The Effects of a Progressive Dehydration Protocol on Glycaemic Response During and Following Exercise in Patients with Type 1 Diabetes

Mr Andrew Wilson

Student ID: 2119697

Physiology, Exercise and Nutrition Research Group,
Faculty of Health Sciences and Sport,
University of Stirling

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To Barney, thanks for the endless walks and paws throughout University, almost all the way to the end. Run free.

*

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Declaration

I confirm that the entire contents of the current thesis are all my own work, apart from where external expertise is specifically acknowledged in the methods section, and I understand the consequences of academic misconduct. I confirm that all data utilised for this thesis has been handled and processed in accordance with Data Protection Act 1998. The contents of this thesis are in accordance with the guidelines and requirements of the Faculty of Health Sciences and Sport, University of Stirling.

Abstract

The aim of this study was to assess whether progressively dehydrating Type 1 Diabetes Mellitus (T1DM) patients via fluid restriction around exercise affected glucose control during and following exercise, compared to euhydrated T1DM patients. It is hoped that this study can aid the formation of T1DM-specific fluid intake guidelines around exercise, based on alterations in glucoregulation and osmoregulation.

The fluid restriction protocol induced a mild level of dehydration (1.1% body mass loss from pre-exercise measurements). Furthermore, dehydrated subjects displayed significantly greater post-exercise serum copeptin concentration ($p<0.01$), compared to resting and euhydrated subjects' values. There was a trend for a greater glycaemic response during exercise and the post-exercise recovery period of the dehydration trial, but there were no significant differences in either the interstitial or blood glucose responses between trials. Although baseline serum glucagon concentrations were significantly different between trials ($p<0.01$), thereafter there were no significant differences between trials at any remaining timepoint. There was a statistically significant effect of trial on serum cortisol concentrations ($p<0.01$), with post-exercise serum cortisol concentrations remaining significantly greater during the dehydration trial, compared to euhydrated subjects'.

Up to 48 hours following the dehydration trial, there was a significantly reduced prevalence of mild hyperglycaemia compared to the same period following the control trial ($p<0.01$), with a trend for a concomitant increase in euglycaemic interstitial glucose measurements. The discrepancies in acute glycaemic control occurred without an increased risk of hypoglycaemia, and were not attributable to any significant differences in total carbohydrate intake or total units of insulin administered. Further research is required to establish the intramuscular and intracellular physiology linking dehydration with alterations in whole-body and tissue-specific glucose metabolism. T1DM patients must therefore balance the potential short-term benefits of mild dehydration on acute glycaemic control, with long-term health consequences associated with regular dehydration and elevated vasopressin concentrations.

Contents

Introduction

Patients with Type 1 (insulin-dependent) Diabetes Mellitus (T1DM) who regularly participate in exercise of varying mode, intensity and duration display improvements in cardio-metabolic health and psychological wellbeing, along with a reduced risk of all-cause mortality and developing disease-related complications (Chimen et al, 2007; Kennedy et al, 2013; Yardley et al, 2014; Riddell et al, 2017). Despite the chronically impaired glucoregulation associated with T1DM, the benefits of regular physical activity outweigh the immediate risks if appropriate glycaemic management strategies are implemented (Riddell et al, 2017). However, there are several challenges and perceived barriers to T1DM patients participating in physical activity, including a fear of losing glycaemic control around exercise (Lascar et al, 2014; Riddell et al, 2017). Stable, regimented glycaemic control is considered by both patients and healthcare professionals to be the primary method of effectively managing T1DM (Kennedy et al, 2013; Fava et al, 2014).

The elevated circulating glucose concentration (hyperglycaemia) symptomatic of poorly controlled T1DM arises due to the immune-mediated destruction of pancreatic islet beta cells, which severely or wholly impairs the regulation of endogenous insulin secretion (McCrimmon and Sherwin, 2010; Younk et al, 2011; Horton and Subauste, 2016). T1DM patients are therefore reliant on administration of exogenous replacement insulin as a method of managing circulating glucose concentrations. Poorly controlled T1DM will result in chronically elevated blood glucose concentrations and elevated glycated haemoglobin (HbA1c) levels- a marker of long-term glycaemic control. There is a significant positive correlation between each of these outcomes and the risk of developing macrovascular complications and long-term disease-specific complications including retinopathy, neuropathy and nephropathy (Cryer et al, 2002; Fullerton et al, 2014). However, attempts to reduce average blood glucose concentrations via intensive glycaemic management may concurrently increase the risk of hypoglycaemia arising (Cryer et al, 2002; Younk et al, 2011, Fullerton et al, 2014). The exogenous insulin administered by T1DM patients is not regulated by the same negative feedback mechanism as the endogenous insulin secreted by healthy, disease-free subjects in response to a rise in blood glucose (Diedrich et al, 2002; McCrimmon and Sherwin, 2010; Younk et al, 2011). As a result, administration of an excessive dose of insulin will likely

result in hypoglycaemia. The risk of hypoglycaemia arising in T1DM patients with tight glycaemic control may be further exacerbated due to defective pancreatic islet alpha cell function, and therefore defective secretion of glucagon (Diedrich et al, 2002; McCrimmon and Sherwin, 2010; Younk et al, 2011). Glucagon is the principle counter-regulatory hormone secreted as part of the overall homeostatic response to hypoglycaemia, whereby the release of glucagon from the pancreatic alpha cells stimulates an increased rate of hepatic endogenous glucose production (Cryer, 2006; Yosten, 2018). However, there is a progressive decline in alpha cell sensitivity to changes in blood glucose concentration in T1DM patients over time, which is thought to arise due to the absence of regulatory signalling pathways from the progressively declining pancreatic beta cell mass (Cryer, 2006; Hughes and Narendran, 2014; Yosten, 2018). Alpha cell function is completely dysregulated with advanced T1DM (>10 years of diagnosis), leading to an attenuated rate of glucagon secretion in response to hypoglycaemia, subsequently increasing the risk of severe hypoglycaemia arising (McCrimmon and Sherwin, 2010; Hughes and Narendran, 2014; Yosten, 2018). Furthermore, there may also be a paradoxical increase in glucagon secretion from the alpha cells in a post-prandial state with advanced T1DM, potentially leading to further glycaemic fluctuations (Hughes and Narendran, 2014; McNeilly and McCrimmon, 2018; Yosten, 2018). In contrast, the overcorrection of hypoglycaemia via carbohydrate supplementation may lead to an increased prevalence of rebound hyperglycaemia. This may subsequently begin a recurring cycle of further glycaemic disturbances with repeated treatment of hypoglycaemia, followed by hyperglycaemia.

