenal system and pancreatic hormones, it has been proposed that glucose itself plays some role in the regulation of hepatic glucose production during exercise. In support of this view, a fall in glucose level during moderate intensity exercise stimulates hepatic glucose production without changes in pancreatic hormone levels or catecholamine release (Coker et al., 2002). These results support the view that decrements in glycaemia may stimulate hepatic glucose production during moderate exercise and therefore maintain euglycaemia. A small decline in glycaemia can also indirectly stimulate hepatic glucose production by stimulating counterregulatory hormone response (Wasserman et al., 1984, 1991), with an increase in growth hormone (Shilo and Shamoon, 1990; Davis et al., 2000e) and cortisol levels being observed (Shilo and Shamoon, 1990; Davis et al., 2000e; Horton et al., 2002). However, growth hormone
and cortisol are unlikely to play an important role as the levels of these hormones change little during moderate intensity exercise, and experimentally induced growth hormone or cortisol deficiency does not affect blood glucose levels during exercise (Hoelzser et al., 1986a, 1986b; Wasserman, 1995). It is important to note, however, that when insulin and glucagon levels are kept constant at basal levels during mild to moderate intensity exercise, glucose production increases rapidly with a decrease in blood glucose level before plateauing despite a decrease in glycaemia (Kjaer et al., 1993a). Hepatic glucose production after prolonged low blood glucose levels thus appears reasonably insensitive to small decreases in glycaemia (Kjaer et al., 1993a). In contrast, exogenous glucose infusion or glucose ingestion affects insulin and glucagon secretion and markedly inhibits hepatic glucose production during exercise (Manzon et al., 1998; Jeukendrup et al., 1999; Jenkins et al., 1985 even in responses to very small changes in blood glucose levels (Berger et al., 1994). This shows that hepatic glucose production is very sensitive to a rise in blood glucose levels.

There is evidence that afferent neural reflex activity originating from exercising muscles may be important for increasing hepatic glucose production during exercise. This is illustrated by the observation that electrical stimulation of cut muscle branches of the femoral nerves increases hepatic glucose production and glycaemia in cats (Vising et al., 1994). However, in humans, epidural blockade to evaluate the effect of neural feedback does not change hepatic glucose production during moderate intensity exercise (Kjaer et al., 1989). Therefore, although afferent neural reflex activity can increase glucose mobilisation during exercise, it is likely to be secondary or only play a minor role comparative to other mechanisms in healthy individuals.

There are other important factors that influence the glucoregulatory response to exercise. In particular, counterregulatory responses to moderate intensity exercise are attenuated by prior hypoglycaemia (Davis et al., 2000d), with blunting of glucagon, catecholamines, GH, cortisol, endogenous glucose production, ketogenesis and lipolytic responses (Davis et al., 2000d). Similarly, antecedent morning exercise of moderate intensity can significantly impair metabolic and neuroendocrine responses during moderate exercise performed three hours later in a gender-specific manner (Galassetti et al., 2001b).

Given that skeletal muscles release IL-6 during exercise, it has been proposed that this might provide a means whereby muscle activity and associated fuel utilisation is related
to the regulation of hepatic glucose production. In support of this view, IL-6 increases hepatic glucose production during exercise (Febbraio et al., 2004), and IL-6 levels increase during exercise, following an almost exponential pattern reaching peak concentration of up to 100-fold from basal levels immediately following exercise (Pedersen and Fischer, 2007). The magnitude of IL-6 response is influenced by the muscle mass recruited during exercise, endurance capacity, and both exercise intensity and duration (Pedersen, 2009). IL-6 also increases both whole-body and intramuscular fatty acid oxidation, thus contributing to the sparing of carbohydrate (Pedersen and Febbraio, 2008). It must be stressed, however, that IL-6 also promotes fuel uptake and utilisation in working muscle, and as a result increases whole body glucose disposal during exercise (Febbraio et al., 2004), thus raising the question of its importance in counterregulation.

1.6 Gluoregulatory Responses to Moderate Intensity Exercise in Individuals with Type 1 Diabetes

In insulin-treated individuals with type 1 diabetes, moderate intensity exercise stimulates an increase in muscle glucose utilisation that is similar in magnitude to non-diabetic individuals (Richter and Galbo, 1986), but that increases with plasma insulin levels (Chokkalingam et al., 2007). However, most but not all studies have reported that hepatic glucose production does not rise to the same degree (Simonson et al., 1984; Richter and Galbo, 1986), resulting in a decrease in blood glucose levels and a rise in hypoglycaemia risk (Schiffрин et al., 1984; Simonson et al., 1984; Zinman et al., 1984; Hübinger et al., 1985; Campagne et al., 1987; Sonnenberg et al., 1990; Oskarsson et al., 1999; Riddell et al., 1999; Francescato et al., 2004; Petersen et al., 2004). Furthermore, during recovery from moderate intensity exercise in type 1 diabetic individuals, blood glucose levels continue to decrease (Hübinger et al., 1985; Campagne et al., 1987) or stabilise (Simonson et al., 1984; Oskarsson et al., 1999) while both hepatic glucose production and glucose utilisation decreasing back to basal levels.

The inability of hepatic glucose production rate to rise to a level that matches glucose utilisation rate during moderate intensity exercise in individuals with type 1 diabetes is due in part to the absence of a fall in circulating insulin levels. As discussed earlier, non-diabetic individuals have the capacity to decrease the secretion of insulin during exercise, thus favouring the stimulation of hepatic glucose production. In contrast, individuals with
type 1 diabetes are unable to control the rate of passive absorption of the insulin from the previously administered insulin bolus, thus increasing their likelihood of exercising in a hyperinsulinaemic state. Hyperinsulinaemia has been reported to increase the rate of exercise-mediated glucose utilisation in type 1 diabetic individuals while having no effect on hepatic glycogen breakdown (Chokkalingam et al., 2007).

Another factor which increases the mismatch between glucose production and utilisation and thus increase further the risk of hypoglycaemia during exercise is the hyperinsulinaemia that may occur as a result of increased insulin mobilisation from subcutaneous depots, especially if insulin is injected near an exercising muscle (Koivisto and Felig, 1978), with the rates of insulin absorption often increasing during exercise (Richter & Galbo, 1986). Significant increases in insulin concentrations is also observed during exercise in insulin-infused diabetic individuals, thus suggesting reduced insulin clearance (Chokkalingam et al., 2007). Overall, since exercise not only increases insulin absorption from subcutaneously administered injection depots, but also decreases insulin clearance, these factors lead to elevated circulating insulin levels during exercise. This state of over-insulinisation combined with exercise itself results in a profound increase in insulin action (Camacho et al., 2005). Indeed, increased insulin level and action can negate the effect of glucagon on hepatic glucose production and amplify glucose utilisation to greater than required levels, thus resulting in a drop in blood glucose level (Camacho et al., 2005). Whether the resulting fall in glycaemia reaches dangerous hypoglycaemic levels greatly depends on pre-exercise blood glucose levels (Richter and Galbo, 1986). Thus, in general, if insulin concentration is elevated, the lack of exercise-induced decrease in insulin levels results in a marked activation of glucose utilisation (Camacho et al., 2005). This together with decreased hepatic glucose production results in a fall in blood glucose levels (Zinman et al., 1977) and a rise in risk of hypoglycaemia. This risk is further exacerbated by the symptoms of hypoglycaemia that may alert an individual to an impending episode such as sweating and tachycardia being difficult to notice as they may be similar to normal responses to exercise.

Aside from the marked difference in insulin response to moderate intensity exercise between diabetic and non-diabetic individuals, the response of counterregulatory hormones to moderate intensity exercise in insulin-treated individuals with type 1 diabetes is relatively similar to that of non-diabetic individuals. Glucagon levels are increased (Oskarsson et al., 1999; Galassetti et al., 2002, 2003, 2004, 2006) or unchanged
(Simonson et al., 1984; Hübinger et al., 1985; Sonnenberg et al., 1990), catecholamines levels are increased (Simonson et al., 1984; Hübinger et al., 1985; Sonnenberg et al., 1990; Oskarsson et al., 1999; Galassetti et al., 2002, 2003, 2004, 2006), and growth hormone levels are elevated (Hübinger et al., 1985; Shilo and Shamoon, 1990; Sonnenberg et al., 1990; Oskarsson et al., 1999; Galassetti et al., 2002, 2004). In contrast, cortisol levels either remain stable (Hübinger et al., 1985; Sonnenberg et al., 1990) or increase later (Oskarsson et al., 1999; Galassetti et al., 2002, 2003, 2004, 2006) during moderate intensity exercise. It is noteworthy that despite the exercise-induced increase in the levels of the circulating counterregulatory hormones being similar between diabetic and non-diabetic individuals, hypoglycaemia risk is increased in insulin-treated diabetic individuals, thus highlighting the glucoregulatory importance of insulin in glucose homeostasis during moderate intensity exercise (Galassetti, et al., 2006).

There are a number of important factors that affect the counterregulatory response to exercise in individuals with type 1 diabetes. In particular, this response is attenuated by prior hypoglycaemia (Galassetti et al., 2003, 2006). This blunting effect appears to be dose-dependent, with hypoglycaemia of increasing depth progressively inducing more acute counterregulatory failure, with a reduction in glucagon, catecholamine, cortisol, endogenous glucose production, and lipolytic responses (Galassetti et al., 2006). Prior aerobic exercise also impairs the counterregulatory response associated with a subsequent bout of moderate intensity exercise (Galassetti et al., 2001b).

Another important factor that influences glycaemic response to moderate intensity exercise and associated risk of hypoglycaemia is the increase in insulin sensitivity that occurs in response to exercise (Richter et al., 1982; Holloszy, 2005; Jensen and Richter, 2012). This together with the exercise-induced increase in glucose utilisation in insulin-treated type 1 diabetic individuals can lead to a rapid decline in blood glucose levels not only during exercise, but also for several hours afterwards (MacDonald, 1987b; Hirsch et al., 1991; Tsalikian et al., 2005; McMahon et al., 2007). For this reason, it is generally recommended that insulin dose should be reduced and carbohydrate intake increased prior to and after exercise to reduce the aforementioned risk of hypoglycaemia (ADA, 2007; Dube et al., 2005; Zinman et al., 2004).

It must be stressed, however, that in cases of severe insulin deficiency exercise provokes a rise in blood glucose levels that increase further during recovery (Wahren et al., 1975).
The increase in blood glucose levels is explained in part on the basis that the magnitude of the increase in muscle glucose utilisation is reduced due to the small additive effect of exercise and insulin. In addition, low insulin is conducive to increased hepatic glucose production at rates greater than rates of glucose utilisation, thus resulting in hyperglycaemia (Riddell and Perkins, 2006). Finally, severe insulin deficiency is accompanied by elevated glucagon levels, thus favouring the activation of hepatic glucose production. Therefore, exercising in a state of severe insulin deficiency is not recommended as the resulting lower insulin to glucagon ratio not only increases glycaemia, but also ketone body levels, thus substantially increasing the risk of severe ketoacidosis (Berger et al., 1980; Richter and Galbo, 1986) and ketoacidotic coma.

**1.7 Glucoregulatory Responses to High-Intensity Aerobic Exercise in Non-Diabetic Individuals**

Although moderate intensity exercise increases risk of hypoglycaemia in insulin-treated type 1 diabetic individuals, it is important to note that not all types of exercise increase this risk. In fact, there are conditions where exercise is actually conducive to hyperglycaemia not only in insulin-treated individuals with diabetes but also in non-diabetic individuals. For instance, blood glucose levels in non-diabetic (Stokes et al., 2013) and diabetic individuals (Turner et al., 2014) increase in response to resistance exercise or when exercise is performed for at least 10 min at an intensity greater than 80% of maximum rate of oxygen consumption ($\dot{V}\text{O}_2_{\text{max}}$; Mitchell et al., 1988; Marliss et al., 1991,1992b; Purdon et al., 1993; Sigal et al., 1994b; Kreisman et al., 2000a; Marliss et al., 2000; Sigal et al., 2000; Harmer et al., 2008; Manzon et al., 1998; Marliss & Vranic, 2002; Wahren & Ekberg, 2007). This rise is glycaemia is followed by a period of hyperglycaemia that can persist for up to an hour post-exercise (Marliss et al., 1991, 1992; Marliss et al., 2000; Sigal et al., 2000).

The glycaemia-rising effect of intense aerobic exercise has been explained on the basis that this type of exercise results in a seven- to eight-fold increase in glucose production (Marliss et al., 1991,1992b; Purdon et al., 1993; Sigal et al., 1994b), the greatest increase in glucose production observed under any physiological or pathophysiological condition (Marliss and Vranic, 2002). This is accompanied by a lesser increase in glucose utilisation rates of approximately 3-4 fold (Marliss et al., 1991, 1992b; Purdon et al., 1993; Sigal et al., 1994b). This greater rate of glucose production in comparison to
glucose utilisation results in an increase in plasma glucose levels. A further increase in glycaemia also occur immediately after the cessation of prolonged intense aerobic exercise in non-diabetic individuals (Mitchell et al., 1988; Marliss et al., 1991, 1992b; Sigal et al., 1994a, 1994b; Kreisman et al., 2000a; Marliss et al., 2000; Sigal et al., 2000), before blood glucose levels return to pre-exercise levels. This is due to glucose utilisation rate initially decreasing more rapidly than glucose production rates at the onset of recovery, thus contributing further to greater hyperglycaemia (Marliss et al., 1992b; Sigal et al., 1994b). This increase in glycaemia is only of short duration, with blood glucose returning to pre-exercise levels by 60 min post-exercise.

Unlike moderate intensity exercise, the significant increase in hepatic glucose production during high intensity exercise is unlikely to be due to changes in pancreatic hormone levels. Insulin levels actually remain unchanged (Kjaer et al., 1986; Mitchell et al., 1988; Marliss et al., 1991, 1992b; Kreisman et al., 2000a), or decline only slightly (Sigal et al., 1994b; Marliss et al., 2000; Sigal et al., 2000), while glucagon levels increase minimally (Marliss et al., 1991, 1992b; Purdon et al., 1993; Sigal et al., 1994b), resulting in a small increase in glucagon to insulin ratio (Sigal et al., 1996; Manzon et al., 1998; Sigal et al., 2000). As mentioned previously, it is important to note that changes in plasma glucagon levels may underestimate the portal levels of this hormone due to hepatic extraction thus suggesting glucagon could play an important role than that suggested by the aforementioned studies. However, experiments involving islet clamps where insulin and glucagon are kept at stable and basal levels show that this has no effect on hepatic glucose production during intense aerobic exercise, thus indicating that these hormones are unlikely to play a major role in increasing hepatic glucose production during high intensity exercise (Sigal et al., 1996; Marliss and Vranic, 2002). A minor role for pancreatic hormones is also suggested by the observation that the pattern of rise in glucose production in response to high intensity exercise persists in glucose-infused individuals despite high plasma insulin levels and insulin/glucagon ratios (Manzon et al., 1998).

The marked catecholamine response to high intensity exercise is thought to play a major role in the activation of hepatic glucose production and rise in blood glucose levels in non-diabetic individuals (Marliss and Vranic, 2002). This is supported by the observation that the increase in glucose production associated with high intensity aerobic exercise is accompanied by a 14- to 18-fold increase in both epinephrine and norepinephrine levels (Kjaer et al., 1986; Marliss et al., 1991, 1992b; Sigal et al., 1994b; Manzon et al., 1998;
Kreisman et al., 2000a; Marliss et al., 2000; Sigal et al., 2000). However, this relationship between catecholamine levels and an increase in hepatic glucose production during high intensity exercise only suggests causality.

One approach to establish causality is to determine whether adrenergic blockade inhibits the rise in hepatic glucose production during intense exercise. Although there is a well demonstrated relationship between exercise-induced changes in glucose output and circulating catecholamine levels, a role for catecholamines is challenged by the observation that the administration of alpha or beta adrenergic blockers during high intensity exercise has no inhibitory effect on the rise in hepatic glucose production (Sigal et al., 1994b; Coker et al., 1997; Sigal et al., 1999, 2000). Indeed, the infusion of propanolol (beta-adrenergic blocker) in non-diabetic individuals (Sigal et al., 1994a) results in an unexpected higher rise rather than a lesser increase in hepatic glucose production, and only a minor inhibition of glucose production is achieved in response to phentolamine infusion (alpha-adrenergic blocker) during intense exercise in humans (Sigal et al., 2000). Moreover, intraportal infusion of propanol and phentolamine to selectively block hepatic and adrenergic receptors in dogs has no effect on endogenous glucose production or net hepatic glucose output during intense exercise (Coker et al., 1997). Overall, these findings do not exclude the possibility that other factors may contribute to the regulation of glucose production during intense exercise, particularly considering the lack of any effect of adrenergic blockade on glucose production (Sigal et al., 1994a, 1999, 2000). One limitation with these studies, however, is that it remains to be determined whether the dose of adrenergic blockers administered was sufficient to counter the effect of the large rise in catecholamines associated with intense exercise.

One of the most convincing pieces of evidence that catecholamines play a role in stimulating hepatic glucose production during intense aerobic exercise is the fact that when epinephrine and norepinephrine are infused during moderate intensity exercise to mimic the levels attained during higher intensity exercise there is a further increase in hepatic glucose production and rise in blood glucose levels that matches those observed during high intensity exercise, therefore suggesting an important role for epinephrine and norepinephrine. When infused alone, epinephrine (Howlett et al., 1999a; Kreisman et al., 2000b) and norepinephrine (Kreisman et al., 2001) are each considered capable of producing a portion of the increase in hepatic glucose production associated with intense exercise. Moreover, blockade of sympathetic nerve activity to liver and adrenal medulla
by local anaesthesia of the celiac ganglion followed by infusion of high physiological doses of epinephrine during exercise resulted in enhanced glucose production in healthy males (Kjaer et al., 1993a). Also, when both catecholamines are infused together to mimic the usual epinephrine and norepinephrine response to high intensity exercise in healthy men, there is a marked and progressive increase in hepatic glucose production and increase in glycaemia, suggesting a role for catecholamines in the regulation of hepatic glucose production during high intensity exercise (Kreisman et al., 2003). However, it is noteworthy that in epinephrine-deficient, bilaterally adrenalectomised humans, the absence of epinephrine does not impair glucose production during high intensity exercise (Howlett et al., 1999b), thus suggesting that norepinephrine might play a more important role here and that other glucoregulatory factors might be involved.

Acute changes in the levels of other glucoregulatory hormones such as growth hormones and cortisol are unlikely to have any effect on the acute increase in blood glucose levels associated with high intensity exercise. This is, in part, because the levels of these hormones change little during a short bout of intense exercise, with their levels increasing mainly during recovery (Marliss & Vranic, 2002). Also, the infusion of octreotide to inhibit insulin, glucagon and growth hormones secretion while glucagon and insulin levels are replaced and maintained at stable levels has no effect on the increase in glucose production during intense exercise (Marlis & Vranic, 2002; Sigal et al., 1996).

In order to understand how intense exercise increases blood glucose level it is important to focus not only on the potential effect of catecholamines and other factors on hepatic glucose production, but also on the factors that influence the rates of glucose utilisation during exercise. On the basis of the findings by some that catecholamines inhibit insulin-stimulated glucose utilisation in resting skeletal muscles (Aftab-Guy et al., 2005; Wasserman, 1995) and glucose Rd during exercise (Howlett et al., 1999; Watt & Hargreaves, 2002; Watt et al., 2001), in part via a glycogenolytically-mediated increase in glucose-6-phosphate level (Watt et al., 2001), a potent inhibitor of hexokinase (Nonogaki, 2000), it is possible that the lesser rise in glucose utilisation relative to glucose production during intense exercise is due to a catecholamine-mediated inhibition of the stimulatory effect of muscle contraction on peripheral glucose utilisation. This issue was investigated in epinephrine-deficient, bilaterally adrenalectomised humans with epinephrine infusion inhibiting glucose clearance during exercise (Howlett et al., 1999b). Similarly, studies in rodents have also shown that epinephrine infusion decreases muscle
glucose transport despite an elevation in glucose transporter (GLUT4) translocation to the plasma membrane, which suggests a reduction in GLUT4 intrinsic activity (Bonen et al., 1992). Despite these findings, other researchers have found that elevated catecholamines in humans stimulate rather than inhibit glucose utilisation (Kreisman et al., 2000b, 2001, 2003), and these investigators proposed that the increase in blood glucose levels during intense aerobic exercise could simply be the result of a greater stimulation of glucose production by catecholamines compared to glucose utilisation rates (Kreisman et al., 2003).

