Effect of a Short Sprint on Glucose Production and Whole Body Glucose Utilisation in Adults with Type 1 Diabetes

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Abstract

Exercise in individuals with type 1 diabetes mellitus (T1DM) can increase the risk of hypoglycaemia. Recently, however, we showed that a 10-second maximal sprint effort performed after moderate intensity exercise in those individuals can prevent blood glucose levels from falling (*Bussau VA, Ferreira LD, Jones TW, Fournier PA. 2006. The 10-s maximal sprint-A novel approach to counter an exercise-mediated fall in glycemia in individuals with type 1 diabetes. Diabetes Care, 29, 601-601*). We hypothesised that this protective effect of sprinting results from a more pronounced post-sprinting rise in the rate of glucose appearance (Ra) compared to the rate of peripheral glucose disappearance (Rd). To test whether sprinting *per se* has such an effect on glucose Ra and Rd, eight T1DM participants completed a 10-second maximal sprint during an euinsulinaemic euglycaemic clamp and were compared to non-diabetic individuals. Immediately after the 10 second sprint, blood glucose levels increased by 1.04±0.58 mM ($p=0.001$) in the diabetic participants and rose transiently by in the non-diabetic group. During the 2 hour recovery, Ra in both groups did not change significantly ($p>0.05$). In contrast, Rd fell significantly by 1.33 $mg/ml^{-1}.kg^{-1}$ and 57% relative to Ra and pre-exercise Rd levels, respectively, before returning within 30 minutes to pre-exercise levels in the diabetic participants, and fell by 38% relative to Ra in the non-diabetic group. In response to sprinting, the levels of epinephrine and norepinephrine in the diabetic group rose by 8 and 4 fold, respectively, and 9.5 and 6 fold in the non-diabetic participants ($p<0.05$), with no significant changes in plasma insulin and glucagon levels. These findings show for the first time that a short sprint can increase glycaemia in T1DM individuals post-exercise and suggest that this results from a transient decline in the rate of peripheral glucose utilisation. These patterns of glucose Ra and Rd responses to exercise are unique and markedly different from those

associated with moderate or intense aerobic exercise. High catecholamines levels might contribute to the early fall in Rd post-exercise in diabetic individuals, but this interpretation must be reconciled with the finding that a similar pattern of change in the levels of these hormones is associated with little post-exercise changes in blood glucose levels, Ra and Rd in non-diabetic individuals. The mechanisms responsible for the unique results described here thus remain to be established.

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Statement of Candidate Contribution

The work involved in designing and conducting the studies described in this thesis has been carried out primarily by Avril Fahey (the candidate). The thesis outline and experimental design of the studies was developed and planned by the candidate in consultation with Professor Paul Fournier (the candidate's 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 analyses and original drafting of the thesis. Professor Paul Fournier has provided feedback for further drafts and completion of the thesis.

Signed:

Avril Fahey	Paul Fournier
(Candidate)	(Supervisor)

Chapter One

Introduction and Literature Review

1.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus is an endocrine disorder affecting 140,000 Australians and 16 million people worldwide, and its incidence is increasing with approximately 2000 new cases every year in Australia (JDRF, 2005) and an expected 40% increase in the incidence worldwide for the period 1998-2010 (Gale, 2002). At present, type 1 diabetes cannot be prevented, can occur at any age, and is believed to be caused by a combination of genetic and environmental factors (Campaigne & Lampman, 1994).

Type 1 diabetes is an autoimmune disease characterised by the progressive destruction of the insulin-producing cells of the pancreas by the body's own immune system, leading to an inability to secrete insulin (Notkins & Lernmark, 2001; Pierce, 1999). Since insulin stimulates glucose utilisation in muscle and adipose tissue and inhibits the hepatic production of glucose and ketone bodies (Rother *et al*., 2008), its absence leads to a large increase in blood glucose (hyperglycaemia) and ketone body levels. This causes symptoms including extreme thirst, frequent urination, nausea, vomiting, sudden and rapid weight loss, blurred vision, confusion, shortness of breath and extreme tiredness. Under extreme conditions, the accumulation of large amounts of ketone bodies combined with hyperglycaemia can lead to severe ketoacidosis, loss of consciousness, sepsis and coma. Prior to the discovery of insulin in the early 1920's, individuals with type 1 diabetes usually died from ketoacidosis or sepsis within 1 to 2 years from diagnosis (Campaigne & Lampman, 1994).

Since insulin deficiency in type 1 diabetes is usually absolute, this condition is treated by regular insulin administration which replaces the endogenous insulin that would normally

be produced by the pancreas. Insulin is delivered either by injection or constant subcutaneous infusion via a pump (Campaigne & Lampman, 1994; CDA, 2008, Chen *et al*., 2003; Laycock & Wise, 1996). There are several types of insulin available, each with different times of onset and duration of action. Although insulin can be obtained from animals (porcine or bovine), the insulin used in therapy is human insulin manufactured synthetically using recombinant DNA technology (Brange, 1997).

A major limitation of insulin therapy is that synthetic insulins do not display the same pharmacokinetic properties as human insulin in either children or adults with type 1 diabetes even with multiple daily injections or continuous subcutaneous insulin infusion (Lenhard & Reeves, 2001, Mortensen *et al*., 2000). In addition, the absence of the natural feedback mechanism whereby plasma insulin levels decrease as blood glucose levels fall results in insulin-treated individuals with type 1 diabetes being at an increased risk of hypoglycaemia. This condition occurs when blood glucose levels fall below normal levels (MacDonald, 1987), and is associated with symptoms including sweating, rapid heart rate, drowsiness, shaking, confusion, poor co-ordination, slurred speech, behaviour changes (including aggression, irritability or anxiety), dizziness, hunger and tingling lips (Colberg, 2001). If left untreated, the symptoms of hypoglycaemia can progress to loss of consciousness, fitting, irreversible brain damage and in rare circumstances, death (Cryer *et al*., 2003). On average, insulin-treated type 1 diabetic individuals experience 1-2 severe episodes of hypoglycaemia per year requiring the assistance of another individual, with 2 – 4% of deaths of people with type 1 diabetes attributable to hypoglycaemia (Cryer *et al*., 2003). In some circumstances, the symptoms of hypoglycaemia may not be detected by individuals with type 1 diabetes due to decreased hypoglycaemic awareness (Laycock &

Wise, 1996), and for these individuals, the risk and likelihood of severe hypoglycaemia are magnified (Cryer, 2008; Cryer *et al*., 2003).

Given the risk and outcomes of hypoglycaemia, it would seem safer for individuals with type 1 diabetes to keep their blood glucose at high levels. However, hyperglycaemia (blood glucose levels >8 mM) in the long-term increases the risk of complications related to diabetes such as microvascular (retinopathy, nephropathy, and neuropathy including motor, sensory, autonomic and sympathetic neurons) and macrovascular disease, (CDA, 2008; Laycock & Wise, 1996). For these reasons, individuals with type 1 diabetes are encouraged to keep their blood glucose levels as close to a normal physiological range as possible. Indeed, the Diabetes Control and Complications Trial in 1993 reported that the benefits of good glycaemic control include reducing the risk of microvascular complications associated with type 1 (The DCCT Reserach Group, 1993: Zinman, 2004). However, maintaining blood glucose levels within a normal range remains an ongoing challenge for individuals with type 1 diabetes as tight glycaemic control is also associated with an increased risk of hypoglycaemic episodes (Camancho *et al*., 2005; Cryer, 2008; Cryer *et al*., 2003) which can be further complicated by other factors including emotional stress, illness, pregnancy and exercise.

1.2 Counterregulatory response to falling blood glucose levels in non-diabetic individuals

Fortunately, in response to falling blood glucose levels in non-diabetic individuals, a number of hormones are released to counter the action of insulin and contribute to maintaining blood glucose levels within physiological range and decrease the risk of severe

hypoglycaemia. In non-diabetic individuals, glucose homeostasis is maintained by a dynamic balance between the glucose lowering effect of insulin and the action of the counterregulatory hormones (glucagon, catecholamines, growth hormone and cortisol) that stimulate endogenous glucose production, inhibit peripheral glucose utilisation, and increase lipolysis (Cryer *et al*., 2003; Roden & Bernoider, 2003). In response to falling plasma glucose levels, there is a characteristic sequence of counterregulatory responses in non-diabetic individuals, beginning with a decrease in circulating insulin levels which occurs at approximately 4.5 mmol/l (Cryer *et al*., 2003). As insulin inhibits hepatic glucose production predominately through inhibition of glycogenolysis (Boden *et al*., 2003) and stimulates peripheral glucose utilisation, a decrease in insulin levels can cause an increase in blood glucose levels (Pierce, 1999). If blood glucose concentrations fall further to 3.6 - 3.9 mmol/l, there is an increase in the secretion of the counterregulatory hormones glucagon, then epinephrine to stimulate hepatic glucose production, followed by cortisol and growth hormone (Cryer *et al*., 2003). The net action of these counterregulatory hormones is to increase hepatic glucose production and to limit glucose uptake to effectively maintain glycaemia within a physiological range. It is important to note, however, that the individual contributions of these hormones in preventing hypoglycaemia is hierarchical, with some hormones being more important than others (Cryer *et al*., 1989; Hirsch *et al*., 1991; Mitrakou *et al*., 1991).

Glucagon is the most important counterregulatory hormones as demonstrated by studies where the inhibition of glucagon secretion leads to impaired recovery from hypoglycaemia (Hirsch *et al*., 1991; Rizza *et al*., 1979). Glucagon acts by stimulating hepatic glucose production via activation of glycogenolysis and gluconeogenesis (Camacho *et al*., 2005;

Drouin *et al*., 1998; Roden *et al*., 1996). It also inhibits the conversion of glucose to hepatic glycogen and therefore prevents blood glucose levels from falling (Myers *et al*., 1991). The importance of this hormone is shown by the relationship between glucagon and insulin in non-diabetic individuals that enables blood glucose levels to remain within a narrow range by keeping a tight balance between glucose production and glucose utilisation (Pierce, 1999; Sandoval *et al*., 2008). Although the absolute levels of these two hormones are important, it is their ratio relative to each other that is vital, as there is a well demonstrated feedback mechanism whereby blood glucose levels are controlled by changes in the glucagon-to-insulin ratio, particularly in the portal circulation (Cherrington et al., 1987; Marliss *et al*., 2000; Petersen *et al*., 2004). When the insulin-to-glucagon ratio is high, blood glucose levels decline as a result of increased glucose utilisation and decreased endogenous glucose production, and vice versa when this ratio is low (Boyle *et al*., 1989; Cherrington et al., 1987).

The catecholamines (epinephrine and norepinephrine) also play an important role in opposing the action of insulin on blood glucose levels. Indeed, circulating levels of the catecholamines are increased in response to hypoglycaemia (Davis *et al*., 2000b; Sotsky *et al*., 1989) and play some role in stimulating hepatic glucose production. It must be stressed, however, that catecholamine deficiency alone has little impact on the counterregulatory response to a fall in blood glucose level. However, in the absence of glucagon, these hormones play a critical role in responding to hypoglycaemia (Boyle *et al*., 1989). Catecholamines are released by the sympathetic nerve endings and by the adrenal medulla upon stimulation by the sympathetic branch of the autonomic nervous system (Deschenes *et al*., 1991) in humans, with close to eighty percent of the catecholamines

released by the adrenals being epinephrine and twenty percent norepinephrine (Maughan *et al*., 1997). A rise in plasma norepinephrine and epinephrine levels causes an increase in blood glucose levels in two ways. Firstly, by stimulating endogenous glucose production (Kreisman *et al*., 2001) via increasing rates of hepatic glycogenolysis and gluconeogenesis, and secondly by directly antagonizing the action of insulin on muscle, thus inhibiting peripheral glucose uptake and utilisation (Coker & Kjaer, 2005; Kjaer *et al*., 1986; Nonogaki, 2000; Sherwin & Sacca, 1984). These effects of epinephrine are increased when released simultaneously with the other counterregulatory hormones; with cortisol acting to sustain the effect of epinephrine (Sherwin & Sacca, 1984). Epinephrine also acts directly on muscle tissue to stimulate lactate production, with lactate acting as gluconeogenic precursor and stimulating the secretion of growth hormone (Deschenes *et al*., 1991).

Growth hormone is a counterregulatory hormone that plays a vital role in the growth and development of bone, connective, visceral, adipose and muscle tissue (Deschenes *et al*., 1991; Doessing & Kjaer, 2005). In general, growth hormone does not play a role in opposing acute hypoglycaemia, but it has an important role during prolonged hypoglycaemia as the pharmacological blockage of growth hormone action in late hypoglycaemia impairs the increase in glucose production observed after ~ 3 hours of hypoglycaemia (Boyle & Cryer, 1991; De Feo *et al*., 1989b). Growth hormone may also have an indirect role in counterregulation during prolonged hypoglycaemia by stimulating lipolysis and increasing blood glycerol levels (gluconeogenic substrates) which contribute to the sparing of glucose and further inhibition of glucose utilisation (De Feo *et al*., 1989b ; Gibney *et al*., 2007). Finally, some recent studies have reported that an increase in growth hormone levels can acutely inhibit peripheral glucose utilisation rate (Gibney *et al*., 2007;

Stokes *et al*., 2002), with constant infusion or repeated bolus of growth hormone inducing impaired insulin sensitivity (Fowelin *et al*., 1991).