The average incidence of symptomatic hypoglycaemia is estimated at twice per week for T1DM patients (McCrimmon and Sherwin, 2010) and negatively affects cognitive and cardio-respiratory function. Hypoglycaemia initially arises due to relative systemic hyperinsulinemia (administration of an excessive dose of exogenous insulin), combined with a defective counter-regulatory physiological response to hypoglycaemia associated with advanced T1DM (Cryer, 2006; McCrimmon and Sherwin, 2010; McNeilly and McCrimmon, 2018). Research has consistently highlighted that following previous hypoglycaemia, including mild asymptomatic episodes, T1DM patients display an impaired neuro-endocrine counter-regulatory response to subsequent episodes of hypoglycaemia (Diedrich et al, 2002; McCrimmon and Sherwin, 2010; McNeilly and

McCrimmon, 2018). The attenuated glucagon response to hypoglycaemia, coupled with the inability to regulate circulating insulin concentrations, is compounded by an attenuated sympathetic neural response to hypoglycaemia- namely the decreased secretion of catecholamines (Cryer, 2006; McCrimmon and Sherwin, 2010; Mcneilly and McCrimmon, 2018). Hypoglycaemia leads to an increase in the glycaemic threshold (lower blood glucose concentration) required to stimulate the magnitude of epinephrine response to prevent a further decline in blood glucose concentrations during any subsequent hypoglycaemic episodes. Furthermore, the sympathetic neural response is closely associated with the symptomatic response to hypoglycaemia, which is comprised of autonomic (e.g. sweating, palpitations) and neuroglycopenic (e.g. confusion, drowsiness) symptoms (Cryer, 2006). T1DM patients may therefore lose the ability to recognise the onset of hypoglycaemia due to less prominent symptoms occurring at a defined hypoglycaemic concentration (Cryer, 2012; McNeilly and McCrimmon, 2018). The delay in recognising and treating the symptoms of hypoglycaemia (Impaired Awareness of Hypoglycaemia- IAH) will increase the risk of severe hypoglycaemia arising (McNeilly and McCrimmon, 2018). This cluster of attenuated physiological responses to antecedent hypoglycaemia is known as Hypoglycaemia Associated Autonomic Failure (HAAF), where there is an increased risk of recurrent and/or severe hypoglycaemia due to the impaired counter-regulatory responses and impaired awareness of hypoglycaemia (Cryer, 2006; Younk et al, 2011). Repeated or severe hypoglycaemic episodes, where external medical assistance is required due to physiological and neural dysfunction, may lead to a coma or death. However, avoidance of hypoglycaemia over a short duration has a protective effect against the development of HAAF and further hypoglycaemia arising (Diedrich et al, 2002; Younk et al, 2011). T1DM patients must therefore balance the avoidance of hypoglycaemia with the aim of attaining the desired level of glycaemic control, which may include participation in exercise.

Longitudinal studies and meta-analyses have shown that regular exercise is positively associated with improvements in indices of glycaemic control, including reductions in daily insulin requirements (via increased insulin sensitivity and glucose tolerance), decreased prevalence of severe hypoglycaemia and increased hypoglycaemia awareness (Kennedy et al, 2011; Fullerton et al, 2014; Riddell et al, 2017). Furthermore, improvements in insulin delivery and glucose monitoring technology, coupled with

differing strategies focusing on improved glucose control via the manipulation of exercise-based nutrition and differing exercise modalities, have aided safer participation in exercise (Riddell and Perkins, 2006; Horton and Subauste, 2016). There is no consensus on the 'optimal' type, duration, intensity or mode of exercise to maintain glucose within a desired range due to the individual nature of the glycaemic response to exercise (Basu et al, 2014; Colberg et al, 2016). There are many differences in the endocrine responses of healthy, non-DM individuals and T1DM patients during various modes of exercise, which may explain some of the glucoregulatory challenges faced by T1DM patients. In non-diabetic individuals, there is an immediate reduction in insulin secretion from pancreatic beta cells, coupled with an increase in glucagon secretion from the pancreatic alpha cells and elevated epinephrine concentrations during the initial stages of moderate intensity exercise. Endogenous insulin secretion in T1DM patients is either dysregulated or wholly absent, and therefore only the rate of absorption and clearance of exogenous insulin will lead to a decline in systemic insulin concentrations. However, the glucagon response during exercise is maintained in T1DM patients, provided there is no threat of hypoglycaemia arising (Younk et al, 2011). The increased glucagon acts synergistically with a gradually increased epinephrine concentration to stimulate an increase in hepatic glucose production beyond the rate of muscle glucose uptake. Higher intensity exercise or resistance exercise leads to the increased secretion of counter-regulatory hormones including cortisol, epinephrine and norepinephrine in both non-DM individuals and T1DM patients. The elevated counter-regulatory hormone concentrations may spare hepatic or muscle glucose stores, while also transiently increasing the circulating glucose concentration. There are a variety of additional exercise-based factors including the insulin dosing regime, nutritional intake and training status that T1DM patients must consider and manage to minimise significant glycaemic excursions (hypoglycaemia and hyperglycaemia), coupled with regularly monitoring circulating glucose concentrations (Riddell et al, 2017).

The fear of hypoglycaemia is the primary barrier to T1DM patients achieving the desired level of glycaemic control, while the risk of post-exercise hypoglycaemia arising negatively affects T1DM patients' participation in exercise (Younk et al, 2011; Colberg et al, 2016; Riddell et al, 2017). The risk of post-exercise hypoglycaemia is elevated up to 24 hours following exercise, particularly during the initial overnight period following evening

exercise, due to the blunting of counter-regulatory responses to hypoglycaemia combined with an increased peripheral insulin sensitivity and glucose uptake respectively (Diedrich et al, 2002; Younk et al, 2011; Riddell et al, 2017). Hypoglycaemia often arises following exercise due to the failure to adequately reduce the administered exogenous insulin dose under conditions of increased insulin sensitivity. T1DM patients must consider the gradual decline in glucose concentrations often observed during continuous, moderate intensity exercise coupled with the elevated peripheral (skeletal muscle) insulin sensitivity due to the contraction-mediated upregulation of GLUT4 translocation (Bussau et al, 2006; Campbell et al, 2013). Furthermore, there is an increased requirement to replenish the depleted glycogen stores with any ingested carbohydrates, which is aided by the increased GLUT4-mediated uptake of glucose (Campbell et al, 2013; Riddell et al, 2017). Hypoglycaemia prior to commencing exercise may also impair subsequent glucoregulation during and following exercise due to the impairments in counter-regulatory hormone secretion (McCrimmon and Sherwin, 2010; Younk et al, 2011). Furthermore, research has also shown that T1DM patients who completed a moderate intensity exercise bout displayed an impaired counter-regulatory neuro-endocrine response to hypoglycaemia up to 24 hours following the initial exercise bout (Diedrich et al, 2002; Younk et al, 2011).