The apparent contradiction with respect to the role of catecholamines on glucose utilisation rate is not surprising given the evidence that the stimulation of muscle adrenoceptors by catecholamines and other adrenoceptor agonists can stimulate glucose transport in skeletal muscle (Abe et al., 1993; Ngala et al., 2013), with the effect of adrenoceptor antagonists having little or a marked effect depending on muscle fibre compositions (Liu et al., 1995). Also, predicting the effect of catecholamines on peripheral glucose utilisation rate is further complicated by the observation that circulating epinephrine inhibits glucose uptake in skeletal muscle by insulin-dependent mechanisms, whereas norepinephrine released from sympathetic nerves increases glucose uptake via insulin-independent mechanisms (Nonogaki, 2000).

The post-exercise transient increase in blood glucose levels and its subsequent fall are explained on the basis of the pattern of change in plasma insulin, glucose and catecholamines levels. Immediately after intense exercise, catecholamine levels are elevated and inhibit insulin secretion, thus contributing to a further transient increase in blood glucose levels. Afterwards, the rapid decrease in catecholamine levels and high blood glucose levels result in a marked rise in plasma insulin level (Marliss et al., 1991; Purdon et al., 1993; Sigal et al., 1994a, 1994b; Kreisman et al., 2000a; Marliss et al., 2000) that helps to decrease glucose back to basal level (Marliss and Vranic, 2002). Indeed, elevated blood glucose and insulin levels enhance the rate of glucose transport during recovery from intense exercise while inhibiting hepatic glucose production. It is noteworthy that this rapid decline in epinephrine and norepinephrine levels during the first few minutes of recovery (Marliss et al., 1991; Kreisman et al., 2000a; Marliss et al., 2000; Sigal et al., 2000) is closely matched with the post-exercise decrease in hepatic glucose production (Marliss et al., 2000; Marliss and Vranic, 2002), supporting the opinion that declining catecholamine levels may potentially mediate the post-exercise fall
in glycaemia in non-diabetic individuals. Other factors might contribute to the fall in hepatic glucose production during recovery. During recovery from high intensity aerobic exercise, lactate (Marliss et al., 1991; Sigal et al., 1994b; Kreisman et al., 2000a; Marliss et al., 2000) levels decline. Since lactate is a potential glucoregulatory precursor, lower lactate levels would be expected to result in a decrease in hepatic gluconeogenesis. Likewise, glucagon levels decline (Marliss et al., 1991; Sigal et al., 1994b) or remain relatively stable during recovery is taking place during high intensity exercise (Marliss et al., 2000), thus producing a further fall in hepatic glucose production. Other counterregulatory hormones such as growth hormone and cortisol are unlikely to be important in the fall in glycaemia observed during recovery from prolonged high intensity exercise.

It is noteworthy that the glucoregulatory response to prolonged high intensity exercise is affected by a number of factors. Firstly, the state of feeding seems to be one of these factors, with a smaller increase in glycaemia observed during high intensity exercise performed postprandially as opposed to in a postabsorptive state (Kreisman et al., 2000a). Unlike moderate intensity exercise, glucose production during high intensity exercise is not as sensitive to the inhibitory effect of hyperglycaemia (Manzon et al., 1998; Wiersma et al., 1993). Gender may also play a role, since women experience a greater rise in glycaemia in the post-exercise period due to a lesser increment in glucose utilisation rate (Marliss et al., 2000) despite men and women experiencing a similar glucoregulatory response to high-intensity exercise. This is also associated with higher insulin levels in women compared to men post-exercise, although glucose levels return to basal within a similar time-frame (Marliss et al., 2000). Finally, training may influence the glucoregulatory response to high intensity exercise, with an increase in exercise-induced epinephrine levels contributing to a greater increase in blood glucose levels due to increased hepatic glucose production in exercise-trained individuals (Kjaer et al., 1986). However, recently it has been reported that the rise in blood glucose levels in response to intense exercise is less in sprint-trained individuals (Harmer et al., 2007).
1.8 Glucoregulatory Responses to High-Intensity Aerobic Exercise in Individuals with Type 1 Diabetes

Not all forms of exercise result in an acute fall in blood glucose levels. For instance, resistance exercise has been shown in some studies to result in a post-exercise increase in blood glucose level (Turner et al., 2014). Also, when exercise is performed at high intensity (>80% \( \dot{V}O_2\text{max} \)) for at least 10 min in insulin-treated patients with type 1 diabetes, blood glucose levels increase (Mitchell et al., 1988; Purdon et al., 1993; Sigal et al., 1994a) in a manner similar to non-diabetic individuals. One major difference, however, is that during recovery, blood glucose level rises and remains elevated for up to several hours post-exercise. This is in marked contrast to non-diabetic individuals where blood glucose levels return to pre-exercise levels within one hour.

The increase in blood glucose levels during intense aerobic exercise in diabetic individuals is due to the approximately 7-fold increase in hepatic glucose production rate (Purdon et al., 1993) being higher than the approximately 4-fold increase in glucose utilisation (Purdon et al., 1993; Sigal et al., 1994a). During early recovery, blood glucose levels continue to rise for a few minutes in these individuals due to glucose production rate being comparatively higher than glucose utilisation (Purdon et al., 1993; Sigal et al., 1994a). Then glucose production and utilisation rate decline, with these processes matching each other as hyperglycaemia remains for at least an hour (Sigal et al., 1999) and up to several hours after the cessation of exercise (Mitchell et al., 1988; Purdon et al., 1993; Sigal et al., 1994a).

The glucoregulatory mechanisms responsible for the increase in blood glucose level during high intensity exercise are similar in both diabetic and non-diabetic individuals. A large catecholamine response to high intensity exercise is also reported in individuals with diabetes, with a similar relative increase of approximately 14-fold for both epinephrine and norepinephrine levels (Purdon et al., 1993). Likewise, glucagon response to high intensity exercise is similar in both diabetic and non-diabetic individuals, together with similar increase in lactate (Mitchell et al., 1988) and cortisol levels (Purdon et al., 1993). However, the pattern of free fatty acid response is different, with an increase during recovery from high intensity exercise in individuals with diabetes, while free fatty levels decrease in non-diabetic individuals (Mitchell et al., 1988).
The sustained rise in glycaemia throughout recovery in diabetic compared to non-diabetic individuals results mainly from the absence of a post-exercise increase in insulin levels (Mitchell et al., 1988; Purdon et al., 1993; Sigal et al., 1994a, 1999). Such a role for insulin is suggested by the observation that the administration of insulin during recovery from high intensity exercise accelerates the return of blood glucose to pre-exercise levels and prevents prolonged hyperglycaemia (Purdon et al., 1993; Sigal et al., 1994a). Due to the absence of a post-exercise increase in insulin release, there is no stimulus to promote glucose uptake by skeletal muscles or to inhibit hepatic glucose production by the liver. For these reasons, blood glucose levels in individuals with type 1 diabetes remain elevated or continues to rise well after the cessation of exercise (Mitchell et al., 1988; Purdon et al., 1993; Sigal et al., 1994a, 1999; Marliss and Vranic, 2002).

1.9 Glucoregulatory Responses to Intermittent High Intensity Exercise in Non-Diabetic Individuals

Although the glucoregulatory responses to both moderate and high intensity exercise have been researched comprehensively as described above, this is not the case for intermittent high intensity, a pattern of activity where moderate intensity is combined with multiple sprints performed in succession. This is unfortunate given that this pattern of physical activity characterises most team sports such as soccer, rugby, Australian Rules football, netball and basketball (Docherty et al., 1988; Bangsbo et al., 1991; Spencer et al., 2004; Spencer et al., 2005; Bishop and Wright, 2006; Davidson and Trewartha, 2008).

This combination of multiple sprints interspersed with active or passive recovery generally results in an increase in blood glucose levels and glucoregulatory responses similar to high intensity exercise. In particular, multiple prolonged sprints of 45-60 sec duration each interspersed with 3-5 min periods result in a progressive rise in glycaemia after each work period in non-diabetic individuals (Hermansen et al., 1970). Blood glucose often continues to rise following the cessation of exercise (Hermansen et al., 1970; Näveri et al., 1985) with a peak in glycaemia reported ~5 min into recovery (Hermansen et al., 1970) before blood glucose declines to pre-exercise levels. Such multiple sprints of 45-60 sec duration are associated with a several-fold increase in the levels of epinephrine, norepinephrine, lactate, glucagon, growth hormone and insulin levels in non-diabetic individuals (Näveri et al., 1985). It is important to note, however, that these prolonged sprints are physically demanding and are not representative of those
observed in team sports where each sprint lasts generally less than 10 sec (Spencer et al., 2005). Sports such as soccer, rugby, hockey, netball, basketball and Australian Rules football have average sprint duration of 2-4 sec (Docherty et al., 1988; Bangsbo et al., 1991; Spencer et al., 2004, 2005; Bishop and Wright, 2006; Davidson and Trewartha, 2008).

Several studies have investigated on the glucoregulatory response to multiple sprint protocols that more closely mimic team sport with shorter duration sprints of 10 sec or less (Brooks et al., 1990; Hamilton et al., 1991; Gaitanos and Williams, 1993; Nevill et al., 1993; Goto et al., 2007; Trapp et al., 2007; Bracken et al., 2009). These patterns of exercise result in an overall increase in glycaemia post-exercise in non-diabetic individuals (Holmyard et al., 1988; Brooks et al., 1990; Hamilton et al., 1991; Gaitanos and Williams, 1993; Nevill et al., 1993). Blood glucose levels typically peak early during recovery (3-5 min; Brooks et al., 1990; Gaitanos and Williams, 1993), with blood glucose levels increasing by approximately 1-2.5 mM (Brooks et al., 1990; Gaitanos and Williams, 1993; Nevill et al., 1993).

This increase in glycaemia in non-diabetic individuals after multiple short sprints is associated with a several-fold increase in epinephrine (Brooks et al., 1990; Gaitanos and Williams, 1993; Goto et al., 2007; Trapp et al., 2007; Bracken et al., 2009), norepinephrine (Brooks et al., 1990; Gaitanos and Williams, 1993; Goto et al., 2007; Trapp et al., 2007; Bracken et al., 2009) and blood lactate levels (Brooks et al., 1990; Hamilton et al., 1991; Gaitanos and Williams, 1993; Nevill et al., 1993; Goto et al., 2007; Trapp et al., 2007; Bracken et al., 2009). When multiple short sprints of less than 10 sec duration are performed in non-diabetic individuals, a decrease in pH is also observed (Brooks et al., 1990; Gaitanos and Williams, 1993; Goto et al., 2007; Bracken et al., 2009) together with an increase in growth hormone (Goto et al., 2007), and glycerol levels (Goto et al., 2007; Trapp et al., 2007).

1.10 Glucoregulatory Responses to Intermittent High Intensity Exercise in Individuals with Type 1 Diabetes

Until quite recently, there has been very limited information on the glucoregulatory responses to intermittent high intensity exercise in individuals with type 1 diabetes. The information that was available came from studies focusing on multiple long sprints of 20
to 60 sec duration. One study involved 30 min of cycling interspersed with successive one-min periods of high intensity cycling at 100% of maximal oxygen uptake, each separated by a one-min rest between (Sills and Cerny, 1983). This resulted in a decline in blood glucose levels during exercise in individuals with type 1 diabetes, but glycaemia stabilised during early recovery (Sills and Cerny, 1983). An increase in growth hormone was also observed while glucagon and cortisol levels remained stable in those individuals (Sills and Cerny, 1983). Another study investigating the glycaemic response of type 1 diabetic individuals to multiple 20-sec sprints at 120% $\dot{V}O_{2max}$ performed every 2 min, with active recovery in between high intensity bouts, shows no significant change in blood glucose levels during the multiple sprints or recovery (Ford et al., 1999). As mentioned above, multiple high intensity bouts of prolonged duration are both physically demanding and do not reflect the activity patterns of repeated sprints in most sports where a typical sprint is usually very short in duration.

Recently, some studies have been performed in individuals with type 1 diabetes to investigate their responses to the multiple short duration sprints typical of many team sports (Guelfi et al., 2007a). Twenty min of repeated 4-sec sprints each interspersed with 2 min of passive recovery result in a decline in glycaemia, but blood glucose levels stabilise during recovery (Guelfi et al., 2005c). This stabilisation was associated with elevated catecholamine and growth hormone levels (Guelfi et al., 2005c). In another study, 30 min of repeated 4-sec sprint cycling were once again performed, each separated by 2 min of active recovery at 40% $\dot{V}O_{2peak}$ was compared to constant moderate intensity cycling at 40% $\dot{V}O_{2max}$ (Guelfi et al., 2005a). In this study, the rate of decline in glycaemia was lower during the multiple sprint protocol compared to sustained exercise, with no further decrease during early recovery. In contrast, blood glucose levels declined during early recovery from sustained exercise (Guelfi et al., 2005a). The lesser rate of fall in blood glucose levels during intermittent compared to sustained exercise was associated with increased catecholamine and growth hormone levels (Guelfi et al., 2005a). Such a multiple short sprint protocol induces a greater increase in glucose production rate during exercise than moderate intensity alone, with glucose utilisation rate being similar in response to both exercise protocols (Guelfi et al., 2007b). The findings of the aforementioned studies and of more recent ones (Dube et al., 2013; Campbell et al., 2014) suggest that the risk of exercise-mediated hypoglycaemia during exercise are lower if moderate intensity exercise is interspersed with repeated short sprints.
(Guelfi et al., 2005a). It is noteworthy that resistance exercise also results in a lesser initial rate of fall in blood glucose level than aerobic exercise (Yardley et al., 2012, 2013). However, the effect of intermittent high intensity exercise on the risk of late onset hypoglycaemia was not examined in these studies.

Two recent studies performed on individuals with type 1 diabetes have investigated the risk of late-onset post-exercise hypoglycaemia (LOPEH) following intermittent high intensity exercise (Maran et al., 2010; Iscoe and Riddell, 2011), but with conflicting findings. One study reported an increased risk of LOPEH with repeated 5-sec sprints compared to moderate intensity exercise (Maran et al., 2010). However, since energy intake post-exercise was not measured and high intensity exercise can suppress appetite (King et al., 1994), it is possible these findings could be explained by a lower carbohydrate intake post-exercise as opposed to the exercise protocol itself. In contrast, another study found that repeated 15-sec sprints as part of an intermittent high intensity exercise protocol was associated with a lower risk of LOPEH in athletes with type 1 diabetes (Iscoe and Riddell, 2011). The discrepancy between the two studies may be explained by differences in fitness levels and training status of the participants together with different sprint durations and the nature of the exercise protocol being performed.

1.11 Glucoregulatory Responses to a Single Short Sprint in Non-Diabetic Individuals

In non-diabetic individuals, the performance of a single short-duration maximal sprint effort generally results in a post-exercise increase in blood glucose level (Hermansen et al., 1970; Schnabel et al., 1983, 1984; Cheetham et al., 1985; Lavoie et al., 1987; Brooks et al., 1988; Nevill et al., 1989; Allsop et al., 1990; Langfort and Zarzeczny, 1997; Bell et al., 2001; Moussa et al., 2003; Vincent et al., 2004; Saraslanidis et al., 2009; Zouhal et al., 2009) across a broad range of sprint duration. For instance, a 6-sec sprint in untrained males results in an increase in glycaemia of approximately 1 to 2 mM, peaking 20 to 30 min after sprinting (Moussa et al., 2003). Similarly, a longer 30-sec sprint results in an increase in blood glucose levels of approximately 1 to 2 mM, but with maximal levels attained approximately 5 to 10 min after the sprint (Cheetham et al., 1985; Brooks et al., 1988; Nevill and Boobis, 1989; Allsop et al., 1990; Langfort and Zarzeczny, 1997; Bell et al., 2001; Moussa et al., 2003; Vincent et al., 2004; Zouhal et al., 2009). Sprinting of even longer duration (40 to 60 sec) also increases blood glucose by approximately 1 to 2
mM, with maximal levels attained 5 to 10 min post-sprint with an increase of (Schnabel et al., 1983, 1984; Saraslanidis, 2009). Likewise, a 90-sec sprint increases glycaemia by approximately 1.5 to 2 mM (Lavoie et al., 1987). Although most studies show that a short sprint increases blood glucose level, one study has reported that glycaemia remains stable during recovery from a single short sprint (Vincent et al., 2004).

Interestingly, Moussa and colleagues (2003) reported significantly higher blood glucose levels following a 6-sec sprint compared to a 30-sec sprint at both 20 and 30 min of recovery in untrained non-diabetic males. The authors could not explain these findings on the basis of changes in the levels of the glucoregulatory hormones measured in their study, so they hypothesised a higher rate of muscle glucose utilisation following the 30-sec sprint than the 6-sec sprint to replenish muscle glycogen stores may explain the smaller increase in glycaemia during recovery (Moussa et al., 2003).

The glucoregulatory responses associated with a single maximal sprint efforts are qualitatively comparable to those associated with high-intensity aerobic exercise. A short duration maximal sprint effort results in a strong sympatho-adrenal response resulting in a several-fold increase in epinephrine and norepinephrine levels in non-diabetic individuals (Kindermann et al., 1982; Schnabel et al., 1983, 1984; Lavoie et al., 1987; Brooks et al., 1988; Nevill et al., 1989; Allsop et al., 1990; Langfort et al., 1997; Zouhal et al., 1998; Bell et al., 2001; Zouhal et al., 2001; Jacob et al., 2002; Moussa et al., 2003; Vincent et al., 2003; Jacob et al., 2004; Vincent et al., 2004; Botcazou et al., 2007; Bracken et al., 2009; Zouhal et al., 2009). This large increase in blood catecholamine levels is observed irrespective of sprint duration. Epinephrine levels double immediately following a 6-sec sprint, with maximal levels of approximately 1.2 nmol/l and 0.6 nmol/l in men and women, respectively (Moussa et al., 2003; Botcazou et al., 2006, 2007; Bracken et al., 2009). The duration of the sprint is one factor influencing the sympathoadrenal activity with significantly higher epinephrine levels recorded after a 30-sec sprint than a 6-sec sprint in untrained males (Moussa et al., 2003). In response to a 30-sec sprint, most studies report a 3 to 7 fold increase in epinephrine levels from resting values (Brooks et al., 1988; Nevill et al., 1989; Langfort et al., 1997; Moussa et al., 2003; Jacob et al., 2004; Vincent et al., 2004) with a peak concentration of approximately 1.4 to 4.5 nmol/l (Brooks et al., 1988; Nevill et al., 1989; Langfort et al., 1997; Zouhal et al., 1998, 2001; Jacob et al., 2002; Moussa et al., 2003; Vincent et al., 2003; Jacob et al., 2004; Vincent et al., 2004; Zouhal et al., 2009). Trained individuals attain even higher
peak epinephrine levels, ranging from approximately 7 to 8 nmol/l (an ~ 8 to 10 fold increase), or at least double those of untrained individuals (Zouhal et al., 1998, 2001). One study in active individuals reported an approximately 25-fold increase in epinephrine levels, peaking at 10.2 nmol/l immediately after a 30-sec treadmill sprint (Allsop et al., 1990). Longer sprint lasting 40 to 60 sec also results in a large increase in epinephrine levels (Schnabel et al., 1983, 1984; Ohkuwa et al., 1984; Schwarz et al., 1990) as is the case for a 90-sec sprint (Kindermann et al., 1982; Lavoie et al., 1987).