Cortisol is another counterregulatory hormone that plays a key role in the body's adaptation to stress (Deschenes *et al*., 1991), and like growth hormone is not considered to be vital to the acute counterregulatory response to hypoglycaemia. However, cortisol is important in the longer-term counterregulation against prolonged hypoglycaemia as it promotes hepatic glucose production and inhibits glucose uptake by skeletal muscles and adipose tissue without affecting glucose uptake in the brain (Boyle & Cryer, 1991; De Feo *et al*., 1989a). For instance, in a study where the action of cortisol was blocked, glucose production was decreased after 3 hours (De Feo *et al*., 1989a). In contrast, Corral and colleagues (1998) showed that blood glucose remained tightly regulated even when cortisol was pharmacologically suppressed. Cortisol, like growth hormone, may also have an indirect effect on counterregulation by having a glucose sparing effect through stimulation of FFA release, lipolysis, ketogenesis and proteolysis (Corral *et al*., 1998; De Feo *et al*., 1989b).

1.3 Other counterregulatory factors

Recently, there has been increased interest in the role that the inflammatory cytokine interleukin-6 (IL-6) may play in the regulation of blood glucose levels and hepatic glucose production (Glund & Krook, 2008; Helge *et al*., 2002; Hoene & Weigert, 2008). IL-6 is a pleiotropic cytokine secreted by adipose tissue, bone marrow and contracting skeletal muscle. During exercise, IL-6 levels can increase by up to 100-fold, and the working muscle may become the primary producing organ as well as the primary target for the metabolic actions of IL-6, due to its role in ensuring adequate substrate supply via effects

on the liver and adipose tissue (Argilés *et al*. 2005). Il-6 plays an important role in the regulation of inflammation and has been shown in recent years to have a role in several metabolic processes (Argilés *et al*., 2005; Scheller *et al*., 2006). There is evidence that IL-6 promotes fuel uptake and utilisation in working muscle, and as a result increases whole body glucose disposal during exercise (Febbraio *et al*., 2004). Exogenous IL-6 administration also increases cortisol and glucagon levels and this is accompanied by concomitant increases in blood glucose levels and may aid in the counterregulatory response to hypoglycaemia (Dotson *et al*., 2008; Glund & Krook, 2008). IL-6 release has also been shown to be stimulated by the release of epinephrine (Helge *et al*., 2002) and to stimulate the mobilisation and utilisation of fatty acids (Glund, 2008). However, the evidence is not yet conclusive for a role in the regulation of glucose and lipid metabolism (Glund, 2008).

Other than the counterregulatory hormones mentioned above, increased levels of circulating free fatty acids, lactate, amino acids and glycerol may increase glucose production by acting as substrates for gluconeogenesis and as a result may help to oppose falling blood glucose levels (Pierce, 1999; Roden & Bernroider, 2003). For instance, free fatty acids are the predominant fuel used by skeletal muscle in a fasted state where they spare blood glucose utilisation in favour of supporting the obligatory glucose requirement for normal brain function (Gibney *et al*., 2007; Wahren and Ekberg, 2007). Lactate is a significant fuel source and gluconeogenic precursor and may have a role in opposing a decrease in blood glucose levels by increasing insulin resistance (Harmer *et al*., 2008), thus playing a part in both increasing glucose production and decreasing glucose utilisation.

There is also evidence that hepatic glucose production responds directly to changes in circulating blood glucose concentrations. However, the mechanisms by which this autoregulation occurs are only partly elucidated (Moore *et al*., 1998; Tonelli *et al*., 2005). It has been shown that such a non-hormonal autoregulatory mechanism could account for approximately 25% of the rise in net hepatic glucose production during hypoglycaemia (Connolly *et al*., 1992). In contrast, hyperglycaemia exerts a direct inhibitory effect on endogenous glucose production through inhibition of glycogenolysis due to the inhibition of glycogen phosphorylase (Tonelli *et al*., 2005; Yki-Järvinen, 1993). In addition, animal models have shown that net hepatic gluconeogenesis is reduced in hyperglycaemia when glycogen levels are depleted (Tonelli *et al*., 2005).

Finally, it is important to note that animal studies have shown through the removal of hormone-producing organs, sympathetic denervation, or the pharmacological blocking of the action of counterregulatory hormones, that there are other mechanisms by which hepatic glucose production is activated (Moore *et al*., 1998). In several species, electrical stimulation of the hepatic nerves results in hyperglycaemia. Similarly, the stimulation of the ventromedial hypothalamus can lead to enhanced gluconeogenesis and glycogenolysis (Moore *et al*., 1998). Finally, denervation of the sympathetic nerves of the liver increases net hepatic glucose uptake, indicating a role in glucoregulation (DiConstanzo *et al*., 2006).

1.4 Counterregulatory response to falling blood glucose levels in type 1 diabetes

Unfortunately for individuals with type 1 diabetes, the counterregulatory responses to both falling blood glucose levels and hypoglycaemia typically found in non-diabetic individuals are either absent or impaired, increasing the risk of severe hypoglycaemia. Several factors

contribute to this impaired counterregulatory response. In the first instance, the levels of circulating exogenous insulin do not decrease when blood glucose levels are falling as they are controlled primarily by the rate of passive insulin absorption from the site of injection. This is unlike the insulin response of non-diabetic individuals whose pancreas can reduce the secretion of insulin in response to a fall in blood glucose levels. In addition, the hypoglycaemia-mediated increase in glucagon secretion is either impaired or absent in individuals with established type 1 diabetes (Galassetti *et al*., 2004; Mevorach *et al*., 2000; Orskov *et al*., 1991) despite normal glucagon secretion in response to other stimuli such as exercise (Cryer, 2008; Cryer *et al*., 1989). This leaves the diabetic individual dependent on the secretion of catecholamines for effective counterregulation (Cryer, 1989). However, it has been reported that diabetic individuals, particularly those with autonomic neuropathy have attenuated epinephrine and norepinephrine secretion in response to hypoglycaemia compared with non diabetic individuals (Cryer *et al*., 2003). Under conditions of advanced neuropathy, this adrenergic response to hypoglycaemia is highly impaired, thus increasing the risk of severe hypoglycaemia (Cryer, 1989). Finally, the rate of fall in blood glucose levels is another factor that can affect the catecholamine response to hypoglycaemia, with a rapid rate of fall eliciting a smaller epinephrine response compared with a slower rate of fall (Fanelli *et al*., 2003).

A prior episode of hypoglycaemia *per se* is a major factor that can reduce the glycaemic thresholds for the activation of the counterregulatory responses to a subsequent episode of hypoglycaemia as well as the magnitude of the counterregulatory response, increasing the risk of further episodes of hypoglycaemia (Cryer *et al*., 2003; Davis *et al*., 2000b). Indeed, previous episodes of mild hypoglycaemia can attenuate the counterregulatory response to

hypoglycaemia, with the deeper the episode of antecedent hypoglycaemia the greater the failure of the counterregulatory response (Galassetti *et al*., 2004). In addition, antecedent exercise can increase the risk of hypoglycaemia for up to 24 hours due to a blunted autonomic counterregulatory response to hypoglycaemia (Sandoval *et al*., 2004, 2006). Finally, recurrent episodes of hypoglycaemia are associated with marked attenuation of the neuroglucopenic symptoms of hypoglycaemia, leading to a condition known as hypoglycaemic unawareness (Cryer, 2008; Cryer *et al*., 2003).

Physical activity is another factor that makes managing blood glucose levels more difficult and further increases the risk of hypoglycaemia in individuals with type 1 diabetes. This risk of hypoglycaemia is increased not only during exercise, but also for several hours afterwards (MacDonald, 1987). For this reason, many individuals with type 1 diabetes are often reluctant to be physically active and can therefore miss out on the many physical and psychological benefits of an active lifestyle (Ludvigsson *et al*., 1980). This is an important issue given that exercise holds many benefits for diabetic individuals including reducing the risk factors for many chronic diseases such as cardiovascular disease and maintaining body mass as well as emotional and psychological well being (Zinman *et al*., 2004). Unfortunately, the challenge of maintaining blood glucose levels within normal ranges and the risk and associated fear of hypoglycaemia may discourage these individuals from attempting any form of exercise including physical training, team games or competitive sports (Dube *et al*., 2006; Horton, 1988).

1.5 Regulation of glucose production during moderate-intensity exercise in non-diabetic individuals

In order to best appreciate how exercise increases the risk of hypoglycaemia, it is essential to understand how blood glucose levels are controlled during exercise in non-diabetic individuals. In these individuals, the commencement of exercise results in a rapid increase in the uptake and utilisation of glucose by skeletal muscles, which during moderate-intensity exercise (40-60% $\dot{V}O_2$ max) can increase by up to 4-fold above resting levels (Camacho *et al*., 2005; Lavoie et al., 1997a; Lavoie et al., 1997b). Fortunately, this is matched precisely by an increase in glucose production, mainly through an increase in hepatic glycogenolysis at the start of exercise, and as a result blood glucose levels remain stable. Then, as exercise continues, gluconeogenesis plays an increasingly important role (Camacho *et al*., 2005; Richter & Galbo, 1986; Wahren & Ekberg, 2007) to keep blood glucose levels within a normal physiological range. However, if the duration of exercise is excessively prolonged (>90 minutes), hepatic glycogen stores become depleted, which can lead to a fall in blood glucose levels (Camacho, 2005; McConnell *et al*., 1999; Wasserman, 1995).

The increase in hepatic glucose production during exercise is believed to result largely from a concurrent decrease in insulin secretion and an increase in the release of glucagon. Such a role for insulin and glucagon in the activation of hepatic glucose production during moderate intensity exercise is suggested by the fall in blood insulin and rise in glucagon levels often associated with this type of exercise. It is important to note that although, in many instances, the levels of these hormones change little or not at all, as is typically the case for glucagon during short duration exercise in humans (Wasserman *et al*., 1993;

Wasserman, 2009), this 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 (Lavoie *et al*., 1997a; Wasserman *et al*., 1989b). In the case of glucagon, its rise in peripheral blood is dampened following its release from the pancreas into the portal circulation because a large proportion of this hormone is extracted by the liver (Lavoie *et al*., 1997a; Wasserman *et al*., 1993). As a result the glucoregulatory importance of this hormone inferred from its level in blood can be underestimated. Also, 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 (Drouin et al., 1998; Wasserman *et al*., 1989b). For these reasons, the importance of the pancreatic hormones is better demonstrated in studies where blocking changes in the levels of these hormones in response to exercise results in a decline in blood glucose levels to hypoglycaemic thresholds in some individuals which eventually is opposed by a rise in counterregulatory hormones (Camacho *et al*., 2005; Hirsch, *et al*., 1991; Marker *et al*., 1991).

A powerful approach to quantify the importance of insulin and glucagon in the activation of glucose production 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 (De Fronzo *et al*., 1979). This way, the importance of these hormones can be evaluated without the confounding effect of falling blood glucose levels. This experimental approach reveals that the rise in glucagon and fall in insulin each

accounts for more than half of the exercise-mediated increment in hepatic glucose production in dogs (Wasserman *et al*., 1989a,b), and that both hormones also play an important role in humans (Lavoie *et al*., 1997a; Marker *et al*., 1991; Wolfe *et al*., 1986). It is important to stress, however, that a number of studies 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; Hoelzer *et al*., 1986a,b; Kjaer *et al*., 1993), thus indicating that changes in the levels of these hormones do not fully explain the increase in hepatic glucose production during moderate intensity exercise in humans and that other factors are involved. Similarly, 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,b; Wasserman, 1995).

Activation of the sympathoadrenergic system also appears to play little role in the rise in glucose production during moderate exercise although the increase in the levels of epinephrine and norepinephrine and those of hepatic glucose production during exercise suggest otherwise. The small role of catecholamines is not this surprising when one considers that epinephrine levels in the portal circulation is 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). More importantly, the small contribution of the sympathoadrenergic system in mediating the increase in glucose production during exercise is suggested by studies reporting 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; Wasserman *et al*., 1984) and humans (Marker *et al*., 1991; Simonson *et al,* 1984). Also, it is unlikely that sympathetic innervations of the liver plays an important role in the activation of hepatic glucose production during moderate intensity exercise because hepatic surgical denervation does not affect the rise in glucose production during exercise in dogs (Wasserman *et al*., 1990). Similarly, the exercise-induced rise in glucose production is little affected in humans with a liver transplant (Kjaer *et al*., 1995). 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). Finally, the role of the sympathoadrenergic system in exercising humans is further questioned on the basis that anaesthesia of the sympathetic celiac ganglion together with an islet clamp where glucose levels are held constant and plasma insulin and glucagon levels altered so as to mimic their responses to exercise has no effect on hepatic glucose production despite a fall in circulating catecholamine levels and even when a substituting dose of epinephrine is infused (Kjaer *et al*., 1993).

Other than the pancreatic hormones and the sympathoadrenal system, there is evidence that glucose plays some role in the regulation of hepatic glucose output during exercise. Given that the activation of hepatic glucose production during moderate exercise serves to maintain euglycaemia, one would expect the presence of a feedback relationship between blood glucose levels and hepatic glucose output. This prediction is supported by the work of Coker and colleagues (2002) who reported that a moderate fall in glucose levels during exercise stimulates glucose production by the liver in the absence of changes in catecholamines or pancreatic hormones levels. However, others have reported that when

insulin and glucagon are maintained at stable and basal levels during exercise, glucose production responds little to small decreases in blood glucose levels (Kjaer *et al*., 1993; Kjaer *et al*., 1996). However, a small decrease in blood glucose levels can stimulate indirectly hepatic glucose production via activation of counterregulatory hormone responses (Wasserman, 1995; Wasserman *et al*., 1984, 1991). In contrast, exogenous glucose infusion or glucose ingestion affects insulin and glucagon secretion and markedly inhibits hepatic glucose production during exercise (Howlet *et al*., 1998; Jeukendrup *et al*., 1999; Manzon *et al*., 1998) even in responses to very small changes in blood glucose levels (Berger *et al*., 1994), thus indicating a high sensitivity of hepatic glucose production to a rise in blood glucose levels.