Inadequate knowledge of strategies to combat any significant changes in glucose concentrations during exercise is also considered to be a significant barrier to T1DM patient's participation in physical activity (Colberg et al, 2016; Riddell et al, 2017). To date, several different pre-exercise and post-exercise interventions have been primarily utilised to prevent post-exercise hypoglycaemia. Bussau et al (2006) showed that completion of a short, supramaximal sprint at the culmination of continuous, moderate intensity exercise prevented a decline in immediate post-exercise glucose concentrations under hyperinsulinemic conditions. Previous research has also successfully manipulated pre- and post-exercise basal and bolus insulin regimens (Rhabasa-Lloret et al, 2001; Campbell et al, 2013), macronutrient intake (West et al, 2011; Campbell et al, 2014) and timing of exercise (Gomez et al, 2015) to prevent post-exercise hypoglycaemia occurring, particularly late-onset (nocturnal) hypoglycaemia. However, no research to date has assessed the effects of variable fluid intake during and following exercise on T1DM patient's acute or longer-term glycaemic control. Furthermore, all exercise-based

interventions to aid post-exercise glycaemic control to date have all advocated adequate hydration during and following exercise, based on current population-wide hydration guidelines, including diabetic patient population cohorts.

There is a dearth of research that has investigated the relationship between fluid intake and glucoregulation. High daily water intake has been shown to have an overall positive effect on management of Diabetes Mellitus symptoms, whereby there was an inverse relationship evident between self-reported daily water intake and the risk of mild hyperglycaemia arising (Roussel et al, 2011). Furthermore, Enhörning et al (2019) showed that 6 weeks of water supplementation (1.5L increased daily water intake) led to a significantly reduced urine osmolality, increased 24 hour urine volume and a significant reduction in fasting blood glucose concentration of non-diabetic subjects. However, there was no significant difference between Type 2 Diabetes Mellitus (T2DM) patients' mean blood glucose concentration following acute water supplementation (additional 3L of water per day), compared to patients who consumed fluids at their habitual rate. The multi-directional relationship between fluid intake and glucoregulation is also highlighted by the effects of chronic hyperglycaemia, and subsequently excess urinary glucose excretion, which stimulates increased glucose-induced osmotic diuresis. The increased glucose-induced fluid losses increase the fluid intake requirements for T1DM patients to avoid dehydration (Buiote-Stella et al, 2018). No research to date has directly assessed the effect of variable fluid intake on changes in T1DM patients' blood glucose concentration at rest or during exercise under euglycaemic conditions. Furthermore, it is unclear whether acute or chronic changes in fluid intake are required to stimulate any significant differences in acute and longer-term glycaemic control. The physiological link between changes in hydration status and glycaemic control is arginine vasopressin, commonly known as antidiuretic hormone.

T1DM and Vasopressin Physiology

Arginine vasopressin (AVP; herein referred to as vasopressin) is primarily synthesised in the hypothalamus of the brain, where it is then transported into and stored within the neurohypophysis (posterior pituitary) for secretion (Ahloulay et al, 1999; Bankir et al, 2001; Rotondo et al, 2016). The primary function of vasopressin is to regulate whole-body fluid balance via the maintenance of serum osmolality within a narrow, defined physiological range (Hew-Butler, 2010). The rate of vasopressin secretion is primarily determined by fluctuations in serum osmolality detected by peripheral osmoreceptors, although relatively greater changes in blood volume or blood pressure may also stimulate vasopressin secretion (Bankir et al, 2001; Hew-Butler, 2010). Zerbe et al (1985) initially highlighted an elevated basal vasopressin concentration in T1DM patients, compared to disease-free subjects. The elevated basal vasopressin concentrations of T1DM patients contrasts the polyuria which is symptomatic of uncontrolled or undiagnosed T1DM, whereby excess glucose is excreted in the urine (glycosuria), stimulating increased fluid losses (Bankir et al, 2001). Elevated vasopressin concentrations have been reported in T1DM patients independent of the level of glycaemic control (Zerbe et al, 1985; Bouby et al, 2014). The increased vasopressin concentration is considered to be a necessary physiological adaptation that stimulates increased fluid conservation and minimises further fluid losses via glucose-induced osmotic diuresis (Ahloulay et al, 1999; Bankir et al, 2001). Vasopressin released from the posterior pituitary binds with V2 receptors- one of three sub-classes of vasopressin receptor, located on the basolateral membrane of the renal collecting ducts (Rotondo et al, 2016). An osmotically-driven increase in serum vasopressin concentration stimulates the endogenous synthesis and insertion of aquaporin-2 channels into the apical membrane of the collecting duct, upregulating the rate of renal fluid reabsorption and maintaining intracellular fluid composition (see Figure 1) (Hew-Butler, 2010; Rotondo et al, 2016; Guelinckx et al, 2016). The increased permeability of the kidney to water and sodium reabsorption by the increase in vasopressin concentration results in the production of a reduced volume of increasingly concentrated urine.

Changes in plasma vasopressin concentration have been associated with differing fluid intake patterns at rest, under free-living conditions and during exercise across previous studies with non-DM subjects. Chronically reduced daily water intake (<1.2L/day

of total fluid intake) led to a significant increase in basal vasopressin concentrations compared to subjects consuming >2L of water per day (Moscogiuri et al, 2018). Furthermore, individuals with habitually low fluid intake display increased circulating vasopressin concentrations, decreased 24 hour urine volume and increased urine concentration (Guelinckx et al, 2016). In contrast, subjects who consumed a small volume of additional water beyond their habitual fluid intake level displayed decreased vasopressin concentrations for up to 4 hours post-water consumption, compared with subjects who only consumed fluids at a habitual rate. Increased total daily water intake over a 1 week period was also associated with a 15% decrease in basal vasopressin concentration (Enhorning et al, 2019), while acute water supplementation (additional 1.5L/day) for 6 weeks led to a significant reduction in plasma vasopressin concentration, along with a reduced 24 hour urine osmolality and increased 24 hour urine volume. During exercise, studies have consistently shown that partial or total fluid restriction around exercise stimulates a significant increase in plasma osmolality, which subsequently stimulates an increase in post-exercise vasopressin concentrations, compared to euhydrated subjects. McConnell et al (1997) and Melin et al (2001) highlighted that a total fluid restriction protocol implemented throughout prolonged, continuous exercise led to >2-fold increase in serum vasopressin concentrations and a significantly increased plasma sodium concentration (indicative of increased serum osmolality), compared to euhydrated subjects. Furthermore, changes in vasopressin concentrations were directly aligned with the largest, statistically significant increase in both plasma osmolality and body mass loss induced via fluid restriction and exercise in the heat, compared to pre-exercise measurements (Montain et al, 1997; Maresh et al, 2004). It is possible that during exercise, increased vasopressin concentrations and plasma osmolality may be more closely related to the exercise intensity rather than an individual's hydration status. However, consumption of 300ml of water prior to maximal exercise was shown to impair the anticipated exercise-induced increase in vasopressin concentration (Wade and Claybaugh, 1980).