Plasma norepinephrine levels also increase markedly in response to a single short sprint, with the magnitude of the increase linked to sprint duration. A single 6-sec sprint results in an approximately 2-fold elevation in norepinephrine levels in men (Moussa et al., 2003; Botcazou et al., 2006, 2007; Bracken et al., 2009) and women (Botcazou et al., 2007), with maximal levels of approximately 5 nmol/l. Significantly higher norepinephrine levels are recorded after a 30-sec sprint, with a 5-fold increase (Moussa et al., 2003) to approximately 13.5 nmol/l in untrained males (Moussa et al., 2003). Numerous other studies have shown that a 30-sec sprint is associated with a 5-6 fold increase in norepinephrine levels (Brooks et al., 1988; Nevill et al., 1989; Zouhal et al., 1998, 2001; Moussa et al., 2003; Vincent et al., 2003, 2004; Jacob et al., 2004; Zouhal et al., 2009) to levels around 11 to 15 nmol/l (Brooks et al., 1988; Nevill et al., 1989; Langfort et al., 1997; Zouhal et al., 1998, 2001; Moussa et al., 2003; Vincent et al., 2003; Jacob et al., 2004; Vincent et al., 2004; Zouhal et al., 2009) in untrained individuals. Trained individuals attain even higher peak norepinephrine levels, ranging from approximately 16 to 18 nmol/l (~ 7 to 10 fold increase) in endurance-trained individuals (Zouhal et al., 2001, 2009) and up to 30 nmol/l (~ 14 fold increase) in sprint-trained individuals (Jacob et al., 2002). One study of active individuals reported an approximately 30-fold increase in norepinephrine levels, which reached 37.1 nmol/l immediately after a treadmill-sprint (Allsop et al., 1990). Longer sprints lasting 40-60 sec (Schnabel et al., 1983, 1984; Ohkuwa et al., 1984; Schwarz et al., 1990) and 90 sec (Kindermann et al., 1982; Lavoie et al., 1987) also result in a large increase in norepinephrine levels. This large rise in catecholamines immediately after short sprints of different durations has been proposed to activate hepatic glucose production and inhibit insulin-mediated glucose uptake in skeletal muscles (Nonogaki, 2000).

The pattern of change in plasma insulin levels in response to a sprint varies across studies in non-diabetic individuals. Plasma insulin levels are unchanged following 6-sec and 30-
sec sprints in one study of untrained males (Moussa et al., 2003). Other studies have also reported no change in insulin levels after a single 30-sec sprint in young active male participants (Vincent et al., 2004; Zouhal et al., 2009). In contrast, a 30-sec sprint has been reported to increase insulin levels during recovery in young active female participants (Vincent et al., 2004), untrained 21 year old males, and trained 34 year old males (Zouhal et al., 2009). Longer duration 45 to 50-sec sprints also result in an increase in insulin levels (Schnabel et al., 1984) as do 90-sec sprints (Kindermann et al., 1982; Lavoie et al., 1987). More recently, a decrease in insulin after a 30-sec sprint was reported in young trained participants (Zouhal et al., 2009). The lack of increase in insulin in some studies in response to the rise in blood glucose levels post-sprint may be due to the inhibition of glucose-mediated stimulation of insulin secretion by the elevated levels of circulating catecholamines (Marliss and Vranic, 2002). In most studies where plasma insulin level increases, insulin helps to bring glucose back to pre-exercise levels by stimulating glucose uptake by skeletal muscles and/or inhibiting hepatic glucose production (Marliss and Vranic, 2002).

Changes in growth hormone level also occur in response to sprinting. Growth hormone levels increases after a sprint, peaking approximately 20 to 60 min post-sprint (Nevill et al., 1996; Stokes et al., 2002, 2003, 2004, 2005, 2006; Gilbert et al., 2008). Growth hormone levels after sprinting remain elevated for at least 60 min (Nevill et al., 1996; Stokes et al., 2002, 2004, 2005) and even for up to 90 to 120 min in some individuals (Stokes et al., 2002). It should be noted that many studies have reported marked inter-individual variability in growth hormone response to sprinting (Stokes et al., 2002; Stokes et al., 2003). Sprint duration may be a factor influencing growth hormone response (Stokes et al., 2003), with the increase in growth hormone levels being significantly less following a 6-sec compared to a 30-sec sprint (Stokes et al., 2002). A sprint duration of 30 to 90-sec also increases markedly growth hormone levels (Gordon et al., 1994; Nevill et al., 1996; Stokes et al., 2002, 2003, 2005; Gilbert et al., 2008).

There are a number of factors that may influence growth hormone response to a single sprint effort. Firstly, the mode of exercise may influence growth hormone response with the larger muscle mass recruited during treadmill sprinting resulting in greater increases in growth hormone than sprint cycling (Stokes et al., 2002). There is also a trend towards faster pedaling rates in cycling at a given workload resulting in greater growth hormone concentrations (Stokes et al., 2002). The increase in growth hormone in response to
sprinting is also larger in sprint-trained compared to endurance-trained athletes (Nevill et al., 1996). However, it is noteworthy that 6 weeks of combined speed- and speed-endurance training blunts the growth hormone response to a single sprint (Stokes et al., 2004). Finally, age may also influence the magnitude of growth hormone response, with the rise in growth hormone levels after a 30-sec sprint being attenuated in older males (Stokes et al., 2006; Gilbert et al., 2008).

There is evidence that the rise in growth hormone level may contribute to the acute increase in blood glucose level post-sprint. Indeed, the administration of a physiological growth hormone pulse in non-exercised individuals results in a rapid fall in muscle glucose uptake (Moller et al., 1990, 1992b, 2003). If this were to be the case in resting individuals recovering from a sprint, growth hormone could potentially contribute to the post-sprint rise in blood glucose levels. However, evidence against this view is the finding that growth hormone levels increase mainly during recovery (Kinderman et al., 1982; Schnabel et al., 1984; Stokes et al., 2002) and the infusion of octreotide (a somatostatin analogue), an inhibitor of growth hormone release, has no acute effects on the magnitude of the hyperglycaemic effect of high intensity exercise (Sigal et al., 1996). Another counterregulatory hormone affected by sprinting is cortisol. Some studies have shown that a sprint of 30 to 90-sec results in an increase in plasma cortisol levels which peak approximately 15 to 20-min after sprinting (Kindermann et al., 1982; Buono et al., 1986; Nevill et al., 1996). In contrast, 45 to 60 sec (Schnabel et al., 1984; Schwarz and Kindermann, 1990) sprints have been reported not to change cortisol levels. Although there is some published evidence that a rise in cortisol levels may play a role in stabilising glycaemia due to its potential acute inhibitory effect on glucose utilisation in skeletal muscles (Shamoon et al., 1980), this hormone is unlikely to contribute to early changes in glycaemia post-sprint because cortisol levels increase after the post-exercise rise in blood glucose level and the effects of cortisol on hepatic glucose production and blood glucose levels require several hours to take place (De Feo et al., 1989a; Heller and Cryer, 1991; Marker et al., 1991; Mitrakou et al., 1991).

Another key glucoregulatory factor that increases after a single sprint effort in non-diabetic individuals is lactate. Sprinting, irrespective of its duration, results in an increase in blood lactate levels that peak approximately 5 to 7 min into recovery. A 6-sec sprint results in an increase in blood lactate levels (Stokes et al., 2002; Moussa et al., 2003;
Botcazou et al., 2006; Bracken et al., 2009). Likewise, a 30-sec sprint causes a large increase in blood lactate levels (Cheetham et al., 1985; Jones et al., 1985; Hardman et al., 1986; Brooks et al., 1988; Nevill et al., 1989; Allsop et al., 1990; Bogdanis et al., 1994, 1995; Granier and et al, 1995; Bogdanis et al., 1996; Nevill et al., 1996; Langfort et al., 1997; Weinstein et al., 1998; Bell et al., 2001; Zouhal et al., 2001; Jacob et al., 2002; Stokes et al., 2002; Moussa et al., 2003; Vincent et al., 2003; Jacob et al., 2004; Gilbert et al., 2008; Chiappa et al. 2009; Zouhal et al., 2009). This increase in lactate is of greater magnitude after a 30-sec sprint, peaking at around 12 to 16 mM (Brooks et al., 1988; Moussa et al., 2003). Longer sprints of 40 to 60 sec (Fujitsuka et al., 1982; Schnabel et al., 1983; Ohkuwa et al., 1984; Schnabel et al., 1984; Saraslanidis et al., 2009) and up to 90 sec (Kindermann et al., 1982; Lavoie et al., 1987) are also associated with very large increases in blood lactate levels. Not surprisingly, the increase in lactate is more pronounced in sprint-trained athletes (Cheetham et al., 1985; Granier et al., 1995). Middle distance runners who complete approximately three anaerobic training sessions per week reach significantly higher peak lactate concentrations of approximately 15 mM after a single sprint than long distance runners and untrained individuals (Jacob et al., 2004). This increase in lactate levels may contribute to the early post-sprint increase in glycaemia by providing gluconeogenic precursors for hepatic glucose production (Miller et al., 2002) and by increasing peripheral insulin resistance (Vettor et al., 1997).

A short duration maximal sprint effort also results in a decrease in blood pH in non-diabetic individuals (Brooks et al., 1988; Nevill et al., 1989; Allsop et al., 1990; Bogdanis et al., 1995; Bogdanis et al., 1996; Stokes et al., 2002; Bracken et al., 2009). This decrease in blood pH occurs after a single sprint of both 6 sec (Stokes et al., 2002; Bracken et al., 2009) and 30 sec (Allsop et al., 1990; Bogdanis et al., 1995; Bogdanis et al., 1996; Stokes et al., 2002). There is evidence that low blood pH can inhibit glucose utilisation by skeletal muscles (Gordon et al., 1994), thus contributing to the early rise in blood glucose levels post-sprint.

Changes in free fatty acid levels are also associated with sprinting. An increase in free fatty acid levels has been reported to continue for at least 4 hours after a single 30-sec cycle sprint (Stokes et al., 2005, 2008). In contrast, a decrease in FFA was recorded early immediately after a longer approximately 45-sec treadmill sprint (Schnabel et al., 1984). Since increases in free fatty acid levels occur hours after a sprint, they are unlikely to play a role in the increase in glycaemia early in recovery from a short maximal sprint effort.
The mechanisms underlying the glycaemic rising effect of a short sprint differ markedly from those associated with high intensity aerobic exercise. Recently, it has been shown that the rise in blood glucose level following a 10-sec sprint results from a transient decline in the rate of glucose utilisation together with the absence of changes in endogenous rates of glucose production (Fahey et al., 2012). This is in marked contrast to the mechanism underlying the post-exercise increase in blood glucose level following a bout of intense aerobic exercise where the rise in blood glucose level results from a disproportionate increase in endogenous glucose production relative to that of glucose utilisation rate (Marliss & Vranic, 2002). Maybe the increases in plasma catecholamines and growth hormone levels associated with short duration sprinting are too small to affect endogenous glucose production rate, whereas the transient fall in peripheral glucose utilisation may result from an exercise-mediated intramuscular rise in glucose 6-phosphate levels resulting in an inhibition of glucose utilisation via a glucose 6-phosphate-mediated inhibition of hexokinase (Fahey et al., 2013).

1.12 Glucoregulatory Response to a Single Short Sprint in Individuals with Type 1 Diabetes

Despite the large amount of research described above on the glucoregulatory responses to a single sprint in non-diabetic individuals, there is a lack of information on the effect of sprinting in individuals with type 1 diabetes. To the best of our knowledge, only a handful of studies have examined the effect of sprinting on blood glucose levels in insulin-treated individuals with type 1 diabetes (Harmer et al., 2006; Fahey et al., 2012). One of these studies showed that a constant load sprint to exhaustion at 130% \( \dot{V}O_2\text{peak} \) lasting approximately 60 to 80 sec increases blood glucose levels, with a peak change in plasma glucose of approximately 3.8 mM after 60 min of recovery in individuals with type 1 diabetes (Harmer et al., 2006). The performance of such a prolonged sprint in these individuals is associated with a sharp elevation in norepinephrine (Harmer et al., 2000, 2006), epinephrine (Harmer et al., 2000, 2006) and plasma lactate (Harmer et al., 2000, 2006, 2008) levels immediately post-sprint, whereas immunoreactive free insulin and glucagon levels did not significantly change during recovery (Harmer et al., 2006). Interestingly, seven weeks of sprint-training did not prevent the rise in blood glucose levels, but reduced its magnitude, and had little effect on catecholamine, insulin or
glucagon response to an identical 60 to 80 sec sprint matched for total work (Harmer et al., 2006).

To the best of our knowledge, only one study other than those described in this thesis has examined the effect of maximal sprint effort on blood glucose levels in individuals with type 1 diabetes (Fahey et al., 2012). This study shows that sprinting for 10 sec in these individuals under basal insulinaemic conditions results in a 1.2 mM increase in blood glucose levels within 30 minutes of recovery, with blood glucose remaining at stable and elevated levels afterwards. The mechanisms underlying this rise in blood glucose levels differ markedly from those associated with prolonged high intensity aerobic exercise (Marliss and Vranic, 2002). Indeed, the rise in blood glucose level in response to sprinting results from a transient decline in the rate of glucose utilisation, with no change in endogenous rate of glucose production (Fahey et al., 2012). This is markedly different from intense aerobic exercise where the post-exercise rise in blood glucose level in type 1 diabetic individuals results from a disproportionate increase in endogenous glucose production relative to the increase in glucose utilisation rate (Marliss and Vranic, 2002). Since the levels of plasma epinephrine, norepinephrine and growth hormone rose transiently and to only a small extent post-sprinting compared to intense aerobic exercise, this suggests they reached levels insufficient to stimulate hepatic glucose production. Interestingly, the effects of such a short sprint on blood glucose levels and counterregulatory hormones are not affected by an episode of antecedent hypoglycaemia (Davey et al., 2014).

### 1.13 Statement of the Problem and Aims

Individuals with Type 1 diabetes are encouraged to participate in regular physical activity (Zinman et al., 2004) due to the numerous physiological and psychological health benefits associated with a physically active lifestyle (Norris et al., 1990; Moy et al., 1993; Laaksonen et al., 2000; Riddell and Iscoe, 2006; Chimen et al., 2012). Unfortunately, exercise increases the risk of hypoglycaemia due, in part, to a contraction-mediated activation of glucose utilisation rate by skeletal muscle (Peirce, 1999) and an increase in insulin sensitivity (Wasserman and Zinman, 1994). This increased risk occurs both during exercise (Riddell et al., 1999; Tuominen et al., 1995; Rabasa-Lhoret et al., 2001), and for several hours during recovery (MacDonald, 1987a; Tsalikian et al., 2005; McMahon et al., 2007; Maran et al., 2010; Iscoe and Riddell, 2011; Davey et al., 2013a).
This is of concern because severe hypoglycaemia can cause brain damage (Suh et al., 2007; Puente et al., 2010), cognitive dysfunction (Northam et al., 2009; Asvold et al., 2010) and even sudden death (Tanenberg et al., 2010). Approximately 6-10% of patients with type 1 diabetes die from hypoglycaemia (Skrivarhau et al., 2006; Jacobson et al., 2007; Feltbower et al., 2008). Consequently, it is not surprising that one of the biggest barriers to regular physical activity in individuals with type 1 diabetes is the fear of hypoglycaemia (Brazeau and et al., 2008). As a result of this legitimate fear, many individuals with type 1 diabetes have been known to avoid physical activity (Ludvigsson et al., 1980; Guelfi et al., 2007a; Brazeau et al., 2008), with ~60-65% of individuals with type 1 diabetes reported to be inactive (Thomas et al., 2004; Plotnikoff et al., 2006).

It is important to remember, however, that not all types of exercise result in elevated risk of hypoglycaemia. In fact, although exercise of low to moderate intensity increases the risk of hypoglycaemia both during and after exercise (MacDonald, 1987a; Tuominen et al., 1995; Riddell et al., 1999; Rabasa-Lhoret et al., 2001), exercise performed at high intensity (>80% of \( \dot{V}O_2\text{max} \)) for approximately 10-15 min is accompanied by an increase in glycaemia during and after exercise (Mitchell et al., 1988; Marliss et al., 1992a; Purdon et al., 1993; Sigal et al., 1994c, 1999; Marliss and Vranic, 2002). The hyperglycaemic effect of prolonged high intensity exercise in individuals with Type 1 diabetes raises the intriguing possibility that this type of exercise might be beneficial if adopted to counter a fall in glycaemia in complication-free individuals with type 1 diabetes, and thus might help to prevent or delay hypoglycaemia if no carbohydrate is readily available. The problem here is that 10-15 min of exercise at intensities above 80% \( \dot{V}O_2\text{max} \) would be unlikely to be well tolerated by most individuals with type 1 diabetes due to the very intense nature of such exercise combined with the impractical duration of the exercise bout. This raises the question of whether a much shorter bout of exercise performed at a higher intensity could be adopted to prevent glycaemia from falling. Although, as discussed above, a 30-90-sec sprint can increase blood glucose levels in individuals with type 1 diabetes, sprint efforts lasting 30-sec or more are associated with physical discomfort (Marquardt et al., 1993; Laurent et al., 2007), undesirable physiological consequences such as nausea, vomiting and dizziness (Inbar et al., 1996; 1998; Laurent et al., 2007) and therefore may be unsafe or impractical for many individuals (Little et al., 2010). However, a maximal sprint effort lasting only 10 sec is known to be well tolerated. For this reason, it was the primary goal of this thesis to determine for the first time whether a 10-sec maximal sprint effort provides a potential means other than
carbohydrate intake to oppose an insulin- or exercise-mediated fall in glycaemia, thus decreasing the risk of hypoglycaemia in complication-free individuals with type 1 diabetes. Also, given that studies concerned with investigating response of individuals with type 1 diabetes to exercise typically determine the $\dot{V}O_2_{\text{max}}$ of their participants using graded exercise testing, another objective of this thesis is to determine whether risk of hypoglycaemia is increased with this type of exercise protocol.

More specifically, our aims are to test the following hypotheses;

1) Graded exercise testing in complication-free individuals with type 1 diabetes will result in a post-exercise increase in blood glucose level, therefore not increasing the risk of hypoglycaemia during early recovery.

2) The performance of a single 10-sec maximal sprint effort after 20 min of moderate intensity exercise performed under hyperinsulinaemic conditions will oppose the exercise-mediated fall in glycaemia, thus decreasing the risk of hypoglycaemia during early recovery in complication-free individuals with type 1 diabetes.

3) The performance of a single 10-sec maximal sprint effort before 20 min of moderate intensity exercise performed under hyperinsulinaemic conditions will lessen the subsequent exercise-mediated fall in glycaemia, thus helping to decrease the risk of hypoglycaemia during exercise in complication-free individuals with type 1 diabetes.

4) The performance of a single 10-sec maximal sprint under hyperinsulinaemic conditions will result in a counterregulatory response typical of sprinting.
1.14 Significance of the Thesis

Unfortunately, individuals with type 1 diabetes are on average less physically active than recommended due to their fear of hypoglycaemia and a lack of information and guidelines on how to safely participate in various sports and recreational activities. It is important for these individuals to enjoy the numerous health and lifestyle benefits of regular physical exercise. Regrettably, there is little research in this area, resulting in a lack of comprehensive guidelines and practical advice on how to safely manage blood glucose levels while performing various types of physical activity. Our findings might identify sprinting as a novel tool for the prevention of hypoglycaemia in individuals with type 1 diabetes.
Chapter 2

Glycaemic Response to Graded Exercise in Individuals with Type 1 Diabetes
2.1 Abstract

**Objective:** To investigate whether the risk of hypoglycaemia in individuals with type 1 diabetes increases in response to the type of graded exercise testing used to determine $\dot{V}O_{2\text{peak}}$.

**Research Design and Methods:** Eight non-diabetic male participants (CON) and seven complication-free type 1 diabetic male individuals in good to moderate glycaemic control (HbA1c = 7.6 ± 0.5%) were recruited for this study. On the morning of testing, the type 1 diabetic participants followed their normal insulin regimen, and both groups ate their usual breakfast to transiently increase their blood glucose levels. Approximately four hours later, graded exercise was commenced on a cycle ergometer. Blood metabolites and hormones were sampled at rest and regularly during recovery.