1.6 Regulation of glucose production during moderate-intensity exercise in individuals with type 1 diabetes.

In insulin-treated individuals with type 1 diabetes, the increase in muscle glucose utilisation rates in response to exercise is similar to that of non-diabetic individuals (Richter & Galbo 1986); however, the rate of hepatic glucose production may not increase to the same extent to match the increased glucose uptake, thus increasing the risk of hypoglycaemia. This is primarily because, unlike non-diabetic individuals in whom insulin secretion decreases in response to exercise, individuals with type 1 diabetes are unable to decrease the level of the the exogenously administered insulin, with the rates of insulin absorption often increasing during exercise depending on the injection site (Richter & Galbo, 1986). This can result in a state of over-insulinisation, which combined with exercise itself results in a profound increase in insulin action (Camacho *et al*., 2005; Wojtaszewski *et al*., 2000). Under these conditions, high insulin levels inhibit glucose production and have an additive effect with

muscle contraction on glucose transport, thus promoting a rapid decline in blood glucose levels. This occurs despite the exercise-induced increase in the levels of the circulating counterregulatory hormones being similar between diabetic and non-diabetic individuals, with increased or unchanged levels of glucagon, elevated levels of catecholamines and growth hormones, and stable or late increases in cortisol concentrations (Galassetti, *et al*., 2006). As with non diabetic individuals, catecholamines but not glucagon, play little role in the activation of glucose production during moderate intensity exercise (Coker *et al*., 2000).

Finally, another factor that increases the risk of hypoglycaemia is the rise in insulin sensitivity in response to exercise, which combined 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 (Hirsch *et al*., 1991; Wasserman, 1995). For this reason, it is generally recommended that insulin dose should be reduced or 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 is noteworthy that in order to decrease the risk of hypoglycaemia associated with physical activity, exercising in a severe insulin deficient state is not a reasonable alternative for individuals with type 1 diabetes, as the exercise-induced release of counterregulatory hormones may result in a disproportionate increase in the glucagon to insulin ratio, a potent stimulus for the hepatic production of glucose and ketone bodies (Riddell & Perkins, 2006). The resulting increase in glycaemia with the presence of elevated ketone body levels could

lead to an increased risk of severe ketoacidosis (Richter & Galbo, 1986) which may require hospitalisation.

1.7 Intense exercise and regulation of glucose production in non-diabetic individuals

Although exercise is associated with an increased risk of hypoglycaemia in type 1 diabetes, what is often overlooked is that not all forms of exercise increases this risk. Indeed, there are conditions where exercise results in an increase in blood glucose levels in individuals with and without diabetes. In non-diabetic individuals, intense aerobic exercise (exercise intensity $\geq$ 80% $\dot{V}O_2$max) of both short and longer duration, stimulates a significant rise in blood glucose levels (Brooks *et al*., 1990; Harmer *et al*., 2008; Kinderman *et al*., 1982; Lavoie *et al*., 1987; Manzon *et al*., 1998; Marliss & Vranic, 2002; Marliss e*t al*., 1991; Marliss *et al*., 2000; Moussa *et al*., 2003; Schnabel *et al*., 1984; Wahren & Ekberg, 2007; peak blood glucose values reported up to 7.11mmol) followed by a period of hyperglycaemia that can persist for up to an hour post-exercise (Marliss *et al*., 1991; Marliss *et al*., 2000; Sigal *et al*., 1994a, 2000). Shorter exercise bouts can also elicit a rise in glycaemia in non-diabetic individuals as shown by Moussa and colleagues (2003) who reported that a 6-second and 30-second sprint result in a significant rise in glycaemia. Furthermore, it has been reported that repeated bouts of high intensity exercise elicit an increase in blood glucose levels (Brooks *et al*., 1990; Näveri *et al*., 1985).

It is important to note that exercise training and the dietary state and gender of the exercising individual are important factors affecting the rise in blood glucose levels in response to intense exercise, with post-absorptive exercise resulting in a greater increment in blood glucose levels compared with postprandial exercise (Kreisman *et al*., 2000) and

the rise in blood glucose levels being less in trained individuals (Harmer *et al*., 2007). 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). Also, although men and women have a similar glucoregulatory response to high-intensity exercise, women experience a greater rise in glycaemia in the post-exercise period (Marliss *et al*., 2000) due to a lesser increment in glucose utilisation, resulting in a greater imbalance between glucose production and utilisation. 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).

The hyperglycaemic effect of high intensity exercise in non-diabetic individuals results from a disproportionately rapid increase in hepatic glucose output which may increase by up to 8-fold (Kreisman *et al*., 2000, Marliss *et al*., 2000; Sigal, *et al*., 1994a; Sigal *et al*., 2000) compared to only a 4-fold increment in glucose disappearance rate from circulation. This effectively creates an imbalance between the rates of glucose production and utilisation, and subsequently a rise in glycaemia (Marliss *et al*., 1992). This increase in blood glucose levels has been described as a feed-forward response to intense exercise which differs from glucoregulation during rest and moderate intensity exercise, where the rate of production and utilisation are balanced (Kjaer, 1986; Marliss & Vranic, 2002).

In contrast to what is observed during moderate intensity exercise, the significant increase in hepatic glucose production during high intensity exercise is unlikely to be attributable to changes in insulin and glucagon levels. This is because plasma insulin levels during intense exercise remain unchanged or decline only very slightly, whereas plasma glucagon

levels increase at most by ~50%, resulting in only a small, physiologically significant change in the glucagon-to-insulin ratio (Marliss *et al*., 1992; Näveri *et al*., 1985; Purdon *et al*., 1993; Sigal, 1994a). It is important to note, however, that the role of glucagon inferred from changes in its levels in blood is likely to be underestimated since, as discussed earlier, the liver may be exposed to more pronounced changes in portal glucagon and insulin levels than suspected from the levels of these hormone measured at a systemic level. More compelling evidence that the marked increase in hepatic glucose production during intense exercise is not mediated by insulin or glucagon is provided from experiments based on islet clamp methodology. When intense exercise is performed while endogenous insulin and glucagon secretions are inhibited via infusion of somatostatin and replaced with insulin and glucagon infused at rates resulting in basal insulin and glucagon levels, exercise-mediated increase in glucose production is not diminished (Marliss & Vranic, 2002; Sigal *et al*., 1996). 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) as well as in the postprandial state (Kreisman *et al*., 2000).

There is evidence that the increase in hepatic glucose production and blood glucose levels during intense exercise in non-diabetic individuals is mediated primarily by catecholamines. In response to bouts of intense exercise (80-130% of $\dot{V}O_{2max}$) of between ~1.5 and 10 minutes duration, both epinephrine and norepinephrine increase by up to 18 fold (Harmer *et al*., 2006; Marliss *et al*., 2000; Marliss & Vranic, 2002; Sigal *et al*., 2000). Botcazou and colleagues (2007) have also demonstrated a rise in the catecholamines in both men and women following a sprint lasting as little as a 6 seconds, with the higher the

intensity the greater the increase in catecholamine levels (Zouhal *et al*., 2008). Although, compared to the pancreatic hormones, there is a tighter relationship between catecholamines levels and rise in hepatic glucose production rate during heavy exercise (Marliss & Vranic, 2002; Sigal *et al*., 1996), this does not necessarily imply causality.

A better 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, all the studies investigating the effect of adrenergic blockade have failed to show any marked impairment in the rise in glucose production during intense exercise (Sigal *et al*., 1994a, 1999, 2000; Cocker *et al*., 1997). For instance, the intraportal simultaneous infusion of propanolol and phentolamine to block catacholamines in dog is without any effect on the rise in hepatic glucose production during heavy exercise (Coker *et al*., 1997). In studies performed on humans, the infusion of propanolol (beta-adrenergic blocker) in non-diabetic (Sigal *et al*., 1994a) and diabetic individuals (Sigal *et al*., 1999) results in an unexpected higher rise rather than a lesser increase in hepatic glucose production. Finally, 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). Whether the dose of adrenergic blockers administered in the studies described above was insufficient to counter the effect of the large rise in catecholamines associated with intense exercise remains to be established.

Currently, the most compelling evidence that catecholamines mediate, at least in part, the rise in hepatic glucose production during intense exercise is the increase in glucose production when catecholamines are infused during exercise in both dogs and humans. The infusion of high levels of epinephrine in islet cell-clamped humans subjected to celiac-ganglion blockade while exercising at moderate intensity results in an increase in glucose production (Kjaer *et al*., 1993), but to a lesser extent than during intense exercise (Howlett *et al*., 1999; Kreisman *et al*., 2000). This is also the case with norepinephrine infusion during exercise of moderate intensity (Kreisman *et al*., 2001). However, when during moderate intensity exercise both norepinephrine and epinephrine are infused following a pattern that results in blood catecholamine levels approximating those of intense exercise ($\geq$ 80% $\dot{V}O_2$max), the resulting increases in hepatic glucose production and rise in blood glucose levels match those associated with intense exercise, thus suggesting that circulating catecholamines are important regulators of glucose production for this type of exercise (Kreisman *et al*., 2003).

In order to understand how catecholamines increase blood glucose levels during intense exercise it is important to examine how these hormones affect not only glucose production, but also the rates of peripheral glucose utilisation. On the basis 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 & Harbreages, 2002; Watt et al., 2001) in part via a glycogenolytically-mediated increase in glucose-6-phosphate (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. However, the recent demonstration that elevated catecholamines during exercise in humans stimulate rather than inhibit peripheral glucose utilisation (Kreisman *et al*., 2000, 2003) suggests 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). These findings on the effect of catecholamines on glucose production and utilisation, however, 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).

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 (Kinderman, *et al*., 1982; Schnabel *et al*., 1984; Stokes *et al*., 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). Whether IL6 released by skeletal muscles could contribute to the hyperglycaemic effect of intense exercise remains to be established. Although it has been proposed that IL-6 may play a role in the production and clearance of glucose (Febbraio *et al*., 2004), to date there have been no studies on the role of interleukin-6 in the glucoregulation during or following intense exercise; however, the research into a possible

role in moderate intensity exercise has pointed toward a role in glucose transport, fuel mobilisation and uptake in working muscle (Hoene &Weigert, 2008).

1.8 Regulation of glucose production during recovery from high intensity exercise in non-diabetic individuals

During recovery from intense exercise in non-diabetic individuals, blood glucose falls progressively and returns to pre-exercise levels within an hour. This has been explained by the rate of glucose utilisation exceeding the rate of glucose production; a process facilitated by the return to basal levels of the catecholamines and an increase in insulin levels mediated by the high post-exercise glucose levels (Lavoie *et al*., 1987; Marliss & Vranic, 2002; Mitchell *et al*., 1988; Sigal *et al*., 1994b). The importance of insulin in this process is supported by the finding that following 10 minutes of an exhaustive bout of intense exercise at approximately 80% of $\dot{V}O_2$ max, post-exercise insulin concentration can rises up to two-fold compared with pre-exercise levels in response to the increase in blood glucose levels (Mitchell *et al*., 1988). As a result, this hyperinsulinaemia can inhibit hepatic glucose production and increase glucose uptake in insulin-sensitive tissue, thus causing a decrease in blood glucose levels during recovery (Marliss *et al*., 1992; Purdon *et al*., 1993; Sigal *et al*., 1994b). In addition, the increase in insulin levels may help to counter the increased insulin resistance and decreased insulin-stimulated glucose uptake associated with high lactate and catecholamine levels (Choi, *et al*., 2002). Since catecholamine levels return to basal levels within the first few minutes of recovery, and their levels are well correlated with the post-exercise decline in glucose production (Marliss & Vranic, 2002), this supports the notion that a fall in catecholamines is possibly another mediator of the post-exercise fall in blood glucose levels.

The role of cortisol and growth hormones in the acute regulation of blood glucose levels early after high intensity exercise is unlikely to be important. Stokes and colleagues (2002) showed that the duration of a sprint affects the magnitude of the growth hormone response, with maximal levels being attained after 40 minutes of recovery, returning to baseline within 60 minutes. A more recent study by Ehrnborg and colleagues (2003) showed that growth hormone peaked directly after a maximal exercise test, with the magnitude of the response being closely related to the intensity rather than total workload, before returning to baseline within 30-60 minutes. Gordon and colleagues (1994) proposed that the acute accumulation of hydrogen ions (H^+) produced during intense exercise is a potential stimulus for the increase in growth hormone levels. In addition, it has been proposed that the early rise in growth hormone is suppressed by high glucose levels, and that the late rise coincides with declining blood glucose concentrations (Schnabel *et al*., 1984). Finally, there is also evidence that growth hormone release may be stimulated by central adrenergic outflow including the catecholamines released during intense exercise (Weltman *et al*., 2000), and that growth hormone secretion is likely to increase with work intensity (Pritzlaff *et al*., 2000). Since a rapid transient increase in growth hormones release can acutely inhibit peripheral glucose utilisation in resting individuals (Gibney *et al*., 2007; Stokes *et al*., 2002), this raises the possibility that elevated levels of this hormones during early recovery might reduce the rate at which blood glucose is falling post-exercise, but this remains to be tested. However, during recovery from intense exercise, the progressive increase in growth hormone levels with time (Gordon *et al*., 1994; Schnabel, 1984; Stokes *et al*., 2008), is unlikely to play an important role in the post-exercise fall in blood glucose levels. This is because the use of blocking agents of growth hormone release, such as

octreotride, has no effect on the plasma glucose post-exercise responses and rates of glucose appearance and disappearance despite different levels of plasma growth hormone (Sigal *et al*., 1996).