While the primary osmoregulatory function of vasopressin has been intensively studied, an osmotically-mediated increase in vasopressin secretion also stimulates several diverse metabolic signalling pathways that may influence glucose homeostasis. Exogenous administration of a physiologically excessive vasopressin dose leads to a

transient increase in circulating glucose concentration (Abu-Basha et al, 2002). The release of vasopressin is not regulated by an increased requirement for elevated glucose production, but the potential increase in glucose output mediated by vasopressin may subsequently affect the osmotically-induced release of vasopressin (Thompson et al, 1989). Figure 1 outlines the various interactions of vasopressin secreted under hypohydrated conditions (increased plasma osmolality, or large increase in blood pressure/reduction in blood volume) with the differing receptors and subsequent proposed effects on whole-body glucose metabolism. V1a receptors are present on both the liver and adrenal gland, where vasopressin binds and stimulates an upregulated rate of hepatic glucose production and increases adrenal cortisol secretion respectively (Perraudin et al, 1993; Mavani et al, 2015). V1b receptors are present on the pancreatic islet alpha cells, where vasopressin secretion stimulates the release of glucagon and subsequently affects hepatic glucose metabolism (Mavani et al, 2015; Rotondo et al, 2016). Furthermore, V1b receptors are also present on the anterior pituitary, where vasopressin either augments the corticotrophin-releasing hormone (CRH)-stimulated increase in adrenocorticotropin-releasing hormone (ACTH), or directly stimulates the secretion of ACTH (Mavani et al, 2015). ACTH secretion ultimately stimulates cortisol release from the adrenal cortex, leading to an increased blood glucose concentration.

The secretion of cortisol from the adrenal cortex leads to both short-term and long-term effects on whole-body glucose metabolism. In response to an acute stress, cortisol exerts various non-genomic effects that lead to a transient increase in circulating glucose concentrations. For example, cortisol stimulates glucose intolerance in peripheral tissues due to the suppression of insulin-mediated skeletal muscle glucose uptake (Zarkovic et al, 2008; Kuo et al, 2015). Furthermore, cortisol secreted in response to an acute stress has also been shown to rapidly regulate the actions of the Hypothalamic Pituitary Axis (HPA) via negative feedback mechanism, leading to the suppression of corticotropin-releasing hormone and ACTH secretion respectively and subsequently impairing a further increase in cortisol (Groeneweg et al, 2012). In contrast, the tissue-specific changes in glucose metabolism mediated by an increase in cortisol secretion tend to arise following transcription-dependent pathways, which may lead to a delay of between 15 minutes to several hours (Groeneweg et al, 2012; Kuo et al, 2013). Cortisol binds with a cytosolic glucocorticoid receptor, which then travels into the nucleus and

associates with cortisol-specific gene sequences that promote the necessary changes in gene transcription to account for the effects of cortisol on glucose metabolism (Groeneweg et al, 2012; Kuo et al, 2013; Kuo et al; 2015). A short-term increase in cortisol secretion stimulates an increase in hepatic gluconeogenesis, concurrently stimulates increased skeletal muscle glycogenolysis with epinephrine and may impair insulin signalling (Andrews and Walker, 1999; Kuo et al, 2013). Research has consistently shown that cortisol activates a transcriptionally-mediated upregulation of the key enzymes and molecules involved in each pathway affecting glucose metabolism (Kuo et al, 2015). However, chronically elevated cortisol concentrations symptomatic of a prolonged stress response may lead to the development of hyperglycaemia and whole-body insulin resistance. Research to date has not elucidated whether vasopressin-mediated alterations in ACTH secretion and subsequent cortisol secretion are non-genomic or transcription-dependent following acute osmotic stress.

Vasopressin receptors (V1a) present on isolated hepatocytes have been shown to stimulate increased rates of gluconeogenesis and glycogenolysis *in vitro* (Whitton et al, 1978; Kirk et al, 1979). Vasopressin mimics the effects of glucagon secretion by stimulating an increase in hepatic glucose production via the V1a receptor subtype, while glucagon binds with a different receptor sub-type on the pancreatic alpha cells before stimulating increased endogenous glucose production. However, no research to date has assessed the changes in the rate of hepatic glucose production at a whole-body level with administration of variable vasopressin concentrations or differences in fluid intake patterns. In contrast, much of the research investigating the effects of V1a receptor activation has focused on the direct release of cortisol from the adrenal cortex following vasopressin secretion (indicative of acute osmoregulatory stress). Perraudin et al (1993) highlighted a dose-dependent increase in cortisol secretion with increased exogenous administration of vasopressin. Furthermore, analysis of the pattern of vasopressin-mediated adrenal cortisol release via V1a receptors highlighted a biphasic response. Vasopressin secretion stimulated a short-term, rapid increase in cortisol concentrations before gradually declining and plateauing at around basal levels. The biphasic cortisol response to the elevated vasopressin concentration is believed to arise due to V1a receptor desensitization rather than an increase in the rate of vasopressin degradation (Perraudin et al, 1993). Previous studies have also postulated that the elevated basal

vasopressin concentration associated with T1DM may lead to a desensitization to the metabolic and/or osmoregulatory functions of vasopressin, including the effects of vasopressin on cortisol secretion and subsequent effect on glucose homeostasis (Ahloulay et al, 1999; Bankir et al, 2001).