**Results:** During graded exercise, there were no significant changes in glycaemia in either group, but blood glucose levels in type 1 diabetic participants increased by more than 2 mM during recovery ($p < 0.05$) and remained elevated for 120 min, while remaining at stable levels in the control group. In both groups, catecholamine and lactate levels increased significantly early in recovery and fell thereafter ($p < 0.05$). Glucagon and cortisol levels also increased during recovery in both groups, while free fatty acid levels increased only in the type 1 diabetic participants ($p < 0.05$). In contrast, the levels of insulin remained stable in both groups ($p > 0.05$).

**Conclusions:** Our graded exercise protocol performed in the post-absorptive state while plasma insulin levels are low increases glycaemia early post-exercise, with no carbohydrate ingestion required for hypoglycaemia prevention early during that time. This type of exercise, therefore, does not increase the risk of early post-exercise hypoglycaemia in individuals with type 1 diabetes. The mechanisms underlying the post-exercise increase in glycaemia remain to be determined.
2.2 Introduction

Regular participation in physical activity is an important component in type 1 diabetes management because it is associated with numerous physiological and psychological health benefits (Norris et al., 1990; Moy et al., 1993; Laaksonen et al., 2000; Steppel and Horton, 2004; Zinman et al., 2004; Riddell and Iscoe, 2006; Chimen et al., 2012, Tonoli et al., 2012). Unfortunately, an active lifestyle may make acute management of blood glucose levels more difficult (Steppel and Horton, 2004; Camacho et al., 2005; Riddell and Perkins, 2006; Guelfi et al., 2007c; Younk and Davis, 2012). For instance, exercise of low to moderate intensity increases the risk of hypoglycaemia both during and after exercise (Camacho et al., 2005; Guelfi et al., 2007c) due, in part, to a contraction-mediated activation of glucose utilisation rate by skeletal muscle and an increase in insulin sensitivity (Steppel and Horton, 2004; Camacho et al., 2005). In contrast, exercise performed at high intensity increases blood glucose levels (Marliss and Vranic, 2002). Given the numerous benefits of an active lifestyle for individuals with type 1 diabetes together with the difficulties and challenges associated with managing blood glucose levels during and after exercise, it is not surprising that these issues have attracted a large volume of research (Chapter 1).

One feature shared by most of the research on exercise in diabetes is the common practice of standardising exercise intensity relative to maximal or peak rate of oxygen consumption (\(\dot{V}O_2\max\) or \(\dot{V}O_2\)peak) or lactate threshold (Chapters 3, 4, and 5 of this thesis; Fremion et al., 1987; Wanke et al., 1992; Purdon et al., 1993; Sigal et al., 1994c, 1996; Nugent et al., 1997; Ford et al., 1999b; Galassetti et al., 2001b; Rabasa-Lhoret et al., 2001; Galassetti et al., 2003; Heyman et al., 2007). The determination of \(\dot{V}O_2\)peak or lactate threshold requires the performance of a graded exercise test whereby exercise intensity increases in a stepwise fashion or continuously until maximal intensity is achieved (volitional exhaustion or attainment of criteria for \(\dot{V}O_2\max/\)peak).

Despite the importance and prevalence of graded exercise testing for \(\dot{V}O_2\)peak determination, it is unclear whether this testing protocol increases the risk of hypoglycaemia in type 1 diabetic individuals, and whether insulin reduction as well as increasing food intake before or immediately after exercise is required to prevent blood glucose levels from falling post-exercise. All studies addressing this issue have performed blood glucose measurements before and immediately after \(\dot{V}O_2\)peak testing (Fremion et
al., 1987; Wanke et al., 1992; Nugent et al., 1997; Ford et al., 1999; Heyman et al., 2007), with some reporting either the absence of changes in blood glucose levels (Fremion et al., 1987; Wanke et al., 1992; Nugent et al., 1997; Ford et al., 1999) or a decrease (Heyman et al., 2007). Since there are conditions where blood glucose level can change markedly after exercise (Purdon et al., 1993; Sigal et al., 1996; Marliss and Vranic, 2002) it is important to assess whether the risk of hypoglycaemia increases during recovery from graded exercise testing. To the best of our knowledge, only one study has examined this issue, but found no significant changes in blood glucose levels 20 min post-exercise (Ford et al., 1999b). However, as pointed out by the authors themselves, the main limitation with their study is that insulin dosage was adjusted and food was ingested one hour prior to exercise (Ford et al., 1999b), thus leaving unanswered the important question of whether blood glucose levels would have fallen during recovery had no food been ingested.

Given that a short bout of intense aerobic exercise under basal insulinaemic conditions can increase blood glucose levels (Marliss and Vranic, 2002), this raises the possibility that graded exercise testing performed when plasma insulin is at near basal level might also be associated with a post-exercise increase in glycaemia, with no carbohydrate ingestion required prior to or after exercise to prevent hypoglycaemia. The primary objective of this study is to test this hypothesis by examining the glycaemic and hormonal response to the type of graded exercise test used for $\dot{V}O_{2peak}$ determination under conditions where food is not ingested soon prior to testing and without adjusting insulin dosage. This is an important issue to address given the widespread use of graded exercise testing in diabetes research and fitness testing, and the absence of information about the risk of hypoglycaemia associated with this exercise protocol.
2.3 Research Design and Methods

2.3.1 Participants
Seven young males with type 1 diabetes (aged 20.4 ± 1.5 years; BMI 27.4 ± 1.0 kg/m$^2$; \(\text{VO}_{2\text{peak}}\) 45.9 ± 2.4 ml/kg/min; duration of diabetes 5.8 ± 1.8 years) and eight age-matched non-diabetic male (CON; aged 20.1 ± 0.8 years; BMI 25.3 ± 1.5 kg/m$^2$; \(\text{VO}_{2\text{peak}}\) 44.6 ± 3.6 ml/kg/min) participants were recruited from Princess Margaret Hospital and the University of Western Australia, respectively. All participants with type 1 diabetes were in moderate glycaemic control (HbA1c = 7.6 ± 0.5%), free from diabetic complications, hypoglycaemia aware, had undetectable levels of C-peptide and were not taking any prescribed medication other than insulin. Participants with type 1 diabetes were on a multiple-injection insulin regime that had not changed for at least three months prior to testing. Following a familiarisation session during which their informed consent was obtained together with anthropometric measurements, participants were required to attend our laboratory on another occasion for testing. Both the University of Western Australia and the Princess Margaret Hospital Ethics Committees approved all the procedures described in this study.

2.3.2 Experimental trials and assays
Participants were not allowed to exercise for 48 h prior to the experimental trial since antecedent exercise has the potential to affect the endocrine response to a subsequent bout of exercise (Galassetti et al., 2001b). Also, testing was rescheduled if they had experienced a hypoglycaemic episode over the previous 48 h because prior hypoglycaemia can also affect the counter-regulatory response to exercise (Galassetti et al., 2003). Each participant was also required to maintain a normal diet and to avoid alcohol for 24 h prior to testing. On the morning of testing, participants were instructed to monitor their blood glucose regularly. They arrived in the laboratory at ~ 7:30 am, and all type 1 diabetic participants were instructed to self-administer their usual dose of morning insulin into their abdomen. Both the control and type 1 diabetes groups were then asked to consume a breakfast, with the meal choice and nutritional content reflecting that normally ingested by the participant.
After breakfast, a catheter was inserted in one of the superficial veins at the back of the subject’s hand prior to heating the hand for the sampling of arterialised venous blood. No physical activity was allowed and blood glucose levels were measured every 15 min. In an effort to comply with the recommendations that exercise should be avoided if insulin levels are peaking, commencement of testing was delayed until four hours after insulin injection, at a time when insulin was near basal levels. During that time, post-prandial blood glucose levels fell, and when glycaemia approached 7 mM (7.24 ± 0.45 mM in the type 1 diabetic participants; 5.27 ± 0.20 mM CON), the graded exercise test to determine $\dot{V}O_{2\text{peak}}$ was initiated on an Evolution cycle ergometer (Evolution, Geelong, Australia).

The graded exercise test described here required participants to initiate cycling at an intensity of 50 watts while breathing through a mouthpiece into an oxygen analyser system. Briefly, every 3 min the exercise intensity was raised progressively by 50 watts until the subject reached his maximal rate of oxygen consumption. A plateau in oxygen consumption (an increase of $<150 \text{ mL} \cdot \text{min}^{-1}$) and/or a respiratory exchange ratio greater than 1.15 during the last min of exercise were the criteria for the achievement of $\dot{V}O_{2\text{peak}}$. The participant’s expired air was monitored continuously, and both oxygen uptake and carbon dioxide production were calculated every 15 sec using a computerized on-line gas analysis system. This comprised of a Morgan Ventilation Monitor (Morgan, Reinham, Kent, U.K.), Ametek S3A Oxygen Analyser and Ametek CD3A Carbon Dioxide Analyser (Ametek, Paoli, PA).

Blood was sampled prior to exercise and then at 0, 5, 10, 15, 30, 60, 90 and 120 min post-exercise. Some of the blood was assayed immediately for glucose, $pO_2$, pH and lactate levels using an ABL 625 blood gas system (Radiometer, Copenhagen) and the remainder of the blood was combined with sodium metabisulphite, polyethylene glycol or aprotinin (Trasylol) for the assays of catecholamines, insulin and glucagon, respectively (Bussau et al., 2006). All samples were centrifuged at 720g for 5 min and the plasma stored at -80°C for later analyses of catecholamines, free fatty acids, insulin, glucagon, cortisol, GH and C-peptide levels.

Heparinized plasma treated with sodium metabisulphate was used for the determination of catecholamine levels by reverse phase high-performance liquid chromatography using a Waters Novapak C18 reverse phase column and a model 5200A Coulochem detector (ESA Biosciences Inc., Chelmsford, MA, USA). Free fatty acids levels were measured
in EDTA-treated plasma using the Roche Half Micro Test Free Fatty Acids Assay Kit (Roche Diagnostic, Mannheim, Germany). Heparinized plasma treated with polyethylene glycol was assayed for free insulin using the Coat-a-Count Insulin Kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Glucagon levels in plasma collected with trasylol were measured from EDTA-treated plasma by radioimmunoassay using a Linco Glucagon RIA Kit (Linco Research, St Charles, Missouri, USA). Cortisol levels were assayed from venous serum by competitive immunoassay on an Immulite 2000 Analyser using the Immulite Cortisol Assay Kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Growth hormone levels were determined from serum by immunometric assay on an Immulite 2000 Analyser using the Immulite Growth Hormone Assay Kit (Diagnostic Products Corporation, Los Angeles, CA, USA). 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 Corporation, Los Angeles, CA, USA).

2.3.3 Statistical analyses
The results within each experimental group were analysed using a mixed model repeated measure analysis of variance (ANOVA) and Fisher’s least significant differences test for \textit{a posteriori} analysis using SPSS 13.0 for Windows software (SPSS, Chicago, IL, USA). Statistical significance was accepted at \( p < 0.05 \). Participants’ characteristics are expressed as means ± S.D. whereas all other results are expressed as means ± S.E.M.
2.4 Results

2.4.1 Blood metabolite response to graded exercise

Before the graded exercise test, blood glucose levels in both experimental groups fell significantly (p < 0.05) (Fig. 2.1). When glycaemia reached ~7 mM in diabetic participants (7.24 ± 0.45 mM in type 1 diabetic participants; 5.27 ± 0.20 mM control participants), the test was initiated. In response to graded exercise (exercise duration ~12 min), there were no significant changes in glycaemia in either experimental group. Immediately following exercise, glycaemia increased significantly (p < 0.05) and remained elevated for the whole duration of the 120-min recovery period (p < 0.05) in the type 1 diabetic participants (Fig. 2.1). In contrast, blood glucose levels remained relatively stable in the control group (Fig. 2.1). Blood lactate levels reached maximal levels at 5 min post-exercise in both experimental groups and decreased throughout recovery (Fig. 2.1). In contrast, blood pH reached minimal levels after 5 min of recovery in both experimental groups before returning to pre-exercise levels within 60 min of recovery (Fig. 2.1). During recovery, free fatty acid levels increased gradually over time and reached maximal levels after 90-120 min of recovery (Fig. 2.1).
**Figure 2.1:** Effect of graded exercise on the levels of blood glucose (A), lactate (B), pH (C) and free fatty acids (D). The exercise testing period is represented by the shaded box. All results are shown as mean ± standard error. Black circles refer to the type 1 diabetic participants whereas white circles refer to the non-diabetic control group. $^a$ represents a statistically significant difference ($p < 0.05$) compared to the rest time point in the type 1 diabetic participants, $^b$ represents a statistically significant difference ($p < 0.05$) compared to the rest time point in the control group.
2.4.2 Hormonal response to graded exercise

In response to graded exercise, catecholamines increased to maximal levels at the onset of recovery in both experimental groups (p < 0.05; Fig. 2.2), with the levels attained being not significantly different between experimental groups (p > 0.05). Norepinephrine levels returned to pre-exercise levels within 15 and 30 min after exercise in the control and type 1 diabetic groups, respectively (Fig. 2.2), whereas epinephrine returned to pre-exercise levels within 5 min post-exercise in both experimental groups (Fig. 2.2). Growth hormone (GH) levels displayed a non-significant transient increase in the type 1 diabetic and control groups, and reached significantly elevated levels in the control group after 15 min of recovery (p < 0.05; Fig. 2.2). Following graded exercise, plasma cortisol levels increased significantly (p < 0.05) and reached maximal levels within 10-30 min post-exercise in both groups (Fig. 2.2). Likewise, glucagon levels increased significantly in the type 1 diabetic group by 30 min post-exercise and by 90 min in the control group (p < 0.05; Fig. 2.2). During exercise, plasma insulin levels remained at relatively stable low levels in both experimental groups (Fig. 2.2).
Figure 2.2: Effect of graded exercise on the levels of epinephrine (A), norepinephrine (B), growth hormone (C), cortisol (D), glucagon (E), and free insulin (F). The exercise testing period is represented by the shaded box. All results are shown as mean ± standard error. Black circles refer to the type 1 diabetic group whereas white circles refer to the control group. \textsuperscript{a} represents a statistically significant difference (p < 0.05) compared to the rest time point in the type 1 diabetic group, \textsuperscript{b} represents a statistically significant difference (p < 0.05) compared to the rest time point in the control group.
2.5 Discussion

Graded exercise testing is integral to $\dot{V}O_{2\text{max/peak}}$ and lactate threshold determination in basic, applied and clinical research. What is still unclear, however, is whether the risk of hypoglycaemia increases in response to this type of exercise in individuals with type 1 diabetes. This is an important issue given that most laboratory-based studies on exercise in diabetes involve the performance of graded exercise for $\dot{V}O_{2\text{max/peak}}$ determination. Here we show for the first time that graded exercise performed when plasma insulin is at near basal level in young adult males with type 1 diabetes increases blood glucose level post-exercise and thus does not increase the risk of early hypoglycaemia. In particular, graded exercise under these conditions results in a rapid post-exercise increase in blood glucose levels (> 2 mM) which remains elevated for the first two hours of recovery (Fig. 2.1), thus suggesting that no carbohydrate ingestion before or after this type of exercise is required to prevent hypoglycaemia. Our findings thus suggest this exercise protocol is safe provided it is performed some time after the injection of rapid acting insulin.

The absence of a significant fall in glycaemia observed here during graded exercise corroborates the findings of most (Fremion et al., 1987; Wanke et al., 1992; Nugent et al., 1997; Ford et al., 1999), but not all studies (Heyman et al., 2007) on graded exercise in individuals with type 1 diabetes, whereas the post-exercise increase in blood glucose levels is described here for the first time. Although differences in experimental design make it difficult to compare our findings with those of others, most earlier studies reported that graded exercise does not affect blood glucose levels, but participants were fasted and omitted their morning insulin to reduce the risk of exercise-induced hypoglycaemia in one of these studies (Nugent et al., 1997) or were fed with excess of food before exercise with (Ford et al., 1999) or without (Fremion et al., 1987) adjusting their insulin dosage prior to exercise, thus making it less likely for blood glucose levels to fall. Although one study reported a fall in glycaemia (Heyman et al., 2007) during $\dot{V}O_{2\text{peak}}$ testing, exercise in that study was performed when plasma insulin levels were near maximal, a time when exercise is usually not recommended for individuals with type 1 diabetes (Rabasa-Lhoret et al., 2001; Steppel and Horton, 2004).

The finding here that blood glucose levels increase during recovery from graded exercise has never been reported before. To the best of our knowledge, only one other study has examined how blood glucose levels change during recovery from graded exercise testing
in individuals with type 1 diabetes, but it only measured glycaemia at 0 and 20 min post-exercise and found no significant change in blood glucose levels (Ford et al., 1999b). However, the main limitation with that study is that insulin was adjusted and food was ingested one hour prior to exercise, thus limiting the relevance of these findings and leaving unanswered the question of whether graded exercise increases the risk of early hypoglycaemia post-exercise if insulin dosage is not reduced or if no carbohydrate is ingested soon before exercise (Ford et al., 1999b). Our findings are consistent with those examining blood glucose response to intense aerobic exercise (>80% of $\dot{V}O_{2\text{peak}}$) performed under basal insulinaemia. Indeed, 10-15 min of sustained high intensity exercise at near 80% of $\dot{V}O_{2\text{peak}}$ in individuals with type 1 diabetes results in a sustained post-exercise increase in blood glucose levels (Purdon et al., 1993; Sigal et al., 1994c; Marliss and Vranic, 2002) similar to that observed here where blood glucose levels increase and remain elevated or continue to rise well after the cessation of exercise (Purdon et al., 1993; Sigal et al., 1994c; Marliss and Vranic, 2002). Similarly, the relatively stable blood glucose levels during graded exercise in the control group (Fig. 2.1) were similar to those reported in previous studies (Nugent et al., 1997; Heyman et al., 2007).

The marked rise in epinephrine and norepinephrine levels immediately following graded exercise might explain, at least in part, the rapid increase in glycaemia during early recovery in the type 1 diabetic group. In support of this view, the increased catecholamine levels during that time coincided with the rapid initial rise in blood glucose levels, with epinephrine and norepinephrine levels increasing by ~10-fold in the type 1 diabetic group (Fig. 2.2). A comparable increase in catecholamines levels (14-18-fold) has also been reported to occur in response to sustained high intensity aerobic exercise in individuals with type 1 diabetes (Purdon et al., 1993; Marliss and Vranic, 2002). It is important to note, however, that others have reported that catecholamines increase by less than 5-fold immediately following graded exercise (Nugent et al., 1997). Although elevated catecholamine levels are acknowledged to oppose insulin-mediated fall in glycaemia (Marliss and Vranic, 2002) via activation of hepatic glucose production and inhibition of insulin-mediated glucose uptake in skeletal muscles (Marliss and Vranic, 2002; Nonogaki, 2000), it is important to note that the importance of catecholamines in the activation of hepatic glucose production in response to intense exercise has been the object of some dispute (Camacho et al., 2005; Coker and Kjaer, 2005). Also, and more importantly, the fact that blood glucose levels remained stable in the control group despite
experiencing a comparable rise in catecholamine levels and no changes in plasma insulin levels in response to graded exercise suggests that other mechanisms both mediate the post-exercise rise in glycaemia in type 1 diabetic participants and explain the different glycaemic responses between the two experimental groups.

During recovery from graded exercise in the type 1 diabetic group, the absence of a post-exercise increase in insulin levels (Fig. 2.2) probably explains, at least in part, the early post-exercise rise in blood glucose level and sustained increased glycaemia as is the case following sustained high intensity aerobic exercise in type 1 diabetic individuals (Purdon et al., 1993; Sigal et al., 1994c; Marliss and Vranic, 2002). Here, however, not only plasma insulin levels did not differ between treatment groups, there was no increase in plasma insulin post-graded exercise test in the control group. This is consistent with the absence of post-exercise increase in blood glucose level in the control group. However, our findings with the control group differ from the transient increase in plasma insulin levels that occurs in non-diabetic individuals recovering from intense aerobic exercise and which have been proposed to explain their lesser post-exercise rise in glycaemia compared to diabetic individuals (Sigal et al., 1994c; Marliss and Vranic, 2002). Our findings thus suggest that other mechanisms are likely to explain the different patterns of glucose response between the control and type 1 diabetic participants.