1.9 Intense exercise and regulation of glucose production in type 1 diabetic individuals

The immediate blood glucose response to high intensity exercise ($\geq$ 80% $\dot{V}O_2$max) in type 1 diabetic individuals with insulin at basal levels is similar to that of non-diabetic individuals, with exercise causing comparable increases in blood glucose levels (Marliss *et al*., 1992; Mitchell *et al*., 1988; Purdon *et al*., 1993; Sigal *et al*., 1994b). However, during recovery, the blood glucose response in diabetic and non-diabetic individuals is dissimilar, with blood glucose levels remaining elevated in individuals with type 1 diabetes. As with non-diabetic individuals, the exercise-mediated increase in glycaemia results from an increase in hepatic glucose production exceeding the rise in glucose utilisation rate (Marliss *et al*., 1992; Mitchell *et al*., 1988; Purdon *et al*., 1993; Sigal *et al*., 1994b). The magnitude and timeframe of this rapid rise in glucose production is similar to that of non-diabetic individuals subjected to a similar bout of intense exercise (Purdon *et al*., 1993).

Consistent with the similar increase in blood glucose levels in diabetic and non-diabetic individuals, the catecholamine response to high intensity exercise is also not affected by diabetes, with an approximate 14-fold increase from resting levels in both epinephrine and norepinephrine (Marliss *et al*., 1992; Purdon, *et al*., 1993) and a similar post-exercise pattern of fall in the levels of these hormones. Also, the glucagon response to intense exercise is similar in non-diabetic and diabetic individuals, along with similar increases in lactate (Mitchell *et al*., 1988) and cortisol levels (Purdon *et al*., 1993). In contrast, FFA

levels increase above basal levels during recovery in diabetic individuals unlike in non-diabetics where FFA levels remain depressed during recovery (Mitchell *et al*., 1988).

The sustained increase in blood glucose levels during recovery from intense exercise in type 1 diabetes has been explained on the basis of the absence of a post-exercise increase in insulin secretion during recovery (Mitchell *et al*., 1988; Purdon *et al*., 1993; Sigal *et al*., 1999). This is because, unlike non-diabetic individuals, insulin secretion does not increase in response to a post-exercise rise in glycaemia, and as a result sustained hyperglycaemia occurs into late recovery (Mitchell, *et al*., 1988; Purdon, *et al*., 1993; Sigal *et al*., 1999). The importance of insulin is also illustrated by the finding that insulin administration post-exercise is accompanied by a pattern of fall in blood glucose level similar to that in non-diabetic individuals. Interestingly, studies in insulin-treated diabetic rats have shown a pattern of sustained hyperglycaemia following a short bout of intense exercise, and this was attributed, in part, to impaired glucose utilisation in muscles rich in oxidative fibres (Ferreira *et al*., 2005).

1.10 Intense exercise as a means to reduce the risk of hypoglycaemia

The observation that intense aerobic exercise ($\geq$ 80% $\dot{V}O_2$max) lasting for more than 10 minutes can result in a sustained increase in blood glucose levels in type 1 diabetes (Marliss & Vranic, 2002) raises the possibility that this type of exercise might provide an effective tool for the prevention of hypoglycaemia. Unfortunately, this type of exercise is physically demanding and thus unlikely to be tolerated by most individuals. This coupled with the impracticality of performing 10-15 minutes of strenuous exercise as a means to oppose a fall in blood glucose levels, prevents this type of exercise from becoming part of standard

guidelines for the prevention of hypoglycaemia. Since performing maximal sprint efforts that last 30-seconds or more is associated with undesirable consequences including cardiovascular discomfort, nausea, dizziness and vomiting, whereas a 10-second sprint is generally well tolerated, Bussau and colleagues (2006) examined whether a 10-second sprint could provide a means to counter an exercise-mediated fall in glycaemia in type 1 diabetic males with moderately elevated plasma insulin levels. They reported that a short sprint performed after a bout of exercise at an intensity of 40% $\dot{V}O_2$ peak opposed a fall in glycaemia post-exercise, compared with passive rest. These findings were explained, in part, by the marked rise in catecholamines and growth hormones levels during early recovery (Bussau *et al*., 2006). These findings have important clinical implications as they suggest that following moderate intensity exercise such as light cycling, it is preferable for complication-free individuals with type 1 diabetes to engage in a 10-second maximal sprint effort than to only rest in order to acutely prevent or slow down a further fall in glycaemia (Bussau *et al*., 2006).

What is still unclear from the findings of Bussau and colleagues (2006) is the issue of whether a short-sprint is capable not only of attenuating an exercise-mediated fall in blood glucose levels, but also of increasing glycaemia when blood glucose levels are stable and insulin at basal levels in individuals with type 1 diabetes. Also, the mechanisms whereby a short sprint might increase blood glucose levels post-exercise have never been investigated. Maybe, a more pronounced increase in the rate of glucose production compared to the rate of glucose utilisation is involved as is the case during intense aerobic exercise ($\geq$ 80% $\dot{V}O_2$max) of prolonged duration (>10 minutes) in insulin-treated diabetic individuals.

1.11 Statement of the problem and research hypotheses

It is unclear whether a 10-second high intensity sprint can increase blood glucose levels in insulin-treated individuals with type 1 diabetes and whether such a postulated rise in glucose levels is due to a disproportionate increase in hepatic glucose production relative to the rise in peripheral glucose utilisation as observed in intense aerobic exercise. For this reason, the primary purposes of this study were (a) to test the hypothesis that a 10-second sprint under conditions of basal insulin levels (insulin levels when Ra matches Rd in a fasted state) results in a sustained post-exercise increase in blood glucose levels in individuals with type 1 diabetes, and (b) that this increase in blood glucose levels after exercise is due to a more pronounced increase in the rate of glucose production compared to the increase in the rate of peripheral glucose utilisation.

1.12 Significance of the Study

The issue of whether a short sprint under the condition of basal insulinaemia can increase blood glucose levels in type 1 diabetes and the mechanism whereby such a short sprint increases blood glucose levels remain to be elucidated. This study might help explain the glucose stabilising effect of a short a short sprint reported by Bussau and colleagues (2006), and contribute to the elaboration of diabetes management guidelines for physically active type 1 diabetic individuals.

Chapter Two

The effect of a short sprint on glucose production and whole body glucose utilisation in adults with Type 1 diabetes

2.1 Introduction

Regular exercise is generally recommended for individuals with Type 1 diabetes mellitus because of its many benefits such as weight control and improvement in risk factors for cardiovascular disease (Tsui *et al*., 2001; Zinman *et al*., 2004). Unfortunately, exercise increases markedly the risk of hypoglycaemia in Type 1 diabetes (Cryer *et al*., 2003; MacDonald, 1987). This is an important issue because the challenge of maintaining blood glucose levels within a normal range during exercise and associated risk and fear of hypoglycaemia may discourage individuals from adopting a physically active lifestyle (Brazeau *et al*., 2008; Dube *et al*., 2006; Ludvigsson *et al*., 1980).

It is important to note, however, that not all forms of exercise enhance the risk of hypoglycaemia as intense aerobic exercise increases blood glucose levels in insulin-treated individuals with Type 1 diabetes (Mitchell, *et al*., 1988; Purdon, *et al*., 1993; Sigal *et al*., 1999). For instance, 10-15 minutes of intense aerobic exercise performed at above 80% maximal aerobic capacity in diabetic individuals treated with basal insulin levels as well as in non-diabetic individuals results in a significant increase in blood glucose levels (Brooks *et al*., 1990; Harmer *et al*., 2008; Kinderman *et al*., 1982; Lavoie *et al*., 1987; Manzon *et al*., 1998; Marliss & Vranic, 2002; Moussa *et al*., 2003; Schnabel *et al*., 1984; Wahren & Ekberg, 2007) that can remain elevated for well in excess of an hour after exercise in diabetic participants (Mitchell, *et al*., 1988; Purdon, *et al*., 1993; Sigal *et al*., 1999). This is an important observation because it raises the possibility that this type of exercise might provide an effective means for the prevention of hypoglycaemia in diabetes. Unfortunately, prolonged aerobic exercise at intensity above 80% $\dot{V}O_2$max is physically demanding and thus unlikely to be well tolerated by most individuals. Moreover, the impracticality of

performing 10-15 minutes of strenuous exercise as a means to oppose a fall in blood glucose level, prevents this type of exercise from becoming an effective tool for the prevention of hypoglycaemia.

Since a maximal sprint effort lasting 30 seconds or less can also elicit a prolonged post-exercise increase in blood glucose levels in non-diabetic individuals (Moussa *et al*., 2003), the possibility that sprinting might be beneficial for the prevention of hypoglycaemia in diabetic individuals was recently examined by Bussau and colleagues (2006, 2007). They reported that a 10-second sprint performed before or after a bout of moderate intensity exercise (40% $\dot{V}O_2$ peak) prevents blood glucose concentration from falling after exercise compared to the rapid fall with a passive rest post-exercise (Bussau *et al*., 2006, 2007). These findings are clinically important as they suggest that following moderate intensity exercise, it is preferable for complication-free individuals with Type 1 diabetes to engage in a 10-second maximal sprint effort, which is generally well tolerated, than to only rest in order to acutely prevent or slow down a further fall in their glycaemia after exercise (Bussau *et al*., 2006).

What still remains without an answer with the findings of Bussau and colleagues (2006, 2007) is the nature of the mechanisms underlying the stabilising effect of sprinting on blood glucose levels with diabetes. Although this has been explained, in part, by the marked rise in catecholamines levels early after exercise (Bussau *et al*., 2006), the effect of sprinting on the post-exercise rates of endogenous glucose production and peripheral glucose utilisation was not examined in that study. Moreover, the issue of whether sprinting by itself can increase blood glucose levels in insulin-treated individuals with Type 1

diabetes still remains to be addressed. Since intense aerobic exercise in diabetic individuals increases blood glucose levels via a more pronounced increase in the rate of glucose production compared to the rise in the rate of peripheral glucose utilisation (Kreisman *et al*., 2000, Marliss *et al*., 2000; Sigal, *et al*., 1994; Sigal *et al*., 2000), maybe a similar mechanism is involved after sprinting in diabetic and non diabetic individuals. For these reasons, the purposes of this study were to explore the effect of a 10-second maximal sprint effort on blood glucose levels in both insulin-treated individuals with Type 1 diabetes and non-diabetic individuals, and more importantly to determine whether the expected increase in blood glucose levels during recovery is due to a disproportionate rise in glucose production rate relative to that of glucose utilisation.

2.2 Materials and Methods

2.2.1 Participants

One group of male and female participants with Type 1 diabetes aged between 16 and 30 years (n=8), together with a group of non-diabetic individuals (n=6) matched for age, fitness and body mass index were involved in the study (Table 2.1). Participants with Type 1 diabetes had been diagnosed for a minimum of 2 years, with glycated haemoglobin <9), and were free from any clinical evidence of macrovascular or microvascular complications associated with diabetes mellitus. Only diabetic participants under an insulin regime of continuous subcutaneous insulin infusion (CSII) were recruited into the study, and all were hypoglycaemic aware. All participants were not on any medication, except insulin for the participants with Type 1 diabetes. Female participants were regularly menstruating and were tested during the follicular phase of the menstrual cycle to account for the influence of female hormones on glucoregulation. All participants were required to visit the Princess Margaret Hospital laboratory on two occasions; the first visit was a familiarisation session and the second visit was the testing trial. Participants signed a consent form and the study was given ethics approval by the Princess Margaret Hospital Ethics Committee.

2.2.2 Familiarisation session

During the familiarisation session, standing height was measured using a stadiometer (Surgical and Medical) with the participant's head aligned in the Frankfort plane. Body mass was measured using an electronic scale (Seca Alpha, model 770), with the participant wearing minimal clothing. The peak rate of oxygen consumption ($\dot{V}O_2$peak) of each participant was determined using an incremental exercise protocol that lasted approximately 15 minutes, and performed on a front access cycle ergometer (Repco,

Melbourne, Australia) connected to a computer running the Cyclemax software (UWA, Australia). Participants cycled at an initial intensity of 50 watts with the intensity increasing progressively at 3-minute intervals by 25-30 Watts until volitional exhaustion. Breath by breath analysis was conducted using a Vmax spectra analysis system (SensorMedics Corporation, USA). $\dot{V}O_2$ peak was defined as the highest minute average $\dot{V}O_2$ with either a respiratory exchange ratio greater than 1.1 or plateau in $\dot{V}O_2$. After a 15-minute rest, participants were instructed to practice the 10-second sprint on the same front access ergometer. Participants stood to start the sprint with both feet level, holding the handles, and were given a short countdown to start the sprint.

2.2.3 Testing Session

Prior to attending the testing session, all participants were required to abstain from consuming caffeine or alcohol for 24 hours before testing, as well as to avoid any physical activity (except light walking) because antecedent exercise can attenuate the counterregulatory hormonal response to a subsequent bout of exercise (Galasetti *et al*., 2006). Participants with Type 1 diabetes were required to monitor blood glucose levels closely and report any period of hypoglycaemia for 48 hours prior to testing because antecedent hypoglycaemia can affect the counterregulatory response to a subsequent bout of exercise (Galasetti *et al*, 2003). In addition, they were contacted prior to arrival to confirm that the testing restrictions had been adhered to. If the participant had experienced any hypoglycaemia (BGL < 4.0mmol) or had been unable to meet these restrictions, testing was rescheduled.