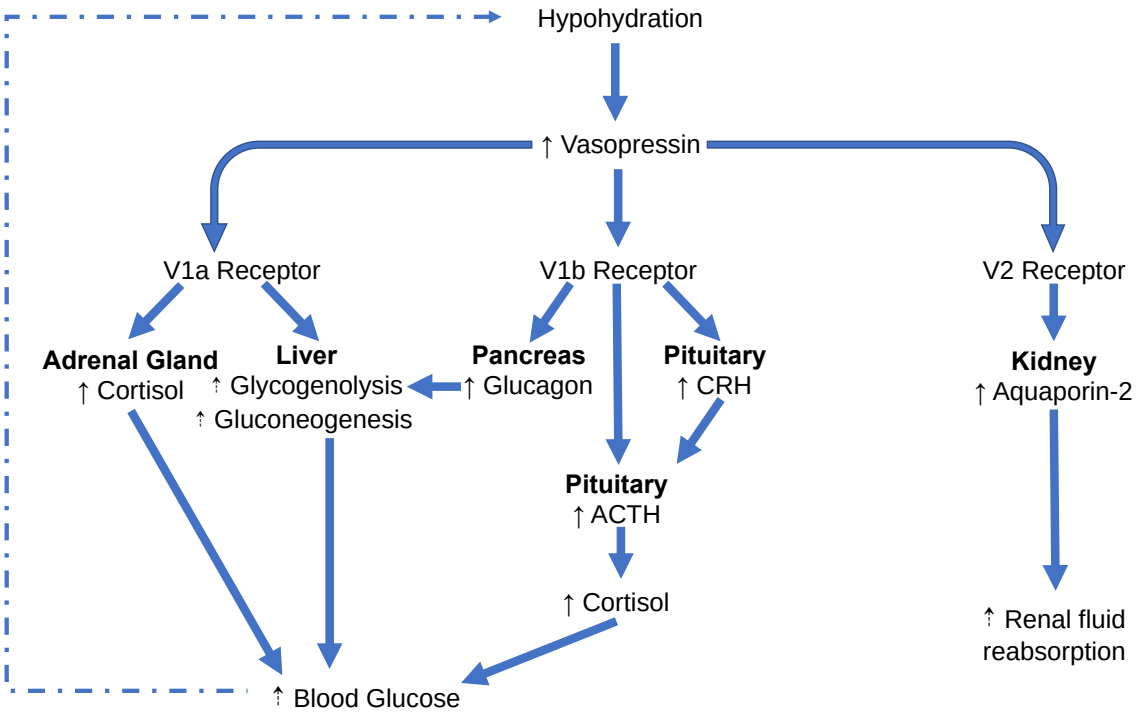

Figure 1: Schematic of the osmoregulatory function and metabolic signalling pathways stimulated by the release of vasopressin under conditions of low fluid intake in Type 1 Diabetes Mellitus patients. Vasopressin is released and subsequently interacts with three specific sub-classes of receptor located on various tissues. Vasopressin's primary function is to promote an increased rate of renal fluid reabsorption, but is also thought to be responsible for elevated blood glucose concentrations through increased hepatic glucose output mediated via increased glucagon and cortisol secretion. Although vasopressin has been shown to stimulate both insulin and glucagon release from the pancreatic alpha and beta cells respectively, endogenous insulin production is absent in Type 1 Diabetes Mellitus patients. Adapted from Rotondo et al (2016) and Moscogiuri et al (2018).

V1b receptors are located on both the pancreatic islet alpha and beta cells respectively, whereby an increase in vasopressin concentration typically stimulates the release of both glucagon (alpha cell) and insulin (beta cell) in healthy, non-DM subjects (Mavani et al, 2015). There is no net effect on glucose metabolism due to the concurrent secretion of the counter-balancing hormones. However, the autoimmune destruction of the pancreatic beta cells, and subsequent absence of endogenous insulin secretion in

T1DM patients, often leads to hyperglucagonemia arising which may further impair T1DM patients' glucose metabolism (Bankir et al, 2001; Yibchock-anun et al, 2004). Glucagon secretion is usually inhibited by the actions of insulin, however the increased endogenous glucose production stimulated by the release of glucagon is unimpaired due to the absence of counter-balancing insulin production (Bankir et al, 2001; Mavani et al, 2015). It is believed that an increase in vasopressin concentration within a 'physiological range' (3-30pmol/L) is likely to stimulate increased glucagon secretion and subsequently increase hepatic glucose output (Mavani et al, 2015). Abu-Basha et al (2002) and Yibchok-anun et al (2004) demonstrated a dose-dependent vasopressin-mediated increase in glucagon secretion from pancreatic alpha cells via V1b receptor activation *in vitro* based on this concentration range. Glucagon release via V1b receptor binding augments the increased hepatic glucose output stimulated independently via vasopressin binding with the V1a receptor subtype on the liver. Vasopressin can therefore stimulate an increased hepatic glucose output even with decreased expression or binding affinity of V1b receptors (Mavani et al, 2015). Studies to date that have investigated the primary effect of the vasopressin-mediated increase in glucagon concentration have shown contrasting results, with administration of pharmacological doses of vasopressin leading to no change in serum osmolality but increased hepatic glycogenolysis via elevated glucagon concentrations (Spruce et al, 1985). In contrast, Ahloulay et al (1999) highlighted an increased rate of hepatic gluconeogenesis associated with an increased vasopressin-mediated glucagon concentration in T1DM patients following a hyperosmotic stimulus at rest. However, no research to date has assessed changes in vasopressin-mediated glucagon concentrations following alterations in whole-body osmoregulation induced by exercise and fluid restriction.

The physiological relationship between vasopressin, glycaemic control and glucagon has proven equivocal in research to date, due to the divergence in methods of altering whole-body fluid balance and recruitment of healthy, non-DM patients. Following the withdrawal of insulin, severe hypohydration via pharmacological treatment and fluid restriction led to a significantly increased fasted glucose response, which was partly attributable to an increase in the fasted glucagon concentration (Burge et al, 2001). In contrast, Enhorning et al (2019) highlighted that prescription of 3L/day of additional water intake for 1 week led to a significantly reduced vasopressin and glucagon

concentration in those with habitually low fluid intake only, but there was no overall effect on plasma glucagon concentration or circulating glucose concentrations. It is therefore unlikely that manipulations in habitual fluid intake and/or hydration status will affect non-DM patients glucoregulation, due to the functional secretion of insulin in response to the elevated hepatic glucose output stimulated by the release of glucagon following vasopressin stimulation (Carroll and James, 2019). The effect of the elevated basal vasopressin concentration in T1DM patients on the vasopressin-mediated glucagon response at rest, around exercise or with variable fluid intake has not been studied to date, therefore there is no defined physiological glucagon concentration range for T1DM patients.