Glucagon and cortisol are unlikely to play a major role in increasing glycaemia during recovery from graded exercise testing in individuals with type 1 diabetes. The levels of plasma glucagon, a potent activator of hepatic gluconeogenesis (Marliss and Vranic, 2002; Camacho et al., 2005), did not increase significantly during early recovery in type 1 diabetes or control participants although they did rise later during recovery (Fig. 2.2). This pattern of response in control participants differs from the increase in glucagon observed in response to sustained high intensity aerobic exercise (Purdon et al., 1993; Sigal et al., 1994c). Assuming that portal glucagon and insulin levels follow patterns of change similar to those of peripheral blood glucose and insulin levels, this suggests that both glucagon and glucagon/insulin ratio play little role in mediating the post-exercise rise in blood glucose levels. Although there is some published evidence that a rise in cortisol levels may play a role in stabilising glycaemia due to its potential acute inhibitory effect on glucose utilisation in skeletal muscles (Shamoon et al., 1980), this hormone is unlikely to play a major role here firstly because the effects of cortisol on hepatic glucose production and blood glucose levels require several hours to take place (McMahon et al.,
1988), and secondly because the increase in glycaemia observed here in type 1 diabetic participants preceded that of cortisol.

Growth hormone is also unlikely to play a major role in the increase in glycaemia during recovery from graded exercise as suggested by the absence of significant changes in the levels of this hormone in type 1 diabetic individuals (Fig. 2.2). It is important to stress, however, that a close inspection of Fig. 3c suggests that significant changes in growth hormone levels in response to graded exercise might have been undetected due to the large inter-individual variability in growth hormone levels. A trend for not only an increase in growth hormone levels in both experimental groups, but also a more pronounced early rise in growth hormone levels in the diabetic compared to non-diabetic group is consistent with the findings of others that graded exercise test results in a lesser post-exercise increase in growth hormone levels in non-diabetic compared to diabetic participants (Coiro et al., 2004). These responses of growth hormone to graded exercise suggest that these hormones could mediate the early increase in glycaemia post-graded exercise. In support of this interpretation, the administration of a physiological growth hormone pulse in non-exercised non-diabetic individuals results in a rapid fall in muscle glucose uptake (Moller et al., 1990). However, even if the increase in growth hormone levels had been significant, it would have probably played a role of lesser importance in increasing glycaemia post-exercise in the type 1 diabetic group. This is because the administration of a growth hormone pulse has been shown not to affect peripheral glucose uptake in insulin-treated individuals with type 1 diabetes (Møller et al., 1992a) and also because the infusion of octreotide, an inhibitor of growth hormone release, has no acute effect on the magnitude of the hyperglycaemic effect of high intensity exercise (Sigal et al., 1996).

Finally, the elevated lactate level after graded exercise (Fig. 2.1) is another factor that may have contributed to the stabilisation of glycaemia early in recovery by providing gluconeogenic precursors for hepatic glucose production (Miller et al., 2002) and by increasing peripheral insulin resistance (Vettor et al., 1997). However, the fact that lactate reached similar levels post-exercise in the type 1 diabetic and control participants suggests that this factor alone is unlikely to explain the post-exercise increase in glycaemia in type 1 diabetes since no change in glycaemia took place in the control participants despite both groups experiencing a similar increase in plasma lactate levels.
Overall, despite the potential role for catecholamines and maybe growth hormone and lactate in mediating, at least in part, the increase in glycaemia during recovery in the type 1 diabetic group, the fact that the patterns of change in plasma insulin and counter-regulatory hormones were similar between the type 1 diabetic and control groups suggest that other factors explain both the different blood glucose responses between these groups and the absence of increase in blood glucose levels in the control group during recovery from graded exercise. The counterregulatory hormone(s) responsible for increasing glycaemia post-exercise in type 1 diabetes thus remain to be identified.

In conclusion, on clinical grounds, our findings suggest that the risks of hypoglycaemia associated with graded exercise testing are minimal when performed under basal/near basal insulin levels, with no carbohydrate administration required just before or early after testing to prevent hypoglycaemia in individuals with type 1 diabetes. This is because this type of exercise protocol increases glycaemia during recovery if performed when insulin levels are not elevated. However, the risk of late onset post-exercise hypoglycaemia during recovery from graded exercise testing needs to be investigated in future studies. In addition, it is our view that health professionals should still regularly monitor glycaemia before, during and after testing since it remains to be established whether similar findings would have been reported with different graded exercise protocols and individuals of different ages, circulating insulin levels or severity of diabetic complications.

2.6 Acknowledgements

This research was funded by a Juvenile Diabetes Research Foundation/ National Health Medical Research Council of Australia program grant to T.W. Jones and P.A. Fournier. L.D. Ferreira is supported by a Juvenile Diabetes Research Fund International Fellowship.
Chapter 3

A 10-second Maximal Sprint Effort: A Novel Approach to Counter an Exercise-Mediated Fall in Glycaemia in Individuals with Type 1 Diabetes

An amended version of this chapter has been published in Diabetes Care:

3.1 Abstract

**Objective:** Our aim is to investigate whether a short maximal sprint effort can provide another means to counter the rapid fall in glycaemia associated with moderate intensity exercise in individuals with type 1 diabetes, and therefore decrease the risk of early post-exercise hypoglycaemia.

**Research Design and Methods:** Seven healthy male participants with type 1 diabetes injected their normal insulin dose and ate their usual breakfast. When their postprandial glycaemia fell to ~11 mM, they pedalled at 40% \( \text{VO}_2\text{peak} \) for 20 min on a cycle ergometer then immediately engaged in a maximal 10-sec cycling sprint (sprint trial) or rested (control trial). Both trials were administered in a counterbalanced order.

**Results:** Moderate intensity exercise resulted in a significant fall (p < 0.05) in glycaemia in both trials (mean ± S.E.M.: 3.6 ± 0.5 vs 3.1 ± 0.5 mM for sprint and control, respectively). The subsequent short sprint opposed a further fall in glycaemia for 120 min, whereas in the absence of a sprint, glycaemia decreased further (3.6 ± 1.22 mM; p < 0.05) after exercise. The stabilisation of glycaemia in the sprint trial was associated with elevated levels of catecholamines, growth hormone, and cortisol. In contrast, these hormones remained at stable or near stable levels in the control trial. Changes in insulin and free fatty acid levels were similar in the sprint and control trials.

**Conclusions:** These results suggest that following moderate intensity exercise, it is preferable for young individuals with insulin-treated, complication-free type 1 diabetes to engage in a 10-sec maximal sprint effort to acutely oppose a further fall in glycaemia than to only rest, thus providing another means to reduce risk of hypoglycaemia in active individuals with type 1 diabetes.
3.2 Introduction

It is well established that exercise of moderate intensity increases the risk of hypoglycaemia both during and after exercise in individuals with type 1 diabetes (Campagne et al., 1987; Riddell et al., 1999) due, in part, to a contraction-mediated activation of glucose utilisation rate in skeletal muscle (Peirce, 1999) and an increase in insulin sensitivity (Wasserman and Zinman, 1994). In contrast, 10-15 min of high intensity exercise (>80% of maximal rate of oxygen consumption [\(\text{VO}_{2\text{peak}}\)]) causes a post-exercise increase in blood glucose levels in insulin-treated individuals with type 1 diabetes, irrespective of their glycaemic control (Mitchell et al., 1988; Marliss et al., 1992a; Purdon et al., 1993; Sigal et al., 1994c, 1999; Marliss and Vranic, 2002). This hyperglycaemic effect of prolonged high intensity exercise raises the intriguing possibility that this type of exercise might provide a means other than carbohydrate intake to counter a fall in glycaemia post-exercise in complication-free individuals with type 1 diabetes, and thus acutely reduce their risk of hypoglycaemia. Unfortunately, 10-15 min of exercise at high intensity is unlikely to be well tolerated by most individuals with type 1 diabetes due to the very intense nature of such exercise combined with its impractical duration.

A more practical way of using intense exercise as a means to prevent glycaemia from falling might be to engage in a much shorter bout of exercise performed at maximal intensity. The main difficulty with this suggestion is that a maximal sprint effort that lasts in excess of 30-sec is associated with unpleasant consequences such as nausea, vomiting and dizziness (Inbar et al., 1996; Van Praagh, 1998). This raises the question of whether a shorter sprint could provide a potential means other than carbohydrate intake to oppose a post-exercise fall in glycaemia. Although individuals at rest are unlikely to engage in a sprint to stabilise their glycaemia, doing so might prove to be an effective and convenient way to counteract a rapid fall in blood glucose level in response to moderate intensity exercise. Because this possibility has never been examined before, it was the primary goal of this study to determine whether a 10-sec maximal sprint effort is preferable to only resting as a means to counter a further fall in glycaemia during recovery from moderate intensity exercise in individuals with type 1 diabetes.
3.3 Research Design and Methods

3.3.1 Participants
Seven young men with type 1 diabetes were recruited to this study (aged 21.0 ± 3.5 years; BMI 26.9 ± 4.0 kg/m²; c 44.5 ± 4.2 ml/kg/min; duration of type 1 diabetes 9.1 ± 3.6 years; HbA₁c 7.4 ± 0.8% ranging between 6.6 to 9.0 %). All participants were free from any diabetic complications, had undetectable levels of C-peptide, and were not taking any prescribed medication other than insulin. They had all been treated with a stable insulin regimen composed of a combination of slow or intermediary acting insulin (eg NPH), and fast acting insulin analogues for at least three months prior to the study. All participants were required to attend our laboratory on three occasions. The first visit was a familiarisation session during which their informed consent was obtained together with anthropometric measurements, and their maximal rate of oxygen consumption was determined as previously described (Fairchild et al., 2002b). The next two visits were the sprint and control rest trials administered in a random counterbalanced order. The Institutional Ethics Committee approved all the procedures described in this study.

3.3.2 Experimental trials
The participants were not permitted to exercise for 24 h prior to the experimental trial, and testing was rescheduled if they had experienced a hypoglycaemic episode over the previous 48 h. Participants were also instructed to maintain a similar diet and avoid alcohol for 24 h before each testing session. On the morning of testing, the participants were required to monitor their blood glucose regularly. They arrived in the laboratory at around 8 A.M. and were instructed to self-administer their usual dose of morning insulin and eat their breakfast as per normal before a catheter was inserted for blood sampling. Participants’ insulin dose and breakfast content (1833 ± 224 kJ total energy; 57 ± 3% carbohydrate; 17 ± 1% protein; 26 ± 4% fat) were kept identical for both trials. Glycaemia was then measured every 15 min, and once blood glucose levels fell after peaking postprandially, no mid-morning snack was allowed so as not to counter the fall in glycaemia. If participants’ blood glucose levels were not decreasing at the commencement of exercise, the testing session was cancelled because the purpose of this study was to determine whether a short sprint could counter an exercise-mediated fall in glycaemia.
When falling blood glucose levels reached ~11 mM (approximately 121 ± 15 and 109 ± 10 min after insulin injection in the sprint and control trials), they engage in 20 min of moderate intensity exercise (40% \( \text{VO}_2\text{peak} \)) on an air-braked Repco front access cycle ergometer (Repco, Sydney, Australia), with the resistance to cycling increasing with cycling rate. An intensity of 40% \( \text{VO}_2\text{peak} \) was adopted because it more closely mimics the intensity of most activity patterns performed under ‘real life’ conditions for the general population. Also, because all participants were tested at a time close to peak plasma insulin levels and insulin-treated individuals with type 1 diabetes are discouraged from engaging in any intense exercise during that time (Zinman et al., 2004), the information gathered from exercising our participants at higher intensity would have been of little practical relevance to most individuals with type 1 diabetes. The 20-min duration was adopted because some preliminary work in our laboratory revealed that had the exercise duration been longer, a large proportion of our participants would have reached hypoglycaemic levels because of the rapid fall in glycaemia when exercising at 40% \( \text{VO}_2\text{peak} \), and testing would have had to end prematurely to avoid a hypoglycaemia-mediated counterregulatory response. On completion of this moderate intensity cycling, participants were instructed to rest or perform a 10-sec sprint, depending on their experimental trial. All participants were instructed to cycle as hard as possible and not to pace themselves for the whole duration of the 10-sec sprint. Venous blood from the arm and arterialised capillary blood from the earlobe were sampled before the moderate intensity exercise, immediately before the cycling sprint (in the sprint trial) and then at 0, 5, 10, 15, 30, 45, 60, 75, 90, 105 and 120 min of recovery or until blood glucose levels declined to 3.5 mM, in which case the trial was ended and the participant was immediately given carbohydrates to prevent hypoglycaemia.

At each sampling point, 35 \( \mu \)l of arterialised capillary blood was taken from the earlobe and assayed immediately for glucose and lactate levels using an ABL 625 blood gas system (Radiometer, Copenhagen). A 15 ml blood sample was also removed from the catheter for measuring hormones. Some of the blood was combined with sodium metabisulphite, poly-ethylene glycol or aprotinin (Trasylol) for the assay of catecholamines, insulin, and glucagon, respectively. All samples were then centrifuged at 720g for 5 min, and the plasma was stored at -80°C for later analysis of catecholamines, free fatty acids, insulin, glucagon, cortisol, growth hormone and C-peptide levels.
3.3.3 Hormones and metabolite assays
Glucose and lactate were analysed using an ABL 625 Blood Gas System (Radiometer, Copenhagen, Denmark). Heparinized plasma treated with sodium metabisulphate was used to determine catecholamine levels by reverse phase high-performance liquid chromatography using a Waters Novapak C18 reverse phase column and a model 5200A Coulochrome detector (ESA Biosciences Inc, Chelmsford, MA, USA). Free fatty acids levels were measured in EDTA-treated plasma using the Roche Half Micro Test Free Fatty Acids Assay kit (Roche, Mannheim, Germany). Heparinized plasma treated with polyethylene glycol was assayed for free insulin using the Coat-a-Count Insulin kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Glucagon levels in plasma collected with aprotinin (Trasylol) were measured from EDTA-treated plasma by radioimmunoassay using a Linco glucagon radioimmunoassay kit (Linco Research, St Charles, Missouri, USA). Cortisol levels were assayed from venous serum by competitive immunoassay on an Immulite 2000 Analyser using the Immulite Cortisol Assay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Growth hormone levels were determined from serum by immunometric assay on an Immulite 2000 Analyser using the Immulite Growth Hormone Assay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). 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 Corporation, Los Angeles, CA, USA).

3.3.4 Statistical analyses
The results were analysed using a two-way (time × trial) repeated-measures analysis of variance (ANOVA) and Fisher least significant differences (LSD) test for a posteriori analysis using SPSS 11.0 software. Statistical significance was accepted at p < 0.05. Participants’ characteristics are expressed as means ± S.D, whereas all other results are expressed as means ± S.E.M.
3.4 Results

3.4.1 Blood metabolite response

Before the bout of moderate intensity exercise, blood glucose levels in both experimental trials fell significantly (p < 0.05; Fig. 1). When glycaemia reached ~11 mM (11.2 ± 0.4 vs 11.9 ± 0.4 mM in the sprint and control trial, respectively), 20 min of cycling at 40% \( \dot{V}O_2 \text{peak} \) was initiated, with the total workload being identical between treatments for each participant (total work of 1176 ± 105 and 1178 ± 104 kJ/kg for sprint and control trials, respectively). This resulted in a further rapid significant decrease in glycaemia in both experimental trials (sprint: 3.6 ± 0.5 mM, p < 0.05; control: 3.1 ± 0.5 mM, p < 0.05; Fig. 3.1). When a 10-sec maximal sprint effort was performed immediately after the moderate intensity exercise, the sprint opposed a further fall in blood glucose levels for the following 120 min. In contrast, blood glucose levels decreased further (3.6 ± 1.22 mM; p < 0.05) in the control trial (Fig. 3.1).

The response of free fatty acid levels to the sprint and control trials was similar, with stable levels observed during moderate intensity exercise followed by a marginal increase later in recovery (Fig. 3.2). In response to moderate intensity exercise, lactate levels increased moderately and rose to a greater extent in response to the sprint, reaching maximal levels after 5 min (p < 0.05) before decreasing to basal levels within 45 min after the sprint. In contrast, in the absence of a short sprint, lactate levels remained at stable basal levels throughout the recovery period.
Figure 3.1: Effect of a 10-sec sprint on blood glucose levels after moderate intensity exercise. The moderate intensity exercise commenced at time-point -20 min. The black line represents the sprint whereas the shaded box represents moderate intensity exercise. Blood glucose levels are expressed relative to those immediately after moderate intensity exercise (time-point= 0). All data are means ± standard error. Black circles refer to the sprint trial whereas white circles refer to the control trial. \(^b\) represents a statistically significant difference (\(p < 0.05\)) compared to the 0 min time point after moderate intensity exercise, \(^c\) represents a statistically significant difference (\(p < 0.05\)) compared to the 0 min time point after moderate intensity exercise.
3.4.2 Hormonal response

In response to the 10-sec maximal sprint effort initiated immediately after moderate intensity exercise, epinephrine and norepinephrine reached maximal levels at the onset of recovery (p < 0.05 for each) and returned to pre-exercise levels within 5 min after the sprint (Fig. 3.2). In contrast, catecholamine levels remained stable in the control trial (Fig. 3.2). Likewise, the response of growth hormone differed between the sprint and control trials, with growth hormone levels increasing progressively after the sprint to reach maximal levels after 15 min of recovery (Fig. 3.2). The response of plasma cortisol levels in the sprint trial also differed from that of the control trial, with cortisol levels increasing significantly during recovery to reach maximal levels 30 min after the sprint (p < 0.05), but remained at stable levels in the control trial (Fig. 3.2). Glucagon increased early in the recovery period in the control trial (p < 0.05) but did not change significantly in the sprint trial. Finally, the pattern of insulin response to exercise was similar in the sprint and control trials, with insulin levels remaining relatively stable throughout exercise and recovery (Fig. 3.2). It is important to note that both trials were performed at a time of the day when insulin levels were elevated (121 ± 15 and 109 ± 10 min after insulin injection in the sprint and control trials, respectively), and plasma insulin levels were similar between trials (Fig. 3.2).
Figure 3.2: Effect of a 10-sec sprint on the levels of lactate (A), free fatty acids (B), norepinephrine (C), epinephrine (D), growth hormone (E), cortisol (F), glucagon (G) and free insulin (H) after moderate intensity exercise. The black line represents the sprint whereas the shaded box represents moderate intensity exercise. Black circles refer to the sprint group whereas white circles refer to the control group. All data are shown as mean ± standard error. a represents a statistically significant difference (p < 0.05) between control and sprint trials, b represents a statistically significant difference (p < 0.05) compared to the 0 min time point after moderate intensity exercise in control, c represents a statistically significant difference (p < 0.05) compared to the 0 min time point after moderate intensity exercise in sprint trial.
3.5 Discussion

Current guidelines for minimising the risks of hypoglycaemia associated with exercise in type 1 diabetes recommend a reduction in insulin dose or increased ingestion of carbohydrates before exercise based on an individual’s previous glycaemic responses to similar exercise (Zinman et al., 2004). This study investigated the intriguing possibility of using a short bout of intense exercise as another means to counter an exercise-mediated fall in glycaemia. In particular, it examined whether a short 10-sec cycling sprint could acutely oppose an exercise-mediated fall in glycaemia during recovery from exercise in individuals with insulin-treated, complication-free type 1 diabetes. Our results suggest that to minimise the risk of a fall in glycaemia after a bout of moderate intensity exercise in young individuals with complication-free type 1 diabetes, it is preferable to engage in a 10-sec maximal sprint effort before resting than to only rest during recovery. Such a sprint opposed a further fall in blood glucose levels for at least 120 min, whereas glycaemia decreased significantly (p < 0.05) by ~ 3.5mM if no sprint was performed (Fig. 3.1). Sprinting is likely to counter the exercise-mediated decrease in blood glucose levels through an increase in catecholamine, lactate, cortisol and growth hormone levels. The ability of the sprint to oppose the fall in glycaemia was more remarkable considering the sprint and control trials were performed when insulin levels were elevated, a time when exercise is not usually recommended (Rabasa-Lhoret et al., 2001).