On the day of testing, all participants arrived in the lab at 0715 hours having fasted since midnight. Upon their arrival, a cannula was inserted in a retrograde fashion in the dorsum of the hand for blood sampling. A second cannula was inserted into a vein in the contralateral antecubital fossa for the infusion of [6,6-^{2}H]glucose in all participants and insulin for the participants with diabetes. In this regard, it was important to standardise the insulin infusion rate so as to take into account the wide range of insulin needs across participants. One approach to do so is similar to that used by others (Marliss & Vranic, 2002; Galassetti *et al*., 2006). The insulin infusion rate adopted for each diabetic participant was that for which the rate of hepatic/renal glucose production is matched by the rate of peripheral glucose utilisation, with no exogenous glucose required to stabilise glycaemia at a level of 4.5 - 5 mmol/l. For this reason, this insulin infusion rate is referred to as basal insulin infusion rate. To achieve this, the CSII of each diabetic participant was disconnected and insulin (Humalog, Eli Lilly Australia Pty Ltd, Australia), at a concentration of 0.1 unit of insulin per 1ml infusate was infused using an Alaris Asena ® GH syringe pump (Alaris Medical Systems, United Kingdom). The infusion rate was adjusted to clamp the blood glucose levels at ~4.5 - 5.0 mmol/l. To this end, blood samples were collected every 15 minutes for glucose assays using a YSI analyzer (YSI, Yellow Springs, Ohio), and depending on the blood glucose result, the rate of insulin infusion was adjusted to maintain blood glucose level at 4.5 - 5 mmol/l. This process required small minor adjustments to the rate of infusion as well as small bolus amounts in some instances, and was accompanied by a waiting period during which the effect of the change to insulin infusion rate was observed and recorded. Insulin infusion rate was considered to have reached basal level when blood glucose levels remained stable for 30 minutes without any

change in insulin infusion rate. Once clamped, the insulin infusion rate remained constant for the duration of the study.

Approximately 30 minutes after the commencement of the insulin infusion in the diabetic participants and immediately after cannulation in the non-diabetic participants, a blood sample was drawn for the determination of background enrichment of [6,6-^{2}H]glucose followed by a priming bolus injection of 3 mg/kg^{-1} of [6,6-^{2}H]glucose. Then, the constant infusion of the isotope was commenced at a rate of 2.4 mg/kg^{-1}hr^{-1} until the end of the study. Blood sampling to determine steady state enrichment was conducted at 15-minute intervals from 90 minutes following the commencement of the isotope infusion and continued for a minimum 2.5 hours (control) or until the participant was considered clamped at basal insulin level. Then, participants were moved from their relaxed seated position onto a front access cycle ergometer (Repco, Melbourne, Australia) connected to a computer running Cyclemax software (UWA, Perth, Australia) approximately 10 minutes before the sprint. The sprint consisted of cycling as hard as possible for 10 seconds while verbal encouragement was provided. Following exercise, participants recovered while comfortably seated.

Before and at predetermined time intervals after exercise, arterialized venous blood was sampled from a dorsal vein on the back of the participant's hand preheated using a hot box (Omega CN 370, Sydney, Australia) at ~50°C to arterialize the venous blood as blood metabolites and hormone levels under these conditions are close to those in arterial blood (Liu *et al*., 1992). Some blood was used to measure [6,6-^{2}H]glucose enrichment and the levels of glucose, insulin and several glucoregulatory hormones including catecholamines,

cortisol, glucagon, and growth hormone. Approximately 2 ml of blood was drawn prior to each blood sample being taken, and a saline was injected to flush and keep the cannula patent. Finally, respiratory gas measurements were collected before and after exercise using a Vmax spectra analysis system (SeonsorMedics Corporation, USA).

At the end of the testing session, both cannulas were removed and the participant was fed. Participants with diabetes were reconnected to their CSII and were asked to monitor their blood glucose levels for the rest of the afternoon. Contact was made with participants to ensure no delayed adverse effects from the testing had occurred.

Figure 2.1 Sprinting on the Front Access Cycle Ergometer

Figure 2.2 Participant resting with hand being warmed in the hotbox during blood sampling

2.2.4 Blood sampling and analysis

Blood samples for free insulin and isotopic enrichment were alliquotted into lithium heparin tubes whereas catecholamines were collected into lithium heparin tubes with 1-2 mg of sodium metabisulphite (Ajax Chemicals Ltd. Sydney, Australia) to prevent oxidation. Blood samples to measure free fatty acids concentrations were alliquotted into an EDTA tube and glucagon into an EDTA tube with added Trasylol (Bayer Pharaceuticals, USA) to prevent proteolysis. Growth hormone and cortisol were aliquoted into a 4 ml serum clot activator tube. All blood samples (except those collected in the 4 ml serum clot activator) were centrifuged for 10 minutes at a speed of 3000 rpm and at 4°C, whereas the blood samples in the 4 ml serum clot activator were left at room temperature to clot, and then centrifuged as above. The resulting plasma or serum supernatants were collected and stored at -80°C for later analysis.

2.2.5 Hormone and metabolite assays

Heparinized plasma treated with sodium metabisulfate was used to determine catecholamine levels using ELISA kit (Bi-cat ELISA, DLD Diagnostika GMBH, Hamburg, Germany). Free fatty acid levels were measured in EDTA-treated plasma using the Roche Half-Micro Test Free Fatty Acids Assay kit (Mannheim, Germany). Heparinized plasma treated with polyethylene glycol was assayed for free insulin using the Coat-a-Count Insulin Kit (Diagnostic Products, Los Angeles, CA). Glucagon levels in plasma collected with aprotinin (Trasylol) were measured from EDTA-treated plasma by radioimmunoassay using the Linco glucagon radioimmunoassay kit (St. Charles, MO). Cortisol levels were assayed from venous serum by competitive immunoassay on an Immulite 2000 Analyser using the Immulite Cortisol Assay kit (Diagnostic Products). Growth hormone levels were determined from serum by immunometric assay on an Immulite 2000 Analyser using the Immulite Growth Hormone Assay kit (Diagnostic Products). 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).

2.2.6 Determination of isotopic enrichment

The enrichment of plasma [6,6-^{2}H]glucose was determined using gas chromatography mass spectrometry (GCMS; Agilent Selective Detector, Agilent technologies, Ryde, NSW, Australis) after converting [6,6-^{2}H]glucose to an aldononitrile derivative as described previously by Hannestad and Lundblad (1997). The measured enrichment was smoothed to minimise the effect of random error of measurements on Ra calculations (Finegood & Bergman, 1983). Glucose rates of appearance (Ra) and disappearance (Rd) were calculated

from the changes in glucose enrichment using the one-compartment fixed-volume non-steady state model of Steele (Wolfe & Chinkes, 2005) as modified for use with stable isotopes (Romijn *et al*., 1993), and a pool fraction of 0.65 (Radziuk et al., 1978).

2.2.7 Statistical analysis

All data were analysed using SPSS 17.0 for Windows computer software. Data were analysed for changes over time and differences between the Control and Type 1 diabetes group. Measures of each variable were subjected to a two-way (time x trial) repeated-measures analysis of variance to determine whether differences existed, followed by Fisher LSD test to determine where the differences lay. Statistical significance was accepted at the $p<0.05$ level.

2.3 Results

2.3.1 Descriptive characteristics of the participants

There were no differences between the descriptive characteristics of the participants with Type 1 diabetes and those without diabetes ($p < 0.05$; Table 2.1).

2.3.2 Blood glucose response to a 10-second sprint

The blood glucose response to a 10-second sprint was different between the Type 1 diabetic group and the Control group (Fig 2.3). There was a significant interaction effect (time by group) observed from 5 minutes post-sprint until the end of the recovery period. In the Type 1 diabetic group, blood glucose levels increased significantly 1.04±0.58 mM ($p=0.001$) after the sprint and then remained stable for the duration of the recovery period. In the Control group, blood glucose levels rose significantly 0.41±0.24 mM in response to exercise and reached maximal levels after 15 minutes of recovery, before returning to stable pre-exercise levels thereafter.

2.3.3 Glucose rate of appearance and rate of disappearance

In response to the 10-second sprint, there was a significant transient fall in glucose Rd early during recovery in the participants with Type 1 diabetes, with glucose Rd returning to pre-exercise levels within 15-30 minutes. There were no significant changes in glucose Ra in response to the sprint in the diabetic group. As a result, early during recovery, glucose Rd was significantly lower than glucose Ra (Fig 2.4). In the Control group, there was no significant change in glucose Ra before and after exercise (Fig 2.5). Early during recovery from the 10-second sprint, glucose Rd was lower than Ra, but returned to similar levels later during recovery.

2.3.4 Blood lactate and free fatty acid response to a 10-second sprint

In the diabetic and control groups, blood lactate levels responded in a similar fashion to exercise (Fig 2.6). There was no significant interaction effect (time by group) recorded between the two groups ($p < 0.05$) in response to the sprint or recovery. Blood lactate levels increased to a small extent in response to exercise and reached maximal levels after 5 minutes of recovery. Blood lactate levels decreased progressively thereafter and eventually reached pre-exercise levels in both experimental groups.

The response of free fatty acids (FFA) levels to sprints was similar in both groups during early recovery (Fig 2.7). However, after 30 minutes of recovery, a significant difference was observed between experimental groups, with free fatty acid levels increasing only in the Type 1 diabetic group (Fig 2.7).

2.3.5 Hormonal responses to a 10-second sprint

In response to the 10-second sprint, there was no significant change in glucagon level over time for each group (Fig 2.8). In contrast, plasma epinephrine levels increased significantly in the diabetic and control groups, and returned to pre-exercise levels within 10 minutes, with plasma epinephrine response being similar in both groups (Fig 2.9). The plasma norepinephrine response to exercise was also similar in both groups as indicated by a lack of an interaction effect (time by group) at any point except 60 minutes ($p <0.05$; Fig 2.10), with norepinephrine levels reaching maximal levels at the onset of recovery. During recovery, norepinephrine levels decreased rapidly in both groups to reach pre-exercise basal levels.

Growth hormone levels also increased in response to exercise, with peak values recorded at 15 and 45 minutes in the diabetic and control groups, respectively (Fig 2.11). Cortisol levels were significantly different at baseline, with no interaction effect (time by group) observed post-exercise ($p < 0.05$; Fig 2.12). In response to exercise, peak cortisol values in both groups occurred at 30 minutes post-sprint in the diabetic and control groups, respectively, and decreased thereafter. Plasma insulin levels did not change significantly in response to exercise and recovery in the diabetic and control groups.

Table 2.1 Descriptive characteristic of the participants

Variable	Type 1 Diabetes (Mean ± SD)	Control (Mean ± SD)
Age (years)	22.9 ± 4.8	24.2 ±3.6
Weight (kg)	79.0 ± 12.6	69.7 ± 11.3
Height (cm)	172.1 ± 12.2	168.9 ± 7.4
BMI (kg/m^2)	26.7 ± 4.0	24.6 ± 4.1
$\dot{V}O_2$ peak ($ml.kg^{-1}.min^{-1}$)	37.6 ± 7.0	41.0 ± 10.8
Total work (J/kg)	91.2 ± 23.0	106.9 ± 21.7
Peak Power Av (w)	976.0 ± 280.5	1078.6 ± 344.5

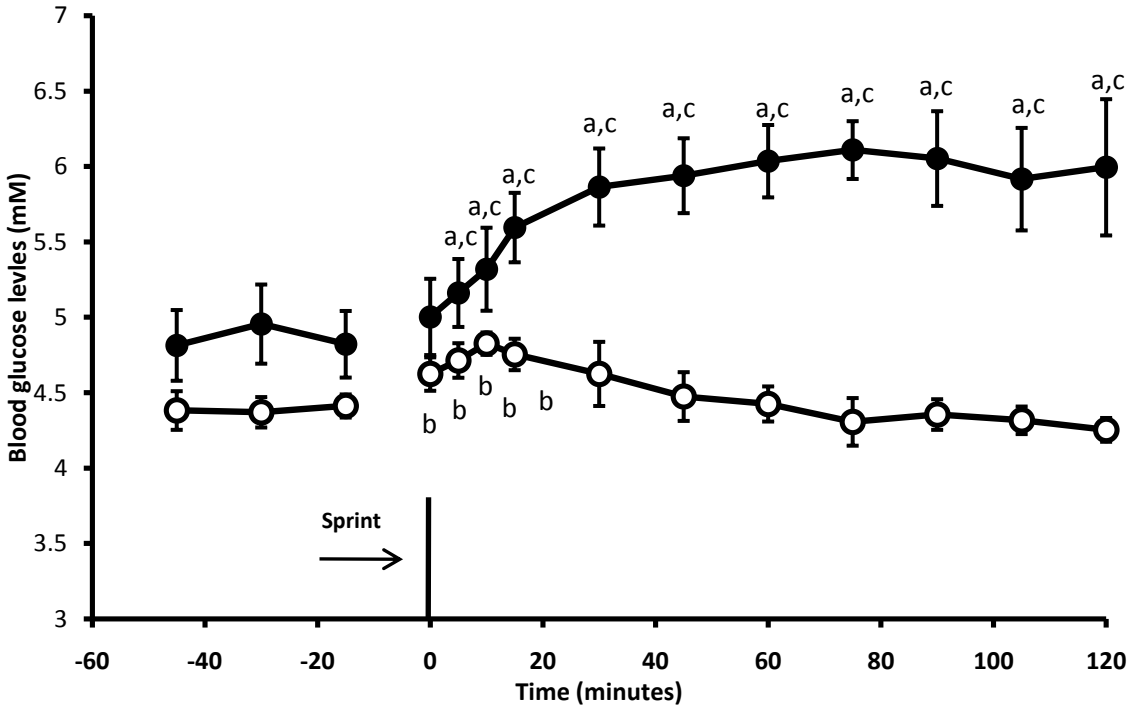

Figure 2.3 Blood glucose response to a 10-second maximal sprint in Type 1 diabetic and control participants. Values represent mean ± SEM. ○ Control, ● Type 1 diabetes. a, significant differences ($p<0.05$) with resting values (-15 minutes) in individuals with Type 1 diabetes. b, significant differences ($p<0.05$) with resting values(-15 minutes) in Control participants. c, significant differences ($p<0.05$) between Type 1 diabetic and Control participants.