V1b receptors are also present at the anterior pituitary, where the secretion and binding of vasopressin has been shown to regulate ACTH release via divergent signalling pathways in response to both acute and chronic stress, that may include changes in hydration status. Vasopressin primarily augments the release of ACTH that is directly stimulated via the actions of CRH, which is synthesized and released from the hypothalamus (Goncharova, 2013; Rotondo et al, 2016). Infusion of CRH and AVP in individuals with reduced glucose tolerance (reduced peripheral insulin sensitivity) led to increased pituitary ACTH secretion, and elevated serum cortisol concentrations that led to hyperglycaemia arising (Mavani et al, 2015). Vasopressin alone may also directly stimulate the release of ACTH from the anterior pituitary via binding with the V1b receptor subtype, but the secretion of CRH does not potentiate the effects of vasopressin on ACTH secretion (Rotondo et al, 2016). ACTH stimulates the adrenal cortex- namely the zona fasciculata, to release adrenocortical glucocorticoids (cortisol). The increase in cortisol secretion subsequently leads to an increased rate of hepatic glucose output due to upregulated glucagon-mediated gluconeogenesis (Moscogiuri et al, 2016; Enhorning et al, 2017). The interaction between CRH, vasopressin and ACTH release is stressor-specific, where an acute or transient stress response to e.g. insulin-induced hypoglycaemia is predominantly mediated by vasopressin and CRH acting synergistically to stimulate ACTH release (Koshimizu et al, 2012; Goncharova, 2013). In contrast, regular/chronic external stress is thought to lead to the upregulation of the actions of vasopressin alone on ACTH release via pituitary V1b receptors (Goncharova, 2013; Rotondo et al, 2016). Furthermore, the vasopressin-induced release of ACTH, and subsequently cortisol, via

direct V1b receptor stimulation is opposed to negative feedback regulation, in contrast to CRH-induced ACTH release (Mavani et al, 2015; Moscogiuri et al, 2016). Pituitary V1b receptor expression is upregulated in the presence of increased cortisol concentrations, which also highlights the importance of the vasopressin-mediated cortisol response to chronic stress. Taken together, it is possible, although unproven in research to date, that the supraphysiological vasopressin concentration associated with T1DM may stimulate excessive ACTH secretion and subsequent cortisol secretion following changes in whole-body osmoregulation. Elevated plasma vasopressin concentrations have been associated with an increased risk of impaired glucose tolerance and development of insulin resistance in T2DM patients due to the excessive stimulation of ACTH release by vasopressin, leading to elevated cortisol concentrations that impair glucose uptake and utilization in peripheral tissues (Moscogiuri et al, 2016).

Evidence of the association between hydration status, cortisol secretion and glycaemic control is based primarily on the results of observational studies, including research highlighting that low (<1.2L water intake/day) habitual water intake has been shown to result in elevated plasma cortisol levels compared to high (>2L water/day) volume drinkers (Moscogiuri et al, 2016). Johnson et al (2017) reported a significantly increased circulating cortisol concentration during an oral glucose tolerance test (OGTT) when T2DM patients were severely hypohydrated, compared to euhydrated subjects completing the OGTT. No measurement of changes in vasopressin or copeptin were undertaken, but the increased cortisol concentration was associated with increased plasma osmolality, increased plasma sodium concentration and increased urine osmolality. Each variable is indicative of increased vasopressin secretion, and is believed to stimulate the release of cortisol. The results of the study concur with those of Burge et al (2001), however subjects in both studies were withdrawn from insulin administration and circulating cortisol concentrations were sampled under fasted conditions. Yadawa et al (2016) is the only study to date that has assessed the vasopressin-mediated HPA neuroendocrine axis response to varying levels of fluid intake. The authors concluded that as CRH-mediated ACTH release was unchanged during water deprivation but vasopressin-mediated ACTH secretion was increased *in vitro*, CRH-mediated ACTH secretion does not have any functional role during osmotic stress including fluid deprivation. While it is important to note that results of *in vitro* research may not be

applicable when assessing the whole-body neuro-endocrine response to e.g. cellular stress following fluid restriction, research to date has consistently shown that chronically elevated ACTH concentrations arise following vasopressin directly stimulating the pituitary V1b receptors, with no increase in CRH-mediated ACTH secretion (Aguilera and Rabadahn-Diehl, 2000; Goncharova, 2013; Rotondo et al, 2016). However, Aguilera and Rabadahn-Diehl (2000) reported a decrease in vasopressin-mediated ACTH secretion following 2% water deprivation, which suggests osmotic stimulation may require a concomitant CRH- and vasopressin-mediated ACTH response. It is currently unclear whether the vasopressin-mediated ACTH/cortisol secretion is solely responsible for the stress response to acute fluid restriction or chronic osmotic-related stress at a whole-body level. Furthermore, the effect, if any, of the elevated basal vasopressin concentration associated with T1DM patients on the endocrine response to variable fluid intake has yet to be investigated.

Effect of Dehydration on Substrate Metabolism

Acute fluid restriction not only results in significant alterations in vasopressin concentration, and subsequently fluctuations in metabolic hormone concentrations, but has also been consistently shown to affect whole-body substrate metabolism. A series of studies by Logan-Sprenger et al (2012, 2015) showed that mild dehydration of up to 3% initial body mass is associated with increased carbohydrate oxidation over the duration of submaximal continuous exercise, as indicated by elevated Respiratory Exchange Ratio (RER) values, the total amount of carbohydrate oxidised and the rate of carbohydrate oxidation respectively. Fallowfield et al (1996) initially highlighted a significantly greater proportion of the total energy expenditure accounted for via carbohydrate oxidation when participants were fluid restricted during submaximal exercise, compared to euhydrated participants. The increased reliance on carbohydrate oxidation throughout exercise was also evident when assessing substrate metabolism responses of a single exercising leg when fluid restricted during exercise, compared to euhydrated participants (Gonzalez-Alonso et al, 1999).The results of research undertaken by Hargreaves et al (1996), Gonzalez-Alonso et al (1999) and Fernandez-Elias et al (2015) respectively, concur with the overall increase in carbohydrate oxidation associated with exercise-induced dehydration, whilst also detailing a significantly greater rate of skeletal muscle glycogenolysis over the duration of exercise with variable degrees of dehydration induced. In contrast, despite Logan-Sprenger et al (2013) also demonstrating a significantly increased rate of intramuscular glycogen utilisation in recreationally active subjects during exercise, there were no significant differences in either the whole-body rate of carbohydrate utilization, RER values or the total amount of carbohydrate oxidised. The discrepancies in results between studies were attributed to varied training status, although the physiological mechanisms responsible for the increased skeletal muscle glycogenolysis under hypohydrated conditions remain unclear (Logan-Sprenger et al, 2012; Logan-Sprenger et al, 2015; Fernandez-Elias, 2015). No study to date has assessed the fluctuations in substrate metabolism of T1DM patients at a whole-body or tissue-specific level during exercise or with variable fluid intake protocols.