It is likely that the marked rise in catecholamine levels at the onset of recovery after the sprint explain how such a sprint counters an exercise-mediated fall in glycaemia, as the levels of the other counterregulatory hormones examined in this study did not change significantly during this time (Fig. 3.2). It is generally acknowledged that high catecholamine levels oppose an insulin-mediated fall in glycaemia (Marliss and Vranic, 2002) via their activation of hepatic glucose production and inhibition of insulin-mediated glucose uptake in skeletal muscles (Nonogaki, 2000). Likewise, elevated lactate levels (Fig. 3.2) may contribute to the stabilisation of glycaemia early in recovery by providing gluconeogenic precursors for hepatic glucose production (Miller et al., 2002). The above interpretation has to be taken with caution given recent findings from this laboratory that a 10-sec sprint by itself has no effect on hepatic glucose production rate but decreases peripheral glucose utilisation rate despite causing a rise in plasma catecholamines and lactate levels comparable to those measured here (Fahey et al., 2012).
In this study, catecholamines returned rapidly to basal levels after the sprint, thus raising the question of whether other hormones opposed the decrease in glycaemia as recovery progressed in the sprint trial. Because insulin levels did not change significantly from pre-exercise levels over the two hours of recovery (Fig. 3.2), they could not explain how sprinting opposed the exercise-mediated fall in glycaemia. Likewise, the absence of an increase in plasma glucagon levels in the sprint trial makes it unlikely that glucagon was responsible for opposing the decrease in glycaemia (Fig. 3.2). Although there is some evidence that the progressive rise in cortisol levels may play a role in stabilising glycaemia due to cortisol’s potential acute inhibitory effect on glucose utilisation in skeletal muscles (Shamoon et al., 1980), it is likely that this hormone played only a minor role because the effects of cortisol on hepatic glucose production and blood glucose levels have been shown to require several hours to take place (McMahon et al., 1988).

The elevated levels of growth hormone after exercise (Fig. 3.2) might play some role in opposing the decrease in glycaemia later during recovery from sprinting. In support of this view, the administration of a physiological growth hormone pulse in non-exercised non-diabetic individuals has been reported to result in a rapid fall in muscle glucose uptake (Møller et al., 1990, 1992b, 2003) and a 1-2 h delayed increase in lipolysis, circulating free fatty acid levels, and fat oxidation rates (Møller et al., 1990, 1992b, 2003), which could contribute further to lowering glucose utilisation rates (Møller et al., 1992b). However, the aforementioned fall in muscle glucose uptake in response to a growth hormone pulse does not occur in insulin-treated individuals with type 1 diabetes (Møller et al., 1992a) and is not associated with a corresponding change in glycaemia and glucose appearance (Ra) and disposal (Rd) rates (Møller et al., 2003), thereby making it unlikely that growth hormone had a role in opposing the post-sprint fall in glycaemia. More importantly, the administration of this hormone after a bout of moderate intensity exercise in growth hormone-deficient individuals has no effect on glucose Ra and Rd (Kanaley et al., 2004), and the administration of octreotide (an inhibitor of growth hormone secretion) in non-diabetic individuals has also no acute effects on the magnitude of the hyperglycaemic effect of high intensity exercise (Sigal et al., 1996). It is important to stress, however, that no study so far has evaluated whether glucose metabolism in response to a short sprint is affected by growth hormone levels. The identity of the counterregulatory hormone(s) responsible for opposing the fall in glycaemia when a sprint is performed following a bout of moderate intensity exercise remains to be established.
In conclusion, this study provides the first evidence that a short maximal sprint effort performed immediately after moderate intensity exercise is preferable to only resting as a means to counter a further fall in glycaemia after exercise, thus decreasing the risk of early post-exercise hypoglycaemia in individuals with type 1 diabetes. On this basis, one might tentatively recommend that following exercise of moderate intensity, young individuals with complication-free type 1 diabetes consider performing a short 10-sec sprint before resting to counter a further fall in their blood glucose levels rather than only resting, particularly if a source of dietary carbohydrate is not readily available. This recommendation does not extend to intermittent high intensity exercise, as a study from our laboratory has shown recently that blood glucose remains at stable levels for at least one hour after this type of exercise (Guelfi et al., 2005a, 2005c). Although the long-term health benefits of regular exercise are generally recognised, to the best of our knowledge these findings provide the first example of a bout of exercise offering immediate short-term benefits (stabilisation of glycaemia). It is important to stress, however, that different results might have been obtained had sprinting been initiated after exercise of higher intensity or longer duration, in younger or older individuals with reduced sprinting capacity, or in individuals with impaired counterregulatory responses. For these reasons, more studies of the kind described here will be required to identify the subpopulation of type 1 diabetic individuals for whom a short maximal sprint effort can be recommended as a safe approach for the short-term stabilisation of blood glucose levels.

3.6 Acknowledgements

This research was funded jointly by a Juvenile Diabetes Research Foundation/National Health Medical Research Council of Australia program grant to T. Jones and P.A. Fournier. L. D. Ferreira is supported by a Juvenile Diabetes Research Fund International Fellowship. The authors acknowledge the technical assistance of Leanne Youngs.
Chapter 4

A 10-second Sprint Performed Prior to Moderate-Intensity Exercise Prevents Early Post-Exercise Fall in Glycaemia in Individuals with Type 1 Diabetes

An amended version of this chapter has been published in Diabetologia:

4.1 Abstract

Objective: We investigated whether a 10-sec maximal sprint effort performed immediately prior to moderate-intensity exercise provides another means to counter the rapid fall in glycaemia associated with moderate-intensity exercise in individuals with type 1 diabetes.

Research Design and Methods: Seven complication-free type 1 diabetic males (21.6 ± 3.6 years; mean ± SD) with HbA1c levels of 7.4 ± 0.7% injected their normal morning insulin dose and ate their usual breakfast. When post-meal glycaemia fell to ~11 mM, participants were asked to perform a 10 sec all-out sprint (sprint trial) or to rest (control trial) immediately before cycling at 40% of peak rate of oxygen consumption for 20 min, with both trials conducted in a random counterbalanced order.

Results: Sprinting did not affect the rapid fall in glycaemia during the subsequent bout of moderate-intensity exercise (2.9 ± 0.4 mM fall in 20 min; p = 0.00; mean ± SE). However, during the following 45 min of recovery, glycaemia in the control trial decreased by 1.23 ± 0.60 mM (p = 0.04) while remaining stable during early recovery in the sprint trial, subsequently decreasing in this latter trial at a rate similar to that in the control trial. The large increase in norepinephrine (p = 0.005) and lactate levels (p = 0.0005) may have contributed to the early post-exercise stabilisation of glycaemia in the sprint trial. During recovery, epinephrine and free fatty acid levels increased marginally in the sprint trial, but other counterregulatory hormones did not change significantly (p < 0.05).

Conclusions: A 10-sec sprint performed immediately prior to moderate-intensity exercise prevents glycaemia from falling during early recovery from moderate intensity exercise in individuals with type 1 diabetes.
4.2 Introduction

Individuals with type 1 diabetes mellitus are faced with the daily challenge of maintaining their blood glucose levels within a narrow physiological range in order to minimise the risks of developing long-term diabetic complications associated with chronic hyperglycaemia (DCCT, 1993). Unfortunately, the achievement of good glycaemic control results in an increased risk of experiencing severe episodes of hypoglycaemia (DCCT, 1993). This is further exacerbated in active individuals, since physical activity of moderate intensity increases the risk of hypoglycaemia both during exercise (Tuominen et al., 1995; Riddell et al., 1999; Rabasa-Lhoret et al., 2001) and for several hours afterwards (MacDonald, 1987). For these reasons, current guidelines aimed at minimising the risk of a fall in glycaemia associated with exercise recommend a reduction in insulin dose or increased ingestion of carbohydrates prior to exercise based on the individual’s previous glycaemic responses to similar exercise (Zinman et al., 2004).

It is well established that prolonged exercise of high intensity (>10 min at >80% peak rate of oxygen consumption; \( \dot{V}O_{2\text{peak}} \)) differs from moderate-intensity exercise in that it causes an increase rather than a fall in glycaemia in insulin-treated individuals with type 1 diabetes (Marliss and Vranic, 2002). For this reason, our laboratory recently examined whether the performance of one or several short maximal sprint efforts could offer an alternative means to counter the fall in glycaemia associated with moderate-intensity exercise or elevated plasma insulin levels in individuals with type 1 diabetes (Chapter 3; Guelfi et al., 2005b, 2005c; Bussau et al., 2006). This research showed that the fall in glycaemia during and after exercise of moderate intensity is not as marked if this type of exercise is interspersed with several 4 sec sprints, despite more work being performed (Guelfi et al., 2005b). Also, as described in Chapter 3 (Bussau et al., 2006), we found that immediately after a bout of moderate-intensity exercise it is preferable to sprint for 10 sec than to only rest as a means of countering a decrease in glycaemia during recovery. It is important to note that the sprints in these studies (Chapter 3; Guelfi et al., 2005b, 2005c; Bussau et al., 2006) were short enough (4–10 sec) to be well tolerated by all participants.

Given the benefits of sprinting during or after moderate-intensity exercise (Chapter 3; Guelfi et al., 2005b; Bussau et al., 2006), this raises the intriguing question of whether performing a short sprint effort immediately prior to, rather than after, moderate-intensity
exercise may offer a novel way of preventing the rapid fall in glycaemia normally observed during and after moderate-intensity exercise. In other words, this study examines whether short-duration sprinting should be an integral part of the preparatory routine of individuals with type 1 diabetes before they engage in sustained physical activities of moderate intensity.
4.3 Methods

4.3.1 Participants

Seven males with type 1 diabetes, (age 21.6 ± 3.6 years; BMI 26.7 ± 4.3 kg/m²; \( \dot{V}O_{2\text{peak}} \) 45.2 ± 5.0 ml kg\(^{-1}\) min\(^{-1}\); diabetes duration 9.5 ± 3.3 years; HbA\(_1c\) 7.4 ± 0.7%; total daily insulin dose 94 ± 38 units; all means ± SD), who were free from diabetic complications, had undetectable levels of C-peptide and were hypoglycaemia-aware, gave informed consent in accordance with both the University of Western Australia and Princess Margaret Hospital Ethics Committees. Participants were not taking any prescribed medication other than insulin and had not changed their insulin regimen for at least 3 months before the study. Following a familiarisation session, during which anthropometric measurements were taken, \( \dot{V}O_{2\text{peak}} \) was determined as described previously (Fairchild et al., 2002b). The next two visits were the sprint and control rest trials conducted in a random counterbalanced order.

4.3.2 Experimental trials and assays

In the 48 h prior to the experimental trial, participants were not allowed to exercise as antecedent exercise could have affected the endocrine response to exercise (Galassetti et al., 2001b). In addition, testing was rescheduled if participants had experienced a hypoglycaemic episode during the 48 h pre-trial phase because prior hypoglycaemia can also affect the counter-regulatory response during exercise (Galassetti et al., 2003). Following their arrival in the laboratory at 08:00 hours, participants self-administered their usual dose of morning insulin into their abdomen, with insulin dosage kept identical between trials (mean dose 15 ± 2 units short-acting insulin, 16 ± 9 units intermediate-acting insulin), and a catheter was inserted for blood sampling. Blood glucose levels were similar (p = 0.843) before insulin injection, with levels of 10.1 ± 1.4 and 10.3 ± 1.5 mM in the sprint and control trials, respectively. Participants then consumed breakfast to increase glycaemia above 11 mM. Breakfast food choice and nutritional content reflected that normally eaten by the participants, with breakfast kept the same (p > 0.05) for both trials (1736 ± 193 kJ total energy, with relative energy content as follows; 56 ± 3% carbohydrate; 17 ± 1% protein; 27 ± 4% fat). However, one of the control participants consumed additional carbohydrate to achieve post-breakfast glycaemia above 11 mM.

After breakfast, no physical activity was allowed and glycaemia peaked at 13.8 ± 0.8 and 13.9 ± 0.50 mM in the sprint and control trials, respectively (p = 0.890). When glycaemia
post-breakfast fell back to ~11 mM (approximately 111 ± 10 and 106 ± 8 min after insulin injection in the sprint and control trials, respectively; p = 0.564), participants either rested or performed a 10-sec maximal sprint effort on an air-braked cycle ergometer (Repco, Sydney, NSW, Australia). This was immediately followed by 20 min of moderate-intensity exercise (40% $\dot{V}O_{2\text{peak}}$), with the rationale underlying the intensity and duration of this bout of moderate-intensity exercise similar to that discussed previously in Chapter 3 (Bussau et al., 2006). Blood sampling and assays of metabolites and hormones were also performed as described in earlier studies from this laboratory (Chapter 3; Guelfi et al., 2005b; Bussau et al., 2006).

### 4.3.3 Statistical analyses

Data were analysed using a two-way repeated-measures ANOVA and Fisher’s least significant differences test for a posteriori analysis with SPSS 13.0 for Windows software (SPSS, Chicago, IL, USA). Statistical significance was accepted at p < 0.05. Participants’ characteristics are expressed as mean ± SD; all other results are mean ± SEM.
4.4 Results

4.4.1 Blood metabolite response

Before the bout of moderate intensity exercise, blood glucose levels in both experimental groups fell significantly ($p = 0.00$; Fig. 4.1). When glycaemia reached ~11 mmol/l (11.4 ± 0.5 and 11.8 ± 0.5 mmol/l in the sprint and control trial, respectively; mean ± S.E., n=7, $p = 0.446$), participants either rested or performed a 10-sec all-out sprint immediately before cycling at 40% $\bar{V}O_2$peak for 20 min. The total workload during the 20-min bout of exercise did not differ between treatments (total work of 295 ± 18 and 281 ± 25 kcal/kg for the sprint and control trials, respectively). Performing either a 10-s all-out sprint prior to exercise or only resting did not affect the rapid fall in glycaemia during the subsequent bout of moderate intensity exercise (2.9 ± 0.4 mmol/l in the sprint trial, $p = 0.00$; 3.2 ± 0.5 mmol/l in the control trial, $p = 0.001$; Fig. 4.1). During the initial 45 min of recovery, glycaemia in the control trial decreased linearly by 1.23 ± 0.60 mmol/l ($p = 0.04$), and fell by 3.80 ± 1.33 mmol/l after 120 min of recovery ($p = 0.03$; Fig. 4.1). In contrast, glycaemia remained stable in the sprint trial during the initial 45 min of recovery before eventually decreasing at a rate similar to that in the control trial, thus resulting in a delayed fall in blood glucose levels post-exercise (Fig. 4.1).

In the sprint trial, blood lactate levels increased significantly immediately post-sprint ($p = 0.0005$) and returned to pre-exercise levels within 30 min of recovery (Fig. 4.2). In contrast, in the control trial, lactate levels only increased immediately post-exercise ($p = 0.031$) before returning to pre-exercise levels after 5 min of recovery (Fig. 4.2). The responses of free fatty acid levels to the sprint and control trials were similar early in recovery; however these levels increased significantly between 90 ($p = 0.0005$) and 120 min ($p = 0.0005$) post-exercise in the sprint trial (Fig. 4.2).

4.4.2 Hormonal response

In response to the 10-s maximal sprint effort initiated immediately before moderate intensity exercise, norepinephrine ($p = 0.005$) reached maximal levels immediately after the sprint before gradually decreasing during the bout of moderate intensity exercise (Fig. 4.2). During recovery, epinephrine and norepinephrine returned to basal levels within 5 and 30 min, respectively. In contrast, epinephrine levels in the control trial remained relatively stable, whereas norepinephrine levels were only elevated immediately
following moderate intensity exercise ($p = 0.013$) and returned to basal levels within 5 min post-exercise (Fig. 4.2). The responses of growth hormone and cortisol in the sprint differed markedly from those of the catecholamines in that growth hormone levels did not change significantly and cortisol levels only increased marginally immediately after the sprint ($p = 0.026$; Fig. 4.2). Glucagon levels also did not change significantly in response to the sprint, but increased transiently ($P < 0.05$) at the onset of recovery from exercise in the control trial (Fig. 4.2). Finally, insulin levels before and after exercise did not change significantly in both trials (Fig. 4.2). It is noteworthy that both trials were performed at a time when plasma insulin levels were elevated (111 ± 10 and 106 ± 8 min in the sprint and control trials, respectively, post-insulin administration).
Fig. 4.1 Effect of a 10 sec sprint on blood glucose levels during and after moderate-intensity exercise. Black box, sprint; hatched box, moderate-intensity exercise; closed circles, sprint trial; open circles, control trial. The moderate-intensity exercise was commenced at the -20 min time-point. Blood glucose levels are expressed relative to those immediately after moderate-intensity exercise (time-point = zero). All values are shown as mean±SEM. \( ^b p < 0.05 \) compared to the 0 min time point after moderate-intensity exercise in the control trial; \( ^c p < 0.05 \) compared to the 0 min time point after moderate-intensity exercise in the sprint trial.
**Fig. 4.2** Effect of a 10-sec sprint on the levels of lactate (A), free fatty acids (B), norepinephrine (C), epinephrine (D), growth hormone (E), cortisol (F), glucagon (G) and free insulin (H) during and after moderate-intensity exercise. Black boxes, sprint; hatched boxes, moderate-intensity exercise; closed circles, sprint trial; open circles, control trial. All values are shown as mean ± SEM.  

\(^a\) p < 0.05 compared with control;  

\(^b\) p < 0.05 compared to the 0 min time point after moderate-intensity exercise in the control trial;  

\(^c\) p < 0.05 compared to the 0 min time point after moderate-intensity exercise in the sprint trial.
4.5 Discussion

It is well established that exercise of moderate intensity increases the risk of hypoglycaemia both during and after exercise in type 1 diabetes (MacDonald, 1987; Tuominen et al., 1995; Riddell et al., 1999; Rabasa-Lhoret et al., 2001). However, in Chapter 3 we reported that a single all-out 10-s sprint performed immediately after moderate intensity exercise provides a simple means to oppose a decrease in glycaemia during recovery (Bussau et al., 2006). Here we show that although a 10-sec sprint initiated immediately before moderate-intensity exercise did not affect the rapid decline in glycaemia during a subsequent bout of moderate-intensity exercise performed when plasma insulin levels were elevated, it did prevent the fall in glycaemia for at least the first 45 min of recovery (Fig. 4.1). This suggests that including a short sprint as part of the preparatory routine of individuals with type 1 diabetes before they engage in sustained moderate-intensity exercise might provide another means of temporarily stabilising glycaemia during early recovery.

The marked rise in norepinephrine and lactate levels immediately after exercise in the sprint trial might explain, in part, how sprinting before moderate-intensity exercise delays the fall in glycaemia early into recovery. Norepinephrine levels during early recovery were elevated in the sprint trial while blood glucose levels were stable, but soon after the return of norepinephrine to basal pre-exercise levels, blood glucose levels in the sprint and control trials fell at comparable rates (Fig. 4.2). Although high catecholamine levels have been generally acknowledged to counter insulin-mediated falls in glycaemia (Marliss and Vranic, 2002) via activation of hepatic glucose production and inhibition of insulin-mediated glucose uptake in skeletal muscles (Nonogaki, 2000), their role as counter-regulatory hormones during exercise in type 1 diabetic individuals has been a controversial issue (Coker and Kjaer, 2005). Elevated lactate levels (Fig. 4.2) are another factor that may have contributed to the stabilisation of glycaemia early in recovery by providing gluconeogenic precursors for hepatic glucose production and by increasing peripheral insulin resistance (Vettor et al., 1997). The above interpretation is challenged, at least in part, by recent findings from this laboratory that sprinting for 10 sec has no effect on hepatic glucose production rate but decreases peripheral glucose utilisation rate despite causing a rise in plasma catecholamines and lactate levels comparable to those measured here (Fahey et al., 2012).
The patterns of change in the levels of insulin, cortisol, glucagon and growth hormones in the sprint and control trials suggest that these hormones are unlikely to explain the delayed fall in glycaemia during recovery in the sprint trial. Insulin levels in both trials did not change significantly during recovery (Fig. 4.2). Also, the levels of glucagon did not increase significantly during and after exercise in the sprint trial. The question of whether portal glucagon levels responded in a similar manner remains to be established (Fig. 4.2). Although there is some published evidence that a rise in cortisol levels may play a role in stabilising glycaemia due to its potential acute inhibitory effect on glucose utilisation in skeletal muscles (Shamoon et al., 1980), this hormone is likely to play only a minor role here not only because of the absence of changes in the levels of this hormone during recovery in both trials, but also because the effects of cortisol on hepatic glucose production and blood glucose levels require several hours to take place (McMahon et al., 1988). Finally, growth hormones are also unlikely to play a role as no significant changes in growth hormone levels were observed in either of the groups (Fig. 4.2). However, a close inspection of Fig. 4.2e suggests that significant changes in growth hormone levels might have been masked by the large inter-individual variability in growth hormone levels. Nevertheless, even if growth hormone levels had increased significantly, this would probably have been of lesser importance because it has been reported that (1) administration of a growth hormone pulse does not affect peripheral glucose uptake in insulin-treated individuals with type 1 diabetes (Møller et al., 1992a); and (2) the infusion of octreotide, an inhibitor of growth hormone release, has no acute effects on the magnitude of the hyperglycaemic effect of high-intensity exercise (Sigal et al., 1996).