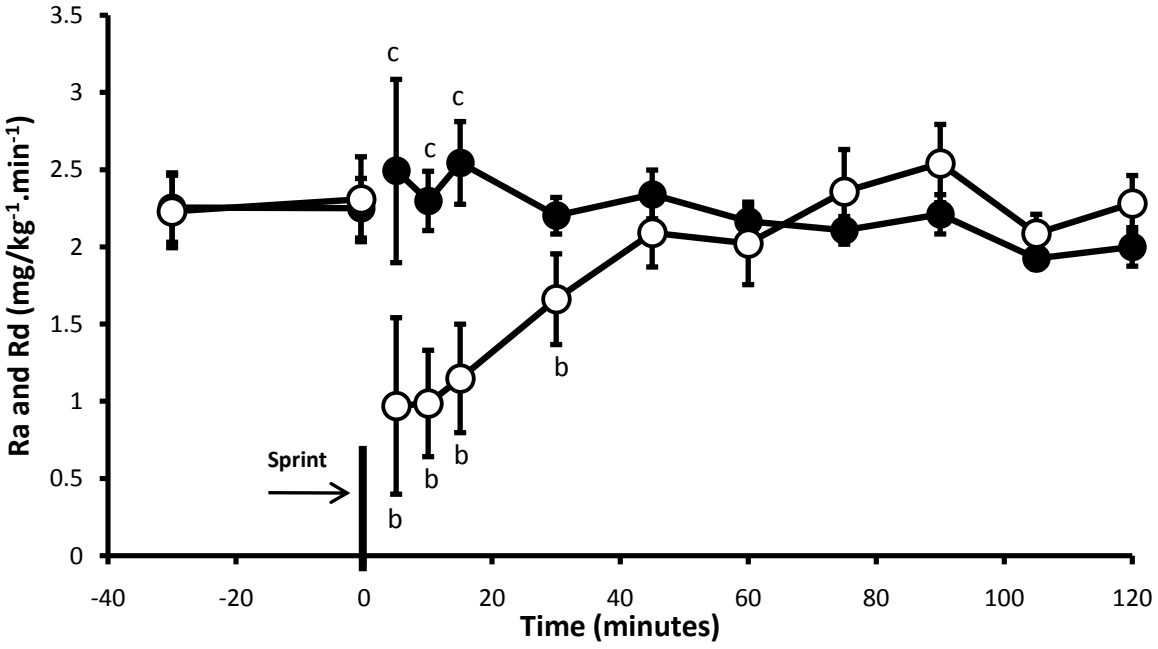

Figure 2.4 Glucose Ra and Rd in response to a 10-second maximal sprint in Type 1 diabetic participants. Values represent mean ± SEM. ● Ra, ○ Rd. b, significant differences ($p<0.05$) with pre-exercise Rd values. c, significant differences ($p<0.05$) between Ra and Rd.

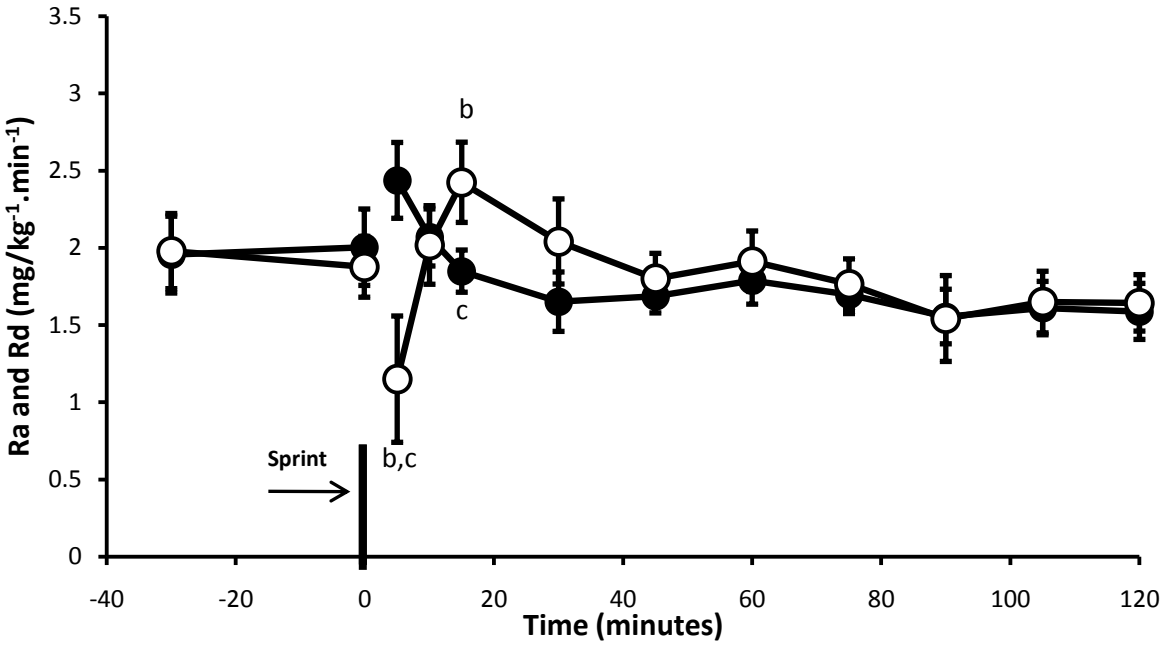

Figure 2.5 Glucose Ra and Rd in response to a 10-second maximal sprint in Control participants. Values represent mean ± SEM. ● Ra, ○ Rd. b, significant differences ($p<0.05$) with pre-exercise Rd values. c, significant differences ($p<0.05$) between Ra and Rd.

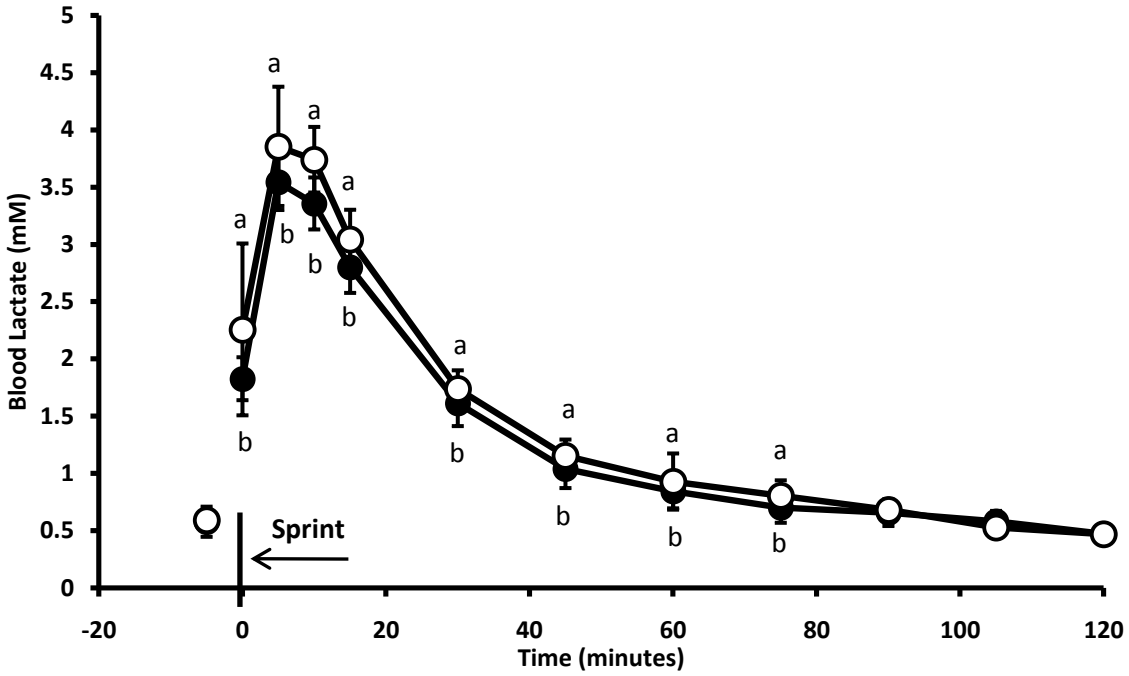

Figure 2.6 Blood lactate response to a 10-second maximal sprint in Type 1 diabetic and control participants. Values represent mean ± SEM. ○ Control, ● Type 1 diabetes. a, significant differences ($p<0.05$) with pre-exercise values in individuals with Type 1 diabetes. b, significant differences ($p<0.05$) with pre-exercise values in Control participants.

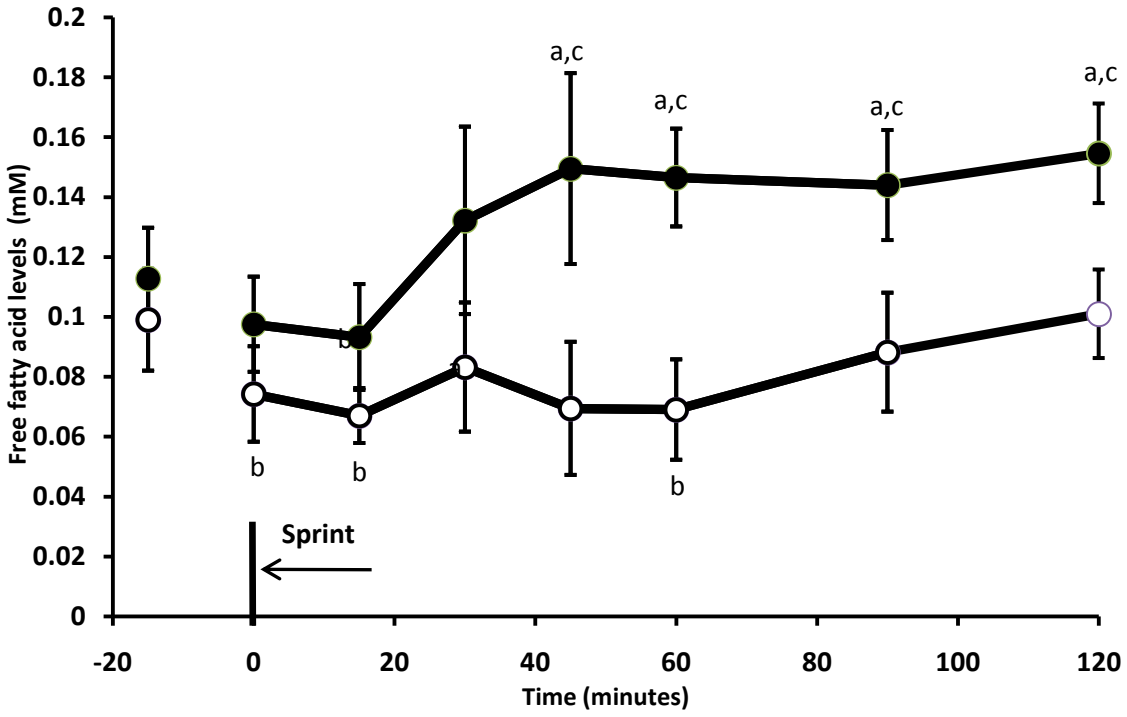

Figure 2.7 Plasma free fatty acid responses to a 10-second maximal sprint in Type 1 diabetic and control participants. Values represent mean ± SEM. ○ Control, ● Type 1 diabetes. a, significant differences ($p<0.05$) with pre-exercise values in individuals with Type 1 diabetes. b, significant differences ($p<0.05$) with pre-exercise values in Control participants. c, significant differences ($p<0.05$) between Type 1 diabetic and Control participants.