Fluid Intake and Copeptin

Although much of the research to date has sought to assess the relationship between glycaemic control and vasopressin-mediated endocrine responses, studies have instead measured circulating copeptin concentrations as an indicator of changes in vasopressin and overall whole-body osmoregulation (Szinnai et al, 2007; Moscogiuri et al, 2016). Analysis of changes in vasopressin concentration are extremely difficult to measure due to the short half-life of vasopressin and rapid clearance rate via urinary excretion, typically resulting in a low plasma vasopressin concentration (Enhorning et al, 2010; Mavani et al, 2015). Vasopressin has a greater molecular weight compared to copeptin, which leads to a reduced rate of renal reabsorption following glomerular filtration and subsequently a greater clearance rate (Bankir, 2001). In contrast, copeptin is released in equimolar amounts from the posterior pituitary with vasopressin, as it synthesised from the pre-cursor vasopressin molecule, and is unimpaired by molecule instability or half-life duration (Enhorning et al, 2010). A validated sandwich immunoluminometric assay has been developed that aids the successful measurement of serum copeptin concentrations, which is present at greater concentrations compared to vasopressin (Fenske et al, 2009). Several longitudinal studies have shown a direct, positive association between plasma copeptin concentrations and prevalence of T2DM, independent of typical diabetes risk factors (Enhorning et al, 2010). Furthermore, plasma copeptin concentrations are statistically correlated with changes in water intake and inversely associated with 24-hour urine volume (Moscogiuri et al, 2016). To date, research has tended to crudely assess glycaemic control via fasted blood glucose concentrations relative to serum copeptin concentrations, but no study to date has detailed the transient fluctuations in copeptin concentration with short-term changes in fluid intake, including around exercise.

Currently, there are no available fluid intake guidelines specific to T1DM patients around exercise. Instead, generalized population-wide guidelines that recommended sufficient replacement of exercise-induced fluid losses and maintenance of a euhydrated status prior to beginning exercise are utilized (Colberg et al, 2016; Riddell et al, 2017). Furthermore, there are no glucoregulatory considerations in the current guidelines, whereby impaired glucoregulation that may arise with differing rates of fluid intake will lead to the development of T1DM-specific health complications. No research to date has directly assessed the effect of variable fluid intake on T1DM patient's osmoregulatory

function, which inhibits the formation of accurate guidelines around fluid intake. Furthermore, despite the elevated basal vasopressin concentration associated with T1DM patients, there has been no research conducted assessing the physiological impact of the previously reported elevated vasopressin at rest or during exercise, and the subsequent fluid intake requirement for patients with T1DM. Based on the results of mostly observational research to date, it is unclear whether the supraphysiological basal vasopressin concentration associated with T1DM may alter the osmotic threshold for vasopressin secretion and subsequently affect the vasopressin-mediated renal and metabolic response to variable fluid intake.

Aim of the Study

The aim of the current study was to assess whether progressively dehydrating Type 1 Diabetes Mellitus patients via restricting fluid intake during and immediately following exercise affects short-term glycaemic control, compared to a euhydrated condition. Furthermore, the secondary aim of this study was to determine whether the anticipated alterations in whole-body osmoregulation and subsequent effects on glucose metabolism between euhydrated and mildly dehydrated trial were evident when assessing each T1DM patient's acute glycaemic control up to 48 hours following each experimental trial.

Study Hypothesis

It was hypothesised that the increased vasopressin concentration associated with fluid restriction during the progressive dehydration trial would stimulate a variety of vasopressin-mediated cellular signalling pathways, resulting in an upregulated endocrine response compared to euhydrated subjects. The osmotically-induced increase in concentrations of metabolic hormones, including glucagon and cortisol, are thought to promote an increased rate of endogenous glucose production and could subsequently lead to an elevated glycaemic response during and following exercise. Furthermore, with reference to the potentially augmented glycaemic response around exercise with progressive dehydration, it was hypothesised that acute glycaemic control in the 48 hours post-dehydration trial would likely be compromised. It is thought that T1DM patients will experience increased glycaemic variability due to compromised glycaemic management regimens following the proposed impairment of glucoregulation with mild dehydration, compared to the same period following the control (euhydration) trial.

Methodology

Eleven patients (n=7 males, n=4 females) with Type 1 Diabetes Mellitus were initially recruited for participation in the study via the National Health Service (NHS) Forth Valley Royal Hospital and the University of Stirling respectively. Participants were eligible to take part in the study if they were between 18-60 years of age, had been diagnosed with Type 1 Diabetes for at least 1 year, regularly completed aerobic exercise (≥3 times per week) and had good-to-moderate glycaemic control. Glycaemic control was assessed via the patient's self-reported HbA1c level (6.4%-9%) within the last 6 months, coupled with the use of the Diabetes Self-Management Questionnaire (DSMQ) (Schmitt et al, 2013; Appendix 3); a multi-component assessment tool which has been utilized as an accurate indicator of glycaemic management. Prospective participants who were pregnant or planning pregnancy, suffered from Type 1 Diabetes related complications; including retinopathy and a diminished ability to recognize the autonomic or neuroglycopenic symptoms of hypoglycaemia arising (Impaired Awareness of Hypoglycaemia), or had been diagnosed with a cardio-respiratory or further metabolic disorder, were excluded from the study. Furthermore, participants who had suffered a major hypoglycaemic episode within the previous 6 weeks were also excluded from participating in the study. A major episode was considered to be a scenario where the patient was unable to self-administer the necessary corrections for hypoglycemic values due to neurological dysfunction (International Hypoglycaemia Study Group, 2017).

Of the participants recruited, both continuous subcutaneous insulin delivery (n=7) and multiple daily insulin injections (n=4) were used as methods of daily short- and long-acting insulin administration. The participants were required to have been consistently employing their chosen insulin administration method for at least 6 months prior to commencing participation in the study. There were no inclusion criteria regarding the exogenous insulin analogues participants were administering as part of their usual basal-bolus insulin regimen.