There is evidence that the aforementioned factors proposed to explain the stabilising effects of a short sprint on post-exercise glycaemia might be similar, irrespective of whether sprinting is performed before, during or after moderate-intensity exercise. Indeed, elevated lactate and catecholamines levels have also been proposed to stabilise glycaemia at the onset of recovery from moderate-intensity exercise interspersed either with several short sprints (Guelfi et al., 2005b) or followed by a 10-sec sprint (Chapter 3; Bussau et al., 2006). It is less clear, however, whether GH is also involved under these conditions because of a lack of consistencies across studies. Clearly, more research is required to identify the factors responsible for the sprint-mediated stabilisation of glycaemia after exercise.
On practical and clinical grounds, our findings suggest that before engaging in moderate-intensity exercise, individuals with type 1 diabetes might consider incorporating into their warm-up routine a short 10-sec maximal sprint effort, especially as a 10-sec sprint is well tolerated (Chapter 3; Bussau et al., 2006). However, before recommending the use of sprinting to prevent an early post-exercise fall in glycaemia, a number of issues must be addressed such as the extent to which the efficacy of prior sprinting is affected by the intensity and duration of the subsequent bout of exercise and the identification of any subpopulations of type 1 diabetic individuals unlikely to benefit from sprinting, such as very young children and older individuals who have limited capacity to engage in a maximal sprint effort.

In conclusion, our findings show that sprinting before a bout of exercise of moderate intensity could be a novel tool of clinical importance for preventing the fall in glycaemia during early recovery in young healthy individuals with type 1 diabetes. These findings provide one of the very few examples (Chapter 3; Guelfi et al., 2005b, 2005c; Bussau et al., 2006) of exercise offering an immediate short-term clinical benefit (delayed fall in glycaemia).

4.6 Acknowledgements

This research was funded jointly by a Juvenile Diabetes Research Foundation (JDRF)/National Health Medical Research Council of Australia program grant to T. W. Jones and P. A. Fournier. L. D. Ferreira is supported by a JDRF Fellowship. The authors acknowledge the technical assistance of L. Youngs and A. Thompson.
Chapter 5

Counterregulatory Response to a 10-second Sprint in Individuals with Type 1 Diabetes Mellitus
5.1 Abstract

**Objective:** A 10-sec sprint performed before or after moderate intensity exercise reduces the risk of post-exercise hypoglycaemia in hyperinsulinaemic fed individuals with type 1 diabetes mellitus. Since the glucoregulatory response to such a short sprint in these individuals has never been examined, this study investigates the response of the counterregulatory hormones to a 10-sec sprint in individuals with type 1 diabetes under hyperinsulinaemic fed conditions.

**Research Design and Methods:** Seven complication-free male individuals with type 1 diabetes (HbA1c = 7.0 ± 0.3%) were recruited to the study. On the morning of testing, the participants followed their normal insulin regimen and ate their usual breakfast. Blood glucose levels were determined regularly and allowed to fall to approximately 6.0 mM. Then, participants pedalled for 10 sec at maximal intensity on a cycle ergometer.

**Results:** Immediately after sprinting, plasma norepinephrine and epinephrine levels rose significantly (p < 0.05) to maximal levels (7.3 ± 1.0 and 0.66 ± 0.09 nmol/L, respectively, p < 0.05). Growth hormone increased to maximal levels (33.1 ± 6.7 mIU/L) 30 min after sprinting (p < 0.05), and remained elevated for the whole duration of the recovery period. Glucagon levels remained at stable levels. Similarly, plasma insulin and cortisol levels did not change significantly. Before sprinting, glycaemia fell significantly and remained stable during recovery.

**Conclusions:** The response of the counterregulatory hormones to a 10-sec sprint in type 1 diabetic individuals in a moderate hyperinsulinaemic and fed state is characterised mainly by marked changes in catecholamines and growth hormone levels.
5.2 Introduction

Individuals with type 1 diabetes mellitus are encouraged to participate in regular physical activity due to the numerous physiological and psychological health benefits associated with an active lifestyle (Norris et al., 1990; Moy et al., 1993; Laaksonen et al., 2000; Zinman et al., 2004; Riddell and Iscoe, 2006; Chimen et al., 2012, Tonoli et al., 2012). Unfortunately, the risk of hypoglycaemia is increased both during exercise (Riddell et al., 1999; Tuominen et al., 1995; Rabasa-Lhoret et al., 2001) and for several hours during recovery (MacDonald, 1987a; Tsalikian et al., 2005; McMahon et al., 2007; Maran et al., 2010; Iscoe and Riddell, 2011; Davey et al., 2013a). Due to this increased risk of hypoglycaemia, it is not surprising that fear of hypoglycaemia is a major barrier to regular physical activity in individuals with type 1 diabetes (Ludvigsson et al., 1980; Guelfi et al., 2007c; Brazeau et al., 2008).

As mentioned in Chapter 1, not all types of exercise increase the risk of hypoglycaemia. Several studies have reported that 10-15 min of high-intensity aerobic exercise (>80% of \( \text{VO}_2\text{peak} \)) under basal insulinaemic conditions is accompanied by an increase in blood glucose levels during and after exercise (Mitchell et al., 1988; Marliss et al., 1992a; Purdon et al., 1993; Sigal et al., 1994c, 1999; Marliss and Vranic, 2002). This is also the case in response to a 10-sec maximal sprint effort (Fahey et al., 2012). This glycaemia-rising effect of aerobic short sprint is such that, in theory, this type of exercise might be beneficial if adopted to prevent or delay hypoglycaemia when no carbohydrate is readily available to exercising individuals with type 1 diabetes.

As indicated in Chapter 3 and 4, when blood glucose level in individuals with type 1 diabetes is decreasing rapidly during moderate intensity exercise, engaging in a maximal 10-sec sprint immediately before or after exercise causes blood glucose levels after exercise to stabilise for 0.5-2 hours, thus decreasing the early risk of hypoglycaemia post-exercise (Chapters 3 and 4; Bussau et al., 2006, 2007). It is noteworthy that sprinting in these studies was beneficial despite elevated plasma insulin levels, a time when physical activity is generally not recommended due to the increased risk of experiencing hypoglycaemia (Rabasa-Lhoret et al., 2001).

In an attempt to identify the endocrine mechanisms likely to mediate the glucoregulatory benefits of sprinting, the levels of several counterregulatory hormones were measured in
the aforementioned studies (Chapters 3 and 4; Bussau et al., 2006, 2007), with our findings suggesting that a post-exercise increase in catecholamines and growth hormone levels might contribute to the protective effect of sprinting in type 1 diabetic individuals (Chapters 3 and 4; Bussau et al., 2006, 2007). However, one difficulty with comparing these findings with the literature has been the lack of information on the effect of short-duration sprinting per se on the levels of counterregulatory hormones in hyperinsulinaemic diabetic individuals in a fed state. Indeed, only one study has examined the effect of a 10-sec sprint on the levels of these hormones in individuals with type 1 diabetes (Fahey et al., 2012). However, this study was performed in overnight fasted individuals and under basal insulinaemic conditions, thus leaving unanswered the question of the effect of sprinting on the counterregulatory hormones responses to short-duration sprinting under combined hyperinsulinaemic and fed conditions. Given the clinical benefits of a 10-sec sprint for the prevention of hypoglycaemia in hyperinsulinaemic fed individuals with type 1 diabetes, and the lack of information about the counterregulatory response to this type of exercise, the purpose of this study was to investigate the effect of a single 10-sec sprint on the levels of the counterregulatory hormones under dietary and insulinaemic conditions mimicking those where sprinting has been shown to be beneficial to individuals with type 1 diabetes. Given that our studies on the benefits of sprinting were performed in hyperinsulinaemic fed individuals (Chapters 3 and 4; Bussau et al., 2006, 2007), this was also the physiological state examined here. The information thus obtained should provide the basis for future studies aimed at elucidating the mechanisms underlying the clinical benefits of sprinting in the prevention of hypoglycaemia in type 1 diabetic individuals.
5.3 Methods

5.3.1 Participants
Seven young males with type 1 diabetes (aged 17.7 ± 0.5 years; BMI 25.9 ± 4.1 kg/m²; \( \dot{V}O_2 \text{peak} \) 43.5 ± 4.8 ml/kg/min; duration of diabetes 5.8 ± 1.8 years) were recruited from Princess Margaret Hospital and the University of Western Australia, respectively. All participants were in moderate glycaemic control (Hb A1c = 7.0 ± 0.3%), free from diabetic complications, were hypoglycaemia aware, had undetectable levels of c-peptide, and were not taking any prescribed medication other than insulin. Moreover, these participants were on a multiple-injection insulin regime that had not changed for at least three months prior to the study. All participants were subjected to a familiarisation session during which their informed consent was obtained as well as their height, body mass and maximal rate of oxygen consumption as described previously (Fairchild et al., 2002a). Then, each participant was required to attend our laboratory on two occasions. Both the University of Western Australia and the Princess Margaret Hospital Ethics Committees approved the procedures described in this study.

5.3.2 Experimental trials
Participants were not allowed to exercise for 48 h prior to the experimental trial since antecedent exercise has the potential to affect the endocrine response to exercise (Galassetti et al., 2001b). Also, testing was rescheduled if they had experienced a hypoglycaemic episode over the previous 48 h because prior hypoglycaemia can also affect the counterregulatory response to exercise (Davis et al., 2000d; Galassetti et al., 2003). Each participant was also required to maintain their normal diet and to avoid alcohol for 24 h prior to testing. On the morning of testing, all participants were required to self-monitor their blood glucose levels regularly before attending the laboratory at ~07.30 h where they were instructed to self-administer their usual morning dose of insulin into the abdomen (mean dose 34.9 ± 10.2 units). Then, they ingested their breakfast as described in Chapters 3 and 4 (1933 ± 287 kJ total energy; 62 ± 3% carbohydrate; 16 ± 1% protein; 22 ± 4% fat), and a catheter was inserted for blood sampling. Following breakfast and when glycaemia started falling, blood glucose levels were measured every 15 min. Then, a 10-sec maximal sprint effort was initiated on a Repco front access cycle ergometer (Repco, Sydney, Australia) when blood glucose levels reached approximately 6.0 mM. The participants were then instructed to cycle as hard as possible for 10 sec and not to pace themselves for the whole duration of the sprint. Blood was sampled prior to
exercise and then at 0, 5, 10, 15, 30, 45 and 60 min post-sprint. At each sampling point, 0.2 ml of blood was first removed for the measurement of blood glucose, pO$_2$, pH and lactate. A second sample of 15 ml was also removed for the measurement of hormones. Some of the blood was combined with sodium metabisulphite, poly-ethylene glycol or trasylol for the assays of catecholamines, insulin and glucagon, respectively. All samples were then centrifuged at 720g for 5 min and the plasma stored at -80°C for later analysis.

5.3.3 Hormones and metabolite assays
Glucose, lactate, pH and pO$_2$ were analysed using an ABL 625 blood gas system (Radiometer, Copenhagen, Denmark). Free fatty acids levels were measured using the Roche half micro test free fatty acids assay kit (Roche Diagnostic, Germany). All hormones were assayed as described previously (Chapters 3 and 4; Bussau et al., 2006, 2007). Plasma catecholamine levels were determined by reverse phase HPLC using a Waters Novapak C18 reverse phase column and a model 5200A Coulochem detector (ESA Inc, USA). Free insulin levels were measured by radioimmunoassay using the Phadeseph insulin RIA kit (Pharmacia, Uppsala, Sweden). Glucagon levels were measured by radioimmunoassay using a Linco glucagon RIA kit (Linco Research, St Charles, Missouri, USA). Cortisol levels were assayed by competitive immunoassay on an Immulite 2000 analyser using the Immulite cortisol assay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Growth hormone (GH) levels were determined by immunometric assay on an Immulite 2000 analyser using the Immulite growth hormone assay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). 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 Corporation, Los Angeles, CA, USA).

5.3.4 Statistical analyses
The results were analysed using a one-way repeated measure analysis of variance and Fisher LSD test for a posteriori comparisons. Statistical significance was accepted at $p < 0.05$ and all analyses were carried out using SPSS 17.0 software. Participants’ characteristics are expressed as means ± S.D whereas all other results are expressed as means ± S.E.M.
5.4 Results

5.4.1 Hormonal response to a 10-sec sprint
In response to sprinting, plasma norepinephrine levels rose significantly (p < 0.05) and reached maximal levels at the onset of recovery (7.3 ± 1.0 nM) before returning to pre-exercise levels within 10 min after exercise (Fig. 5.1). Similarly, plasma epinephrine reached maximal levels at the onset of recovery (0.66 ± 0.09 nM, p < 0.05) and remained elevated above pre-sprint levels during the first 10 min of recovery before returning to pre-sprint levels (Fig. 5.1). Plasma growth hormone levels increased progressively during recovery from sprinting to reach maximal levels of 33.1 ± 6.7 mIU/L after 30 min of recovery (p < 0.05; Fig 5.1), with these levels still remaining above pre-sprint levels 60 min after exercise. In response to sprinting, plasma glucagon, cortisol and insulin levels did not change significantly (p > 0.05; Fig. 5.1). However, it must be noted that there was a non-significant trend for cortisol levels to increase and reach maximal levels after 30 min of recovery.
Figure 5.1: Effect of a single 10-sec sprint on the levels of norepinephrine (A), epinephrine (B), growth hormone (C), cortisol (D), free insulin (E), and glucagon (F) in participants with type 1 diabetes. All graphs are shown as mean ± standard error. The sprint is represented by the black line. * represents a statistically significant difference (p < 0.05) compared to the rest time point in the type 1 diabetic participants.
5.4.2 Blood metabolite response to a 10-sec sprint

In response to sprinting, blood lactate levels reached maximal levels after 5 min of recovery (p < 0.05; Fig. 5.2) before decreasing to pre-sprint levels within 60 min after exercise. Blood pH fell to minimal levels at 5 min after sprinting (p < 0.05; Fig. 5.2) before returning to pre-sprint levels within 60 min of recovery. Free fatty acid levels increased progressively after exercise to reach maximal levels after 60 min of recovery (p < 0.05; Fig. 5.2). Before sprinting, blood glucose levels fell significantly (p < 0.05; Fig. 5.2) and remained stable for 60 min after sprinting.

5.4.3 Work load and peak power associated with a 10-sec sprint

The total work performed during the 10-sec sprint was 82.8 ± 8.4 kJ/kg and the peak power achieved was 10.7 ± 0.9 W/kg.
Figure 5.2: Effect of a single 10-sec sprint on blood glucose (A), free fatty acids (B), pH (C) and blood lactate (D) levels in participants with type 1 diabetes. The sprint is represented by the black line. All results are shown as mean ± standard error. Black circles refer to the type 1 diabetic participants. \textsuperscript{a} represents a statistically significant difference (p < 0.05) compared to the rest time point in the type 1 diabetic participants.
5.5 Discussion

In chapters 3 and 4, we showed that even when plasma insulin levels are elevated, a 10-sec sprint performed either immediately before or after moderate intensity exercise can prevent blood glucose levels from falling after exercise (Bussau et al., 2006, 2007), thus providing a novel clinical tool to acutely decrease the risk of post-exercise hypoglycaemia. Although, the combined effects of sprinting and moderate intensity exercise on counterregulatory hormones levels were examined in these studies (Chapters 3 and 4; Bussau et al., 2006, 2007), surprisingly the responses of these hormones to sprinting *per se* in type 1 diabetic individuals who are fed and under hyperinsulinaemic conditions has never been investigated before, thus making it impossible to compare our earlier findings with those of others. Here, for the first time, the effect of short-duration sprinting *per se* on the levels of several counterregulatory hormones was examined in hyperinsulinaemic fed individuals with type 1 diabetes. We found that a 10-sec maximal sprint in those individuals resulted in marked changes in plasma catecholamines and growth hormone levels, with no significant effect on plasma cortisol, insulin and glucagon levels. In addition, the responses of growth hormones and cortisol levels differed from those observed when a sprint is performed immediately before or after a bout of moderate intensity exercise in individuals with type 1 diabetes (Chapters 3 and 4; Bussau et al., 2006, 2007).

Our findings show that a 10-sec sprint results in an early rise in plasma epinephrine and norepinephrine levels in hyperinsulinaemic fed individuals with type 1 diabetes. The magnitude of this rise, however, was submaximal and comparable to that observed when a 10-sec sprint is performed immediately before or after a bout of moderate intensity exercise under hyperinsulinaemic fed conditions (Chapters 3 and 4; Bussau et al., 2006, 2007). Only one other study, published from this laboratory, has investigated the effect of a 10-sec sprint *per se* on plasma catecholamines levels in type 1 diabetic individuals and also reported an increase in the levels of these hormones, but all participants were in an overnight fasted state and under basal insulinaemic conditions (Fahey et al., 2012). Interestingly, the rise in catecholamines levels reported here was more pronounced than those reported by others in non-diabetic individuals subjected to a 6-sec sprint (Moussa et al., 2003; Botcazou et al., 2007; Bracken et al., 2009), but generally of a lesser magnitude compared to longer duration sprinting (Brooks et al., 1988; Nevill et al., 1989;
Langfort et al., 1997; Zouhal et al., 1998, 2001; Jacob et al., 2002; Moussa et al., 2003; Vincent et al., 2003; Jacob et al., 2004; Vincent et al., 2004; Zouhal et al., 2009).

The pattern of change in growth hormone levels in response to sprinting in hyperinsulinaemic type 1 diabetic individuals in the fed state differed in some respects from that when sprinting is performed immediately before or after a bout of moderate intensity exercise (Chapters 3 and 4; Bussau et al., 2006, 2007). Indeed, growth hormones levels remained elevated for longer in response to sprinting alone. Only one other study has investigated the effect of a 10-sec sprint per se on plasma growth hormones levels in type 1 diabetic individuals, and showed that the levels of this hormone increased post-sprinting (Fahey et al., 2012). However, as mentioned earlier, the participants involved in that study were in an overnight fasted state and under basal insulinaemic conditions (Fahey et al., 2012) rather than being in a hyperinsulinaemic fed state. It is noteworthy that sprinting for as little as 6 sec has been found to result in a small significant increase in growth hormone levels in non-diabetic individuals (Stokes et al., 2002), with the magnitude of growth hormone response increasing with sprint duration (Stokes et al., 2002). Other factors are also likely to affect the sprint-mediated increase in growth hormone levels. For instance, GH secretion increases in response to both high catecholamines levels and acidosis (Stokes, 2003), but is opposed by elevated plasma insulin (Lanzi et al., 1997; Frystyk, 2004) and fatty acids levels (Godfrey et al., 2003).

Although sprinting for 10 sec did not change significantly plasma cortisol levels, there was a trend towards a small increase after exercise. This trend is consistent with earlier work from our laboratory where sprinting performed immediately before or after a bout of moderate intensity exercise has been shown to result in a significant but small increase in cortisol levels in hyperinsulinemic fed individuals with type 1 diabetes (Chapters 3 and 4; Bussau et al., 2006, 2007). Only one other study has investigated the effect of a 10-sec sprint per se on plasma cortisol levels in type 1 diabetic individuals and shown that sprinting results in a small increase in plasma cortisol levels after exercise (Fahey et al., 2012). However, as mentioned earlier, the participants in that study were in an overnight fasted state and under basal insulinaemic conditions (Fahey et al., 2012) rather than being in a hyperinsulinaemic fed state.