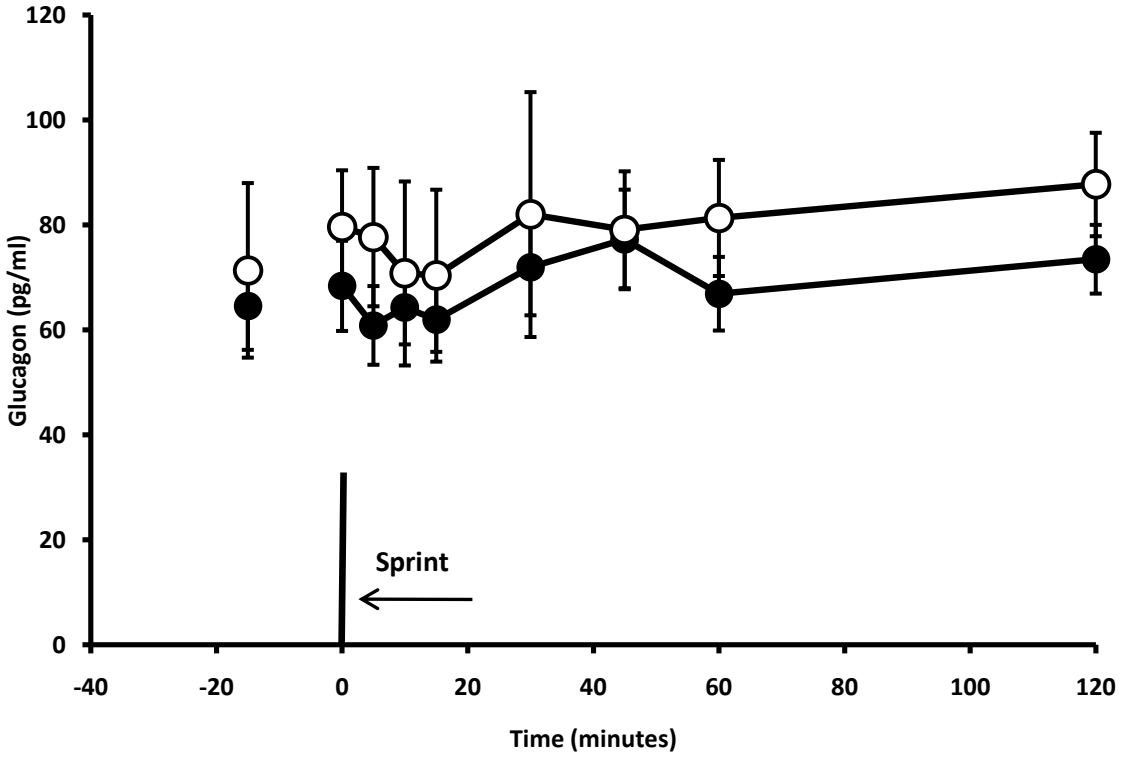

Figure 2.8 Plasma glucagon responses to a 10-second maximal sprint in Type 1 diabetic and control participants. Values represent mean ± SEM. ○ Control, ● Type 1 diabetes.

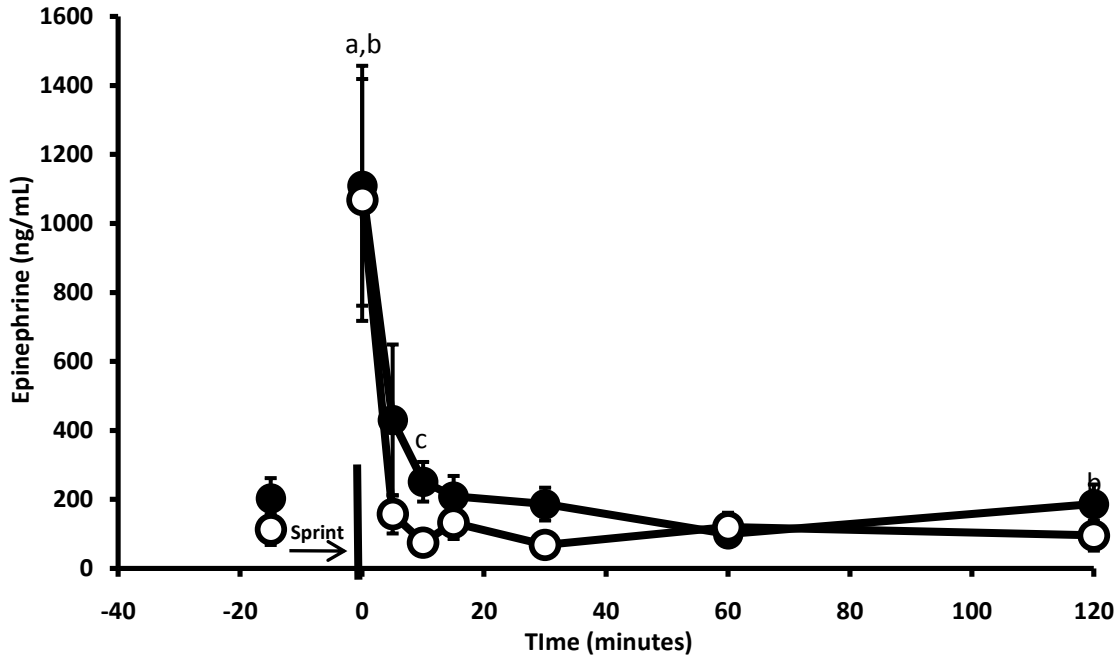

Figure 2.9 Plasma epinephrine responses to a 10-second maximal sprint in Type 1 diabetic and control participants. Values represent mean ± SEM. ○ Control, ● Type 1 diabetes. a, significant differences ($p<0.05$) with pre-exercise values in individuals with Type 1 diabetes. b, significant differences ($p<0.05$) with pre-exercise values in Control participants. c, significant differences ($p<0.05$) between Type 1 and Control participants.

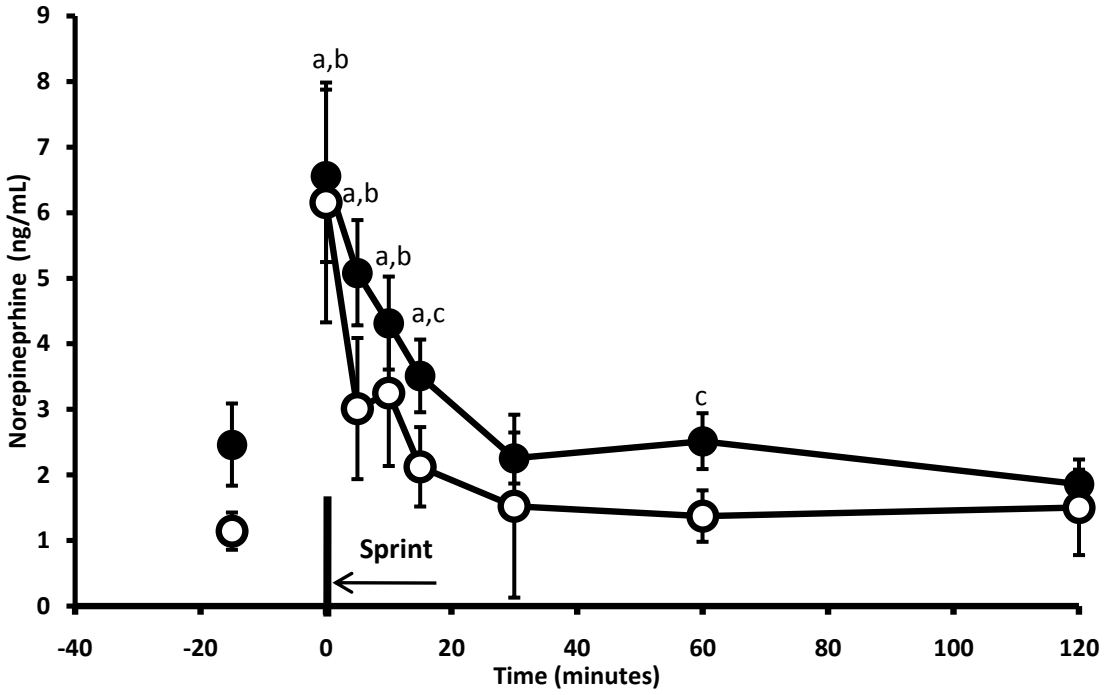

Figure 2.10 Plasma norepinephrine responses to a 10-second maximal sprint in Type 1 diabetic and control participants. Values represent mean ± SEM. ○ Control ●, Type 1 diabetes. a, significant differences ($p<0.05$) with pre-exercise values in individuals with Type 1 diabetes. b, significant differences ($p<0.05$) with pre-exercise values in Control participants. c, significant differences ($p<0.05$) between Type 1 and Control participants.

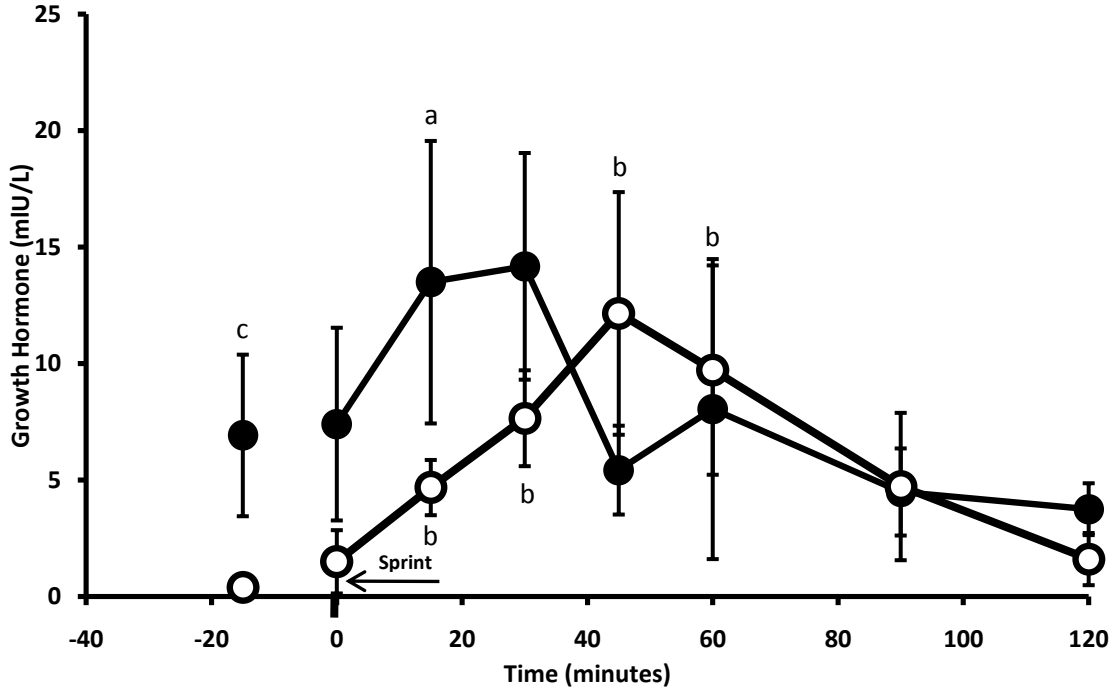

Figure 2.11 Plasma growth hormone responses to a 10-second maximal sprint in Type 1 diabetic and control participants. Values represent mean ± SEM. ○ Control, ● Type 1 diabetes. a, significant differences ($p<0.05$) with pre-exercise values in diabetic participants. b, significant differences ($p<0.05$) with pre-exercise values in Control participants. c, significant differences ($p<0.05$) between Type 1 diabetic and Control participants.

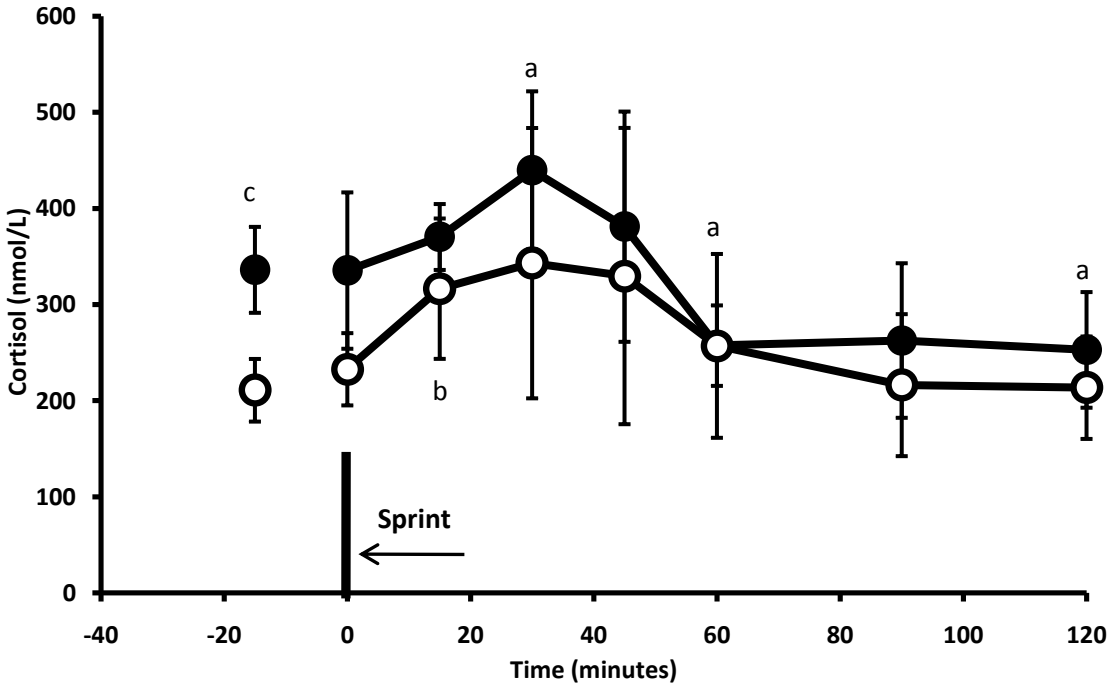

Figure 2.12 Plasma cortisol responses to a 10-second maximal sprint in Type 1 and control participants. Values represent mean ± SEM. ○ Control, ● Type 1 diabetes**.** a, significant differences ($p<0.05$) with pre-exercise values in individuals with Type 1 diabetes. b, significant differences ($p<0.05$) with pre-exercise values in Control participants. c, significant differences ($p<0.05$) between Type 1 diabetic and Control participants.