The absence of any effect of a 10-sec sprint on plasma glucagon levels in hyperinsulinaemic fed participants with type 1 diabetes is consistent with earlier
observations from our laboratory that a 10-sec sprint performed immediately before or after moderate intensity exercise has no effect on plasma glucagon levels (Chapters 3 and 4; Bussau et al., 2006, 2007). In the absence of any change in plasma insulin levels, our findings also suggest that the plasma glucagon/insulin ratio is not affected by a sprint of short duration. The main limitation with this interpretation, however, is that it assumes that portal glucagon and insulin levels follow patterns of change similar to those of peripheral blood glucose and insulin levels. The only other study that has examined the effect of a 10-sec sprint on plasma glucagon levels has also reported no changes in plasma glucagon levels, but all participants were in an overnight fasted state and under basal insulinaemic conditions (Fahey et al., 2012). Since catecholamines and insulin stimulate and inhibit glucagon secretion (Ahren, 2000; Gromada et al., 2007) respectively, maybe the rise in epinephrine levels was not sufficient to stimulate glucagon release. 

The post-exercise rises in plasma lactate and free fatty acids levels in response to sprinting in hyperinsulinaemic type 1 diabetic individuals in the fed state were similar to those observed when a 10-sec sprint is performed immediately before or after a bout of moderate intensity exercise (Chapters 3 and 4; Bussau et al., 2006, 2007). These findings are also comparable to those obtained in a study examining the effect of a 10-sec sprint on plasma lactate and free fatty acid levels, but all participants were in an overnight fasted state and under basal insulinaemic conditions in that study (Fahey et al., 2012). Finally, others have reported similar plasma lactate levels in response to a 6- or 10-sec sprint in non-diabetic individuals (Zajac et al., 1999; Moussa et al., 2003). Plasma pH, another potential glucoregulatory variable with the capacity to affect hepatic glucose production and muscle glucose transport (Kashiwagura et al., 1985; Kristiansen et al., 1994) fell in response to the 10-sec sprint, consistent with the decrease in blood pH that occur in non-diabetic individuals after a sprint lasting 6 sec or more (Allop et al., 1990; Bogdanis, 1995; Bogdanis and Nevill, 1996; Stokes et al., 2002, 2005).

It is important to note that the comparison made here between the findings of this study and those obtained in participants were sprinting was performed before or after moderate intensity exercise (Chapters 3 and 4; Bussau et al., 2006, 2007) should be performed with caution. Although the work load was well matched between these studies, as indicated by their similar total work and peak power, and blood glucose levels were falling, it is important to note that pre-sprint plasma insulin levels were not fully matched at the time of the sprint. Indeed, the hyperinsulinaemic state of our participants in this study was not
as marked as in those other studies. Since the release of growth hormone and glucagon are opposed by elevated plasma insulin levels (Lanzi et al., 1997; Ahren, 2000; Frystyk, 2004; Gromada et al., 2007), it is possible that a less marked increase in plasma growth hormone level would have occurred under more pronounced hyperinsulinaemic conditions.

In conclusion, this study is the first one to examine the counterregulatory responses to a maximal sprint effort of short duration in fed type 1 diabetic individuals under hyperinsulinaemic conditions. What remains to be established in future studies is the extent to which the changes in the levels of each of the counterregulatory hormones examined here affects blood glucose levels, hepatic glucose production and peripheral glucose utilisation in response to sprinting. Overall, this study provides the basis for more research to understand better the mechanisms underlying the clinical benefits of sprinting in hypoglycaemia prevention.

5.6 Acknowledgements

This research was funded by a Juvenile Diabetes Research Foundation/National Health Medical Research Council of Australia program grant to T. Jones and P.A. Fournier. L. D. Ferreira was supported by a Juvenile Diabetes Research Foundation International Fellowship.
Chapter 6

General Discussion
6.1 General Discussion

Maintaining blood glucose levels within physiological range is a constant challenge for individuals with type 1 diabetes. As a result of the lack of an acute response of plasma insulin levels to changes in glycaemia, blood glucose levels are usually managed by combining daily exogenous insulin administration, carbohydrate intake, and regular glucose monitoring. Unfortunately, despite the numerous physiological and psychological health benefits of exercise (Norris et al., 1990; Moy et al., 1993; Laaksonen et al., 2000; Zinman et al., 2004; Riddell and Iscoe, 2006; Chimen et al., 2012), physical activity can make the acute management of blood glucose levels even more difficult for individuals with type 1 diabetes (Riddell and Perkins, 2006; Guelfi et al., 2007c; Younk and Davis, 2012). In fact, the risk of hypoglycaemia is increased both during (Tuominen et al., 1995; Riddell et al., 1999; Rabasa-Lhoret et al., 2001) and after exercise (MacDonald, 1987a; Tsalikian et al., 2005; McMahon et al., 2007; Maran et al., 2010; Iscoe and Riddell, 2011; Davey et al., 2013). Consequently, it is not surprising that the biggest barrier to regular physical activity in individuals with type 1 diabetes is fear of hypoglycaemia (Brazeau et al., 2008). As a result, many people with type 1 diabetes avoid physical activity (Ludvigsson et al., 1980; Fremion et al., 1987; Guelfi et al., 2007c; Brazeau et al., 2008) with ~60-65% of individuals with type 1 diabetes found to be inactive (Thomas et al., 2004; Plotnikoff et al., 2006). It is also concerning that young individuals are discouraged from performing vigorous physical activity by physicians, school staff or parents due to their fear of exercise-induced hypoglycaemia (Fremion et al., 1987).

As discussed at length in Chapter 1 of this thesis, not all types of exercise result in an elevated risk of hypoglycaemia. Aerobic exercise performed at high intensity (>80% of \( \dot{V}\text{O}_2\text{max} \)) as well as sprinting or intermittent high intensity exercise are all associated with an increase in glycaemia during and after exercise. This raises the intriguing possibility that these types of exercise might be beneficial if adopted to counter a fall in glycaemia in complication-free individuals with type 1 diabetes, and thus might help to prevent or delay hypoglycaemia if no carbohydrate is readily available. The problem here is that adopting such a strategy to prevent hypoglycaemia is unlikely to be well tolerated by most individuals with type 1 diabetes due to the very intense nature and impractical duration of these types of exercise. This raises the primary aim at the core of this thesis which was to determine whether a much shorter bout of exercise performed at maximal intensity...
could be adopted to prevent glycaemia from falling. Since a single maximal intensity sprint effort lasting 30-sec or more is associated with undesirable physiological consequences such as nausea, vomiting and dizziness (Inbar et al., 1996; 1998; Laurent et al., 2007; Stickley et al., 2008; Little et al., 2010), we undertook to explore the glucoregulatory benefits of a maximal sprint effort lasting only 10 sec. For this reason, the primary goal of this thesis was to determine whether a 10-sec maximal sprint effort performed after (Chapter 3) or before (Chapter 4) moderate intensity exercise provides a possible means other than carbohydrate intake to prevent glycaemia from falling when exercise is performed under hyperinsulinaemic conditions by complication-free individuals with type 1 diabetes, thus decreasing acutely their risk of hypoglycaemia.

Also, given that for this type of study, it is common practice to subject participants to a graded exercise test to determine their \( \dot{V}O_2 \text{peak} \) not only to determine their aerobic fitness, but also to set exercise intensity relative to \( \dot{V}O_2 \text{peak} \), a secondary objective of this thesis was to determine whether the risk of hypoglycaemia is increased early during recovery from this exercise protocol (Chapter 2). Finally, given that the counterregulatory response to sprinting has not been examined in hyperinsulinaemic fed individuals with type 1 diabetes, thus making it difficult to compare the findings of Chapters 3 and 4 with the literature, our last aim was to examine the counterregulatory responses to sprinting in type 1 diabetic individuals under hyperinsulinaemic conditions (Chapter 5).

Given that most laboratory-based studies on exercise in diabetes involve the performance of graded exercise for \( \dot{V}O_2 \text{peak} \) and lactate threshold determination, the primary aim of the study described in Chapter 2 was to determine whether the risk of hypoglycaemia increases in response to graded exercise in individuals with type 1 diabetes. For this study, eight non-diabetic control male participants and seven complication-free type 1 diabetic male individuals in good glycaemic control were recruited. On the morning of testing, the type 1 diabetic participants followed their normal insulin regimen, and both groups ate their usual breakfast. Then, participants were subjected to graded exercise testing approximately four hours later. We found that this type of exercise result in a rapid post-exercise increase in blood glucose levels (> 2 mM), which remain elevated for the first two hours of recovery. On clinical grounds, these findings suggest for the first time that the early post-exercise risks of hypoglycaemia associated with graded exercise testing are minimal when performed under near basal plasma insulin levels, with no carbohydrate administration required after testing to prevent hypoglycaemia. However, it is important
to stress that the risk of late onset post-exercise hypoglycaemia associated with this type of exercise was not examined and should be investigated in future studies. In addition, it is our view that health professionals should still regularly monitor glycaemia before, during and after testing since it remains to be established whether similar findings would have been obtained with different graded exercise protocols and individuals with different circulating insulin levels.

The primary goal of the study described in Chapter 3 was to determine whether a short 10-sec maximal sprint effort is preferable to only resting as a means to counter a further fall in glycaemia during recovery from moderate intensity exercise in hyperinsulinaemic individuals with type 1 diabetes, thus providing another clinical tool for the prevention of early post-exercise hypoglycaemia. To meet our objective, seven healthy complication-free male participants with type 1 diabetes injected their normal insulin dose and ate their usual breakfast. Then, when their postprandial glycaemia fell to ~11 mM they pedalled at 40% VO$_2$peak for 20 min on a cycle ergometer followed immediately by either a maximal 10-sec sprint or a rest. Our results show that during exercise blood glucose levels fell rapidly. However, sprinting immediately after exercise opposes a further fall in blood glucose levels for at least 120 min while glycaemia decreases significantly (p < 0.05) by ~3.5mM when no sprint was performed. Our findings also suggest that sprinting is likely to counter the exercise-mediated decrease in blood glucose levels through an increase in catecholamine, lactate, and growth hormone levels. These glucoregulatory benefits of sprinting are remarkable considering the sprint trial was performed when insulin levels were elevated, a time when exercise is not usually recommended. On the basis of these findings, one might tentatively recommend that in order to minimise the risk of early hypoglycaemia post-moderate intensity exercise, it is preferable for complication-free young individuals with type 1 diabetes to engage in a 10-sec maximal sprint effort before resting than to only rest during recovery, particularly if a source of dietary carbohydrate is not readily available. It is important to stress, however, that our findings do not exclude the possibility that different results might have been obtained if sprinting had been initiated after a bout of exercise of higher intensity or longer duration, in younger or older individuals with reduced sprinting capacity, or in individuals with impaired counterregulatory responses. For these reasons, more studies of the kind described here are required in order to identify the conditions and subpopulation of individuals with type 1 diabetes for whom a short maximal sprint effort can be recommended as a safe approach for the short-term stabilisation of blood glucose levels post-exercise.
Given the glycaemia stabilising effect of sprinting performed after moderate-intensity exercise (Chapter 3), this raises the issue examined in the study described in Chapter 4 of whether performing a short sprint effort immediately prior to, rather than after, moderate-intensity exercise may offer a novel way of preventing the rapid fall in glycaemia normally observed both during and after moderate-intensity exercise performed under hyperinsulinaemic conditions. In other words, this study examined whether short-duration sprinting should be an integral part of the preparatory routine of individuals with type 1 diabetes before they engage in sustained physical activities of moderate intensity. To this end, seven complication-free type 1 diabetic males injected their normal morning insulin dose and ate their usual breakfast. When post-meal glycaemia fell to ~11 mM, they were asked to perform a 10 sec all-out sprint (sprint trial) or to rest (control trial) immediately before cycling at 40% of peak rate of oxygen consumption for 20 min. We found, against expectations, that sprinting for 10 sec immediately before moderate-intensity exercise performed under hyperinsulinaemic conditions does not affect the rapid decline in glycaemia during exercise. However, sprinting rather than resting before moderate intensity exercise did prevent glycaemia from falling for at least the first 45 min of recovery in individuals with type 1 diabetes. This suggests that including a short sprint as part of the warm-up routine of individuals with type 1 diabetes before they engage in sustained moderate-intensity exercise might provide another means of temporarily stabilising glycaemia during early recovery. However, before recommending such a use of sprinting, a number of issues must be addressed such as the extent to which the efficacy of prior sprinting is affected by the intensity and duration of the subsequent bout of exercise and the identification of any subpopulations of type 1 diabetic individuals unlikely to benefit from sprinting.

In our attempt to identify the endocrine mechanisms mediating the glucoregulatory benefits of sprinting, the levels of several counterregulatory hormones were measured in the studies described in Chapters 3 and 4. Unfortunately, one difficulty with comparing these findings with the literature has been the lack of information on the effect of short-duration sprinting per se on the responses of counterregulatory hormones in diabetic individuals in a hyperinsulinaemic fed state. For this reason, the purpose of the study described in Chapter 5 was to investigate the effect of a single 10-sec sprint on the levels of the counterregulatory hormones in type 1 diabetic individuals under hyperinsulinaemic fed conditions approaching those reported in Studies 3 and 4. In this final study, we found
that performing a 10-sec maximal sprint resulted in patterns of change in plasma catecholamines, growth hormone, cortisol and glucagon levels comparable to those observed when a sprint is performed immediately after a bout of moderate intensity exercise in individuals with type 1 diabetes (Study 2) and also comparable to those observed in response to a sprint performed after an overnight fast (Fahey et al., 2012). What remains to be established in future studies is the extent to which the changes in the levels of each of the counterregulatory hormones examined here affects blood glucose levels, hepatic glucose production and peripheral glucose utilisation in response to sprinting. Overall, this final study provides the basis for more research to understand better the mechanisms underlying the clinical benefits of sprinting in hypoglycaemia prevention.

### 6.2 Clinical Implications, Limitations with our Findings and Direction for Future Studies

The counter-intuitive findings described in this thesis that the risk of hypoglycaemia associated with exercise can be decreased by sprinting before or after moderate intensity exercise have the potential to cause a significant shift in the way blood glucose levels are managed in active individuals with type 1 diabetes. Some authors have mentioned the potential of incorporating sprints to help oppose an exercise-mediated decline in glycaemia in young healthy adults with type 1 diabetes (Tonoli et al., 2012; Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2013; Robertson et al., 2014; Yardley & Sigal, 2015). It is important to stress, however, that before recommending the general adoption of short-duration sprinting as a safe and reliable clinical tool for the short-term stabilisation of blood glucose levels in exercising type 1 diabetic individuals, a number of issues must be addressed as in our view it would be premature and irresponsible at this stage to advocate its widespread adoption. Indeed, it is of paramount importance to determine whether there are conditions likely to impair the efficacy of sprinting in hypoglycaemia prevention. One must remember that the findings arising from this thesis are based on data obtained from a very specific population of healthy complication-free young adults with type 1 diabetes, and for this reason may not apply to the wider population of individuals with type 1 diabetes. For instance, it would be ill advised to recommend the use of a short sprint as a means to reduce the risk of exercise-mediated hypoglycaemia for individuals with an impaired capacity to engage in a maximal sprint effort, such as very young children or old sedentary individuals with or
without pre-existing medical conditions, or for diabetic individuals with advanced neuropathy, or for individuals with diabetic complications for whom intense exercise is contra-indicated. But what about the large population of complication-free type 1 diabetic individuals who have the capacity to engage in an all-out sprint effort?

Even with the population of type 1 diabetic individuals who would be responsive to the benefits of sprinting, a number of important issues must also be addressed before recommending the adoption of short-duration sprint as a safe and reliable clinical tool for the short-term management of blood glucose levels. For instance, it remains to be determined whether our findings obtained on a cycle ergometer in a well-controlled laboratory environment would extend to all modes of all-out sprinting including running and swimming. It is also unclear whether performing an ‘all out’ sprint and reaching maximal power output early in the sprint is important as opposed to ‘pacing’ for the duration of the sprint. The effect of the fitness and training status of the participants on the glucoregulatory response to sprinting also remains to be investigated. Since the rate of fall in glycaemia during moderate intensity exercise increases with both exercise intensity and plasma insulin levels, it is possible that the efficacy of sprinting at preventing hypoglycaemia post-moderate intensity exercise might be reduced in response to more intense aerobic exercise or more severe hyperinsulinaemic conditions than those tested in this thesis. Given our findings that catecholamines and growth hormones might mediate the effect of sprinting on glycaemia (Chapters 3, 4), this raises the possibility that a defect in the secretion of these hormones could impair the ability of a sprint to counter glycaemia in exercising individuals. Moreover, it remains to be established whether the glucoregulatory benefits of sprinting increase with one’s maximal power output since the catecholamine response to exercise increases with exercise intensity. Finally, it would be interesting to investigate the effect of gender and phase of menstrual cycle on the glucoregulatory responses to a single 10-sec sprint.

Several questions related to the counterregulatory status of diabetic individuals prior to exercise must also be answered before recommending the adoption of short duration sprinting as a safe and reliable tool for the short-term management of blood glucose levels. For instance, the depletion of hepatic glycogen stores by a prolonged fast, and associated changes in counterregulatory hormone levels prior to exercise might impair the glucoregulatory benefit of sprinting. However, this prediction is not supported by the finding that intense exercise results in comparable hyperglycaemic effect in fed and
fasted non-diabetic individuals (Lavoie et al., 1987). Given that the counterregulatory response to exercise is reduced following antecedent hypoglycaemia (Galassetti et al., 2003, 2004) or antecedent exercise performed hours earlier (Galassetti et al., 2001a), this raises the intriguing possibility that the efficacy of sprinting as a means to prevent glycaemia from falling post-exercise might be impaired if preceded hours earlier by either an episode of hypoglycaemia or exercise. Such a potential detrimental effect of antecedent hypoglycaemia on the glycaemia-stabilising effect of sprinting might also be more pronounced in men than in women given that antecedent hypoglycaemia has been reported to result in a relatively greater fall in the responses of counterregulatory hormones (catecholamines, growth hormone, glucagon) and glucose production in men compared with women in response to a subsequent prolonged bout of moderate intensity exercise (Galassetti et al., 2004). However, the possibility that antecedent hypoglycaemia impairs the benefit of sprinting is not supported by a study recently published from this laboratory where a one-hour hypoglycaemia period was reported to have no effect on the glycaemia-rising effect and counterregulatory response of a sprint performed hours later (Davey et al., 2014).

Finally, another important clinical issue to address is whether sprinting impairs the counterregulatory responses to a subsequent hypoglycaemia episode, thus increasing the risk of late onset hypoglycaemia. That this might be an important issue is suggested by the work of Galassetti and colleagues who showed that the rise in catecholamines, growth hormone and cortisol levels in response to hypoglycaemia is impaired if preceded several hours earlier by a bout of moderate intensity exercise, with more glucose being required to prevent hypoglycaemia (Galassetti et al., 2001b; Sandoval et al., 2004). In addition, this impairment is more pronounced in male compared to female participants. However, given the short duration of the sprint investigated in this thesis and the small amount of carbohydrate expected to be mobilised by during such a sprint, we predict that the effect of sprinting on the counterregulatory responses to a subsequent episode of hypoglycaemia should be minimal. This prediction is supported by recent findings from this laboratory that a 10-sec sprint does not affect the glucose demands to maintain blood glucose at stable levels for several hours post-exercise (Davey et al., 2013b).

Overall, it is important to answer the aforementioned questions before recommending the adoption of short duration sprinting as a safe and reliable tool for the short-term management of blood glucose levels in individuals with type 1 diabetes as this clinical
tool has the potential to be integral to future glucose management guidelines aimed at helping diabetic individuals exercise more safely and take advantage of the many physiological and psychological health benefits of a physically active lifestyle.
Chapter 7

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