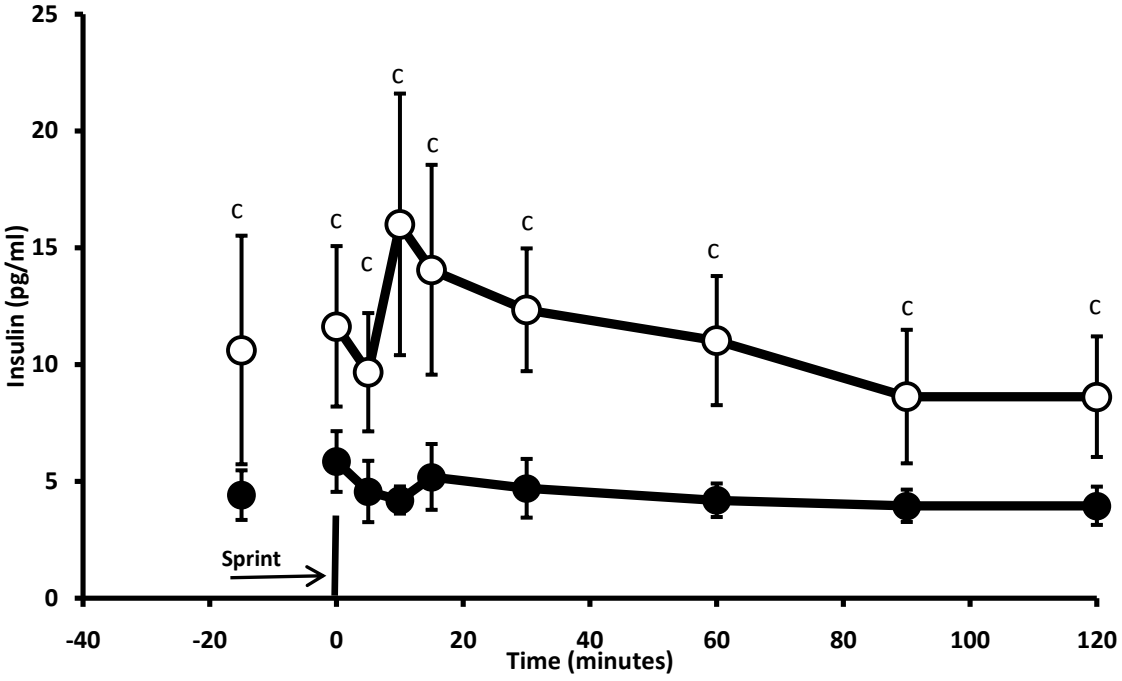

Figure 2.13 Plasma insulin response to a 10-second maximal sprint in Type 1 diabetic and control participants. Values represent mean ± SEM. ○ Control, ● Type 1 diabetes. c, significant differences ($p<0.05$) between Type 1 and Control participants.

2.4 Discussion and Conclusion

It is well established that due in part to a disproportionate increase in glucose Ra relative to glucose Rd, intense aerobic exercise (intensity $\geq$ 80% $\dot{V}O_2$max) can cause a rapid and sustained increase in blood glucose levels in individuals with Type 1 diabetes (Marliss *et al*., 1992; Mitchell *et al*., 1988; Purdon *et al*., 1993; Sigal *et al*., 1994b). These findings together with the recent observation that an exercise-mediated decrease in blood glucose level can be opposed by a single 10-second sprint (Bussau *et al*., 2007) led us to examine whether a 10-second sprint under basal insulinaemia (insulin levels where the rate of glucose production matches the rate of glucose utilisation under euglycaemic conditions) may be sufficient to cause a rapid increase in blood glucose levels in individuals with Type 1 diabetes. Here we show for the first time that this is the case, with a single 10-second maximal sprint effort under basal insulinaemia leading to a significant 1.04 ± 0.58 mmol/l ($p<0.001$) sustained post-exercise increase in blood glucose levels in diabetic individuals, and a smaller and transient rise in blood glucose levels in non-diabetic control individuals. This post-exercise rise in glycaemia in both diabetic and non-diabetic individuals is due to a decrease in glucose Rd in the presence of stable glucose Ra. Such a post-exercise pattern of glucose kinetics has never been reported before and differs markedly from that seen in response to prolonged intense aerobic exercise.

During recovery from a 10-second sprint, the patterns of increase in blood glucose levels in diabetic and non-diabetic individuals share some similarities with those associated with prolonged aerobic exercise of high intensity. Indeed, in response to heavy aerobic exercise at >80% $\dot{V}O_2$max, diabetic individuals experience a rise in glycaemia, albeit larger than that reported here, which remains elevated for several hours afterwards (Howlett *et al*., 1999b;

Kreisman *et al*. 2003; Manzon *et al*., 1998; Marliss *et al*., 1991; Mitchell *et al*., 1988; Purdon *et al*., 1993; Sigal *et al*., 1994b; Sigal *et al*., 1996; Sigal *et al*., 1999). Moreover, in agreement with others, non-diabetic individuals experience a similar early rate of increase in blood glucose levels compared to diabetic participants followed by a return to pre-exercise levels (Kreisman *et al*., 2000; Marliss *et al*., 2000; Purdon *et al*., 1993; Sigal *et al*, 1994b; Sigal *et al*., 2004). However, the change in mean maximal blood glucose level attained during recovery in non-diabetic individuals was less here (0.41 mmol/l) than that in the diabetic participants (1.04 mmol/l). Others have also reported such a transient increase in blood glucose level in response to sprinting in non-diabetic individuals (Moussa *et al*., 2003; Schnabel *et al*., 1984; Vincent *et al*., 2004).

The mechanism whereby glycaemia increases in response to a 10-second sprint in Type 1 diabetic and non-diabetic individuals is markedly different from that associated with intense aerobic exercise. Here, the 10-second sprint did not result in any significant post-exercise change in glucose Ra in the diabetic and non-diabetic participants, with glucose Ra remaining stable throughout the duration of the recovery period in both groups. Instead, it is the decrease in glucose Rd below both pre-exercise Rd and glucose Ra levels that explains the modest (albeit significant) post-exercise rise in blood glucose levels associated with sprinting in diabetic individuals. Similarly, the significant decrease in glucose Rd relative to glucose Ra during early recovery in the non-diabetic participants explains their small transient rise in blood glucose levels. The mechanisms underlying the increase in blood glucose levels in response to a 10-second sprint in diabetic and non-diabetic individuals thus differ from those associated with intense aerobic exercise where the rise in blood glucose levels during exercise has been shown to be due to a disproportionate

increase in glucose Ra relative to the rise in glucose Rd (Kreisman *et al*., 2003; Marliss *et al*., 1991; Marliss & Vranic, 2002; Purdon *et al*., 1993; Sigal *et al*., 1994b). It is important to stress, however, that although the sprint-mediated decrease in glucose Rd reported here has not been reported elsewhere in the literature on humans, mainly because this issue has never been examined before, a similar finding has been reported in streptozotocin-induced diabetic rats where a short bout of high intensity exercise has been shown to cause a fall in the rate of glucose utilisation in skeletal muscles (Ferreira *et al*., 2005).

A few mechanisms may be proposed to explain how a 10-second sprint inhibits glucose Rd during early recovery in diabetic and non-diabetic individuals. Firstly, the increase in catecholamines levels immediately following sprinting together with earlier published evidence that catecholamines inhibit muscle glucose uptake at rest (Aftab Guy *et al.,* 2005) and during exercise (Howlett et al., 1999a; Watt and Hargreaves, 2002; Watt *et al*., 2001) might explain the post-exercise decrease in glucose Rd in the diabetic and control groups. However, it is important to note that others have shown that catecholamines infusion during exercise has either little effect on glucose Rd (Howlett *et al*., 1999b) or enhances rather than inhibits glucose Rd (Kreisman *et al*., 2000, 2001, 2003), thus suggesting that other mechanisms might be involved to explain the sprint-mediated fall in glucose Rd. Maybe, the rise in growth hormone levels early during recovery in diabetic but not in control non-diabetic individuals might contribute to the early post-exercise decrease in Rd and rise in blood glucose levels, since a sudden increase in growth hormone levels can acutely inhibit glucose Rd in non-diabetic individuals (Møller *et al*., 1990, 1992b). Unfortunately, the finding that glucose Rd in our non-diabetic participants also fell to a similar extent after sprinting despite no early increase in growth hormone levels together with the observation

that a growth hormone bolus in diabetic individuals is without any effect on glucose Rd (Møller *et al*., 1992a) brings into question this interpretation. Similarly, the increased cortisol levels post-exercise are unlikely to mediate the fall in glucose Rd because this fall precedes the rise in cortisol levels, and cortisol-mediated inhibition of muscle glucose utilisation may take several hours to occur (Shamoon *et al*., 1980). Finally, one metabolic factor that may have played a role in mediating the decrease in glucose Rd is the likely build-up of intramuscular glucose-6-phosphate normally associated with rapid glycogen breakdown, since high levels of this metabolite can inhibit muscle glucose utilisation via inhibition of hexokinase (Wasserman, 1995, Watt *et al*., 2001). However, in the absence of any measurement of this metabolite, its role in the fall in glucose Rd post-sprint remains to be established.

One interesting finding was the absence of an increase in glucose Ra in both experimental groups despite elevated post-exercise catecholamines levels. This is surprising relative to the evidence that catecholamines play an important role in the activation of glucose Ra associated with intense aerobic exercise (Marliss & Vranic, 2002; Kreisman *et al*, 2001, 2002, 2003). Maybe the increase in catecholamines levels was too small for the effective stimulation of glucose Ra. In support of this view, a much larger increase in catecholamines levels has been reported to accompany the increase in glucose Ra during intense aerobic exercise (Kreisman *et al*., 2003; Marliss & Vranic, 2002), and the lesser rise in catecholamines levels associated with moderate intensity exercise plays little or no role in the stimulation of glucose Ra (Coker & Kjaer, 2005; Kjaer et al., 1993; 1995; Watt & Hargreaves, 2002). Alternatively, our findings might be taken as further evidence in support of the view that catecholamines contribute little to the rise in glucose Ra during

intense exercise (Coker & Kjaer, 2005; Howlett *et al*., 1999; Wahren & Ekberg, 2007), thus suggesting the involvement of other factors.

The fact that insulin levels in the diabetic group were maintained at basal and stable levels makes it unlikely that insulin would have had an inhibitory effect on glucose Ra, particularly considering that high insulin levels have little inhibitory effect on the rise in hepatic glucose production associated with intense aerobic exercise (Sigal *et al*., 1996). Arguably, the absence of changes in plasma glucagon and insulin levels in response to sprinting would explain the lack of increase in glucose Ra if these hormones were to be the main regulator of hepatic glucose production during a short sprint and if a similar pattern of change in the levels of these hormones were to occur in the portal circulation. However, the main problem with this interpretation is that although insulin and glucagon play an important role in the stimulation of glucose Ra during moderate intensity exercise (Wasserman *et al*., 1989a,b; Marker *et al.*, 1991; Wolfe *et al.*, 1986), these hormones play a role of minor importance compared to catecholamines in the stimulation of glucose Ra during intense aerobic exercise (Marliss & Vranic, 2002; Manzon *et al*., 1998; Sigal *et al*., 1996). Clearly, the mechanism underlying the absence of increase in glucose Ra in response to a 10-second sprint remains to be elucidated.

As recovery progresses, the absence of a fall in blood glucose levels after exercise in the diabetic participants might be explained on the basis of their lack of post-exercise increase in plasma insulin levels. In support of this view, are the findings that following a prolonged bout of intense aerobic exercise ($\geq$ 80% $\dot{V}O_2$max), plasma insulin levels in non-diabetic individuals increase together with the rise in blood glucose levels (Manzon *et al*.,

1998; Marliss *et al*., 1991; Purdon *et al*, 1993) thus causing a subsequent fall in blood glucose to pre-exercise levels. A non-significant trend here for a post-exercise increase in insulin levels in our non-diabetic participants supports further this interpretation and may explain the rapid return of their blood glucose exercise to pre-exercise levels. In contrast, since insulin levels in our diabetic participants were not affected by exercise and remained at stable and low levels throughout recovery, this most probably explains the absence of a fall in their blood glucose level post-exercise.

In conclusion, this study is important as it shows that a sprint as short as 10 seconds can increase blood glucose levels in Type 1 diabetic individuals when insulin are at near basal levels as is normally the case during night-time, before morning insulin injection, or late in the afternoon. It is also noteworthy that the mechanisms underlying this increase in glycaemia is unique and unlike that associated with intense aerobic exercise. Although the increase in blood glucose levels is modest, it is important to note the potential to protect against hypoglycaemia as observed in earlier findings that a 10-second sprint can oppose an exercise-mediated decrease in blood glucose levels (Bussau *et al*., 2006), thus providing an effective tool for the management of blood glucose levels and the prevention of hypoglycaemia in individuals with complication free Type 1 diabetes.

2.5 Recommendations and areas for further study

Whilst the results of this study show that a ten-second sprint can increase blood glucose levels in diabetic and non-diabetic individuals, it must be noted that the diabetic participants involved in our study were young and free of diabetic complications. For this

reason, before this type of exercise is incorporated into guidelines for the prevention of hypoglycaemia, further research on other segments of our population is required, particularly on children, the elderly, and those individuals with diabetic complications and with variable levels of glycaemic control. Of further interest is the influence of increasing the levels of circulating insulin on the hyperglycaemic effect of sprinting, and whether the response observed in the current study would be similar during hypoglycaemia in diabetic individuals.

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