Following written and verbal explanation of the study design and procedures to be undertaken during each experimental trial, all participants provided written, fully informed consent to participate. All prospective participants were required to complete the DSMQ along with a standardized Pre-Participation Health Screening Questionnaire (P-PHSQ), which was adapted for the purposes of this study. This questionnaire allowed for

the identification of individuals who may be at risk of aggravating pre-existing medical conditions through study participation. The study received approval from the National Health Service West of Scotland Research Ethics Committee 4 (Rec no. 17/WS/0003; ID: 217607) and the University of Stirling NHS, Invasive and Clinical Research Ethics Committee. All study procedures were conducted in accordance with the guidelines laid down in the 2013 Declaration of Helsinki.

The study required participants to attend the laboratory 4 times. First, an incremental ramp exercise test to exhaustion was completed, followed by a familiarisation session and two main experimental trials; one control trial and one progressive dehydration trial. The familiarization session and the respective experimental trials consisted of a one hour exercise protocol at a continuous workload on a stationary cycle ergometer, followed by a two-hour post-exercise recovery period. The study was a within-subject design, with trial order assigned in a randomized, crossover manner. The trial order was randomized using an online latin square crossover-based randomization scheduling tool, with participants matched to the trial based on their individually assigned participant ID when written consent to participate was obtained. There was at least 48 hours between the VO_2max test and familiarization session, with the subsequent experimental trials separated by at least 7 days. The overall study timeline can be seen in Figure 2.

Figure 2: Overall study timeline including the initial aerobic capacity test, familiarisation session and each of the two main experimental trials. Details of the flash interstitial glucose monitor fittings (FreeStyle Libre™; Abbott Technologies, California) are also outlined. Each monitor was applied 48 hours prior to the respective experimental trials and continuously collated interstitial glucose concentrations during, and up to 48 hours following, each experimental trial. Each interstitial glucose monitor was removed 5 days following each experimental trial.

Study Design

Visit 1- Ramp Test to Exhaustion

Participants were requested to arrive to the laboratory for their initial incremental ramp test to exhaustion in a well hydrated state (~500ml water 60 minutes prior to arrival) and having fasted for at least 4 hours. Upon arrival, the participant was asked to void the contents of their bladder and bowels ahead of recording their near-nude (underwear only) initial body mass. Participants were fitted with a heart rate monitor (Polar, Sweden) and the seating position on the stationary electronically-braked cycle ergometer (Lode Excalibur, Netherlands) was also adjusted and recorded to ensure the ergometer setup was consistent throughout the remaining trials. Participants were only cleared to commence the ramp test protocol if their capillary glucose value; assessed via finger prick technique, was within a specified range (5-11.9mmol/L), based on the guidelines for exercise detailed in Colberg et al (2016).

The test protocol itself was similar to that described by Nevill et al (2005). Briefly, participants began exercising at a gender-specific pre-selected wattage (50 watts for females, 100 watts for males). Throughout the test, participants were fitted with a mouthpiece for continued breath-by-breath online expired gas analysis (OxyCon Pro, Jaeger, Germany). Following a 5 minute warm-up and allowing the participant to regulate their breathing rate using the mouthpiece, participants were then asked to begin pedaling at their desired cadence which they could maintain throughout the duration of the test. Following the completion of each one minute stage, the workload increased in gender-specific increments (20 watts/min for females, 25 watts/min for males) until the participant could no longer complete the stage workload. The online expired gas analysis data were subsequently used to determine each participant's maximal aerobic capacity (VO_2max), defined as the highest average 30-second oxygen uptake (VO_2 ml/kg/min^{-1}) value. These data were also used to calculate the workload requirements for the remaining experimental trials, where the aim was for participants to complete the exercise protocol at a set workload (60% of their maximal aerobic capacity).

Pre-trial Standardization

Participants were asked to complete a 48-hour dietary intake and exercise diary before arriving at the laboratory for their familiarisation session and return this to the

investigator. Ahead of the remaining experimental trials, the participants were asked to replicate this dietary intake and exercise pattern as closely as possible, and to include any necessary hypoglycaemia corrections. Participants arrived to the familiarisation session, and for all subsequent experimental trials, in an overnight fasted state (from 10 hours prior to arrival) and having refrained from alcohol, tobacco and caffeine consumption and moderate-vigorous exercise in the previous 24 hours. Participants were also asked to disclose whether they had suffered from a major hypoglycaemic episode between trials, which was defined as a blood glucose concentration <3mmol/L, leading to neurological or physiological dysfunction that required immediate medical assistance. If the participant was found to have suffered from such an episode, they were excluded from further participation in the study. Furthermore, details of any additional insulin or carbohydrate requirements to treat either mild hypoglycaemia or hyperglycaemia in the hours immediately preceding the trial, which may have accounted for discrepancies in glycaemic response as the experimental trial progressed, were collected prior to commencing each trial. The laboratory temperature was maintained at a constant value throughout all experimental trials.

48 hours before each of the two main experimental trials commenced, participants were fitted with a flash interstitial glucose monitor (FreeStyle Libre™) on their left arm, as per manufacturer instructions. The flash glucose monitors were utilized to collate both within-trial interstitial glucose data and ambulatory interstitial glucose data prior to, immediately following, and in the two days following each experimental trial.

Visit 2- Familiarisation session

At least 48 hours following the VO_2max test, participants arrived at the laboratory for their familiarization session. The familiarization session was primarily employed to ensure that participants could effectively complete the exercise protocol at the calculated workload. Furthermore, it allowed the participants to ensure that the required adjustments to their basal insulin and the insulin bolus administered for the breakfast provided (26g carbohydrate; ~0.4 g/kg^{-1} body mass, 164kcals) were appropriate for the maintenance of stable glycaemia during the upcoming exercise period. Participants voided their bladder and bowels before their near-nude body mass was measured. This

body mass value, along with a post-familiarisation trial body mass measurement, was used to estimate fluid requirements to match sweat losses when completing the exercise protocol. This was to ensure that participants remained euhydrated on the control trial.

The exercise protocol consisted of a 60 minute continuous exercise workload with venous blood samples, glucose monitoring and expired gas analysis completed at 15 minute intervals. During the familiarisation session, only capillary glucose concentration was obtained, via finger prick technique. Water was also provided in a fixed volume every 15 minutes based on the participant's estimated sweat rate. Upon completing the exercise protocol, participants removed all equipment, before towel drying and voiding their bladder prior to a post-exercise near-nude body mass measurement. Following the completion of the familiarization session, the volume of fluid was adjusted for the subsequent control experimental trial if the participant's body mass had significantly changed from the pre-exercise value after completion of the exercise protocol.

Participants remained in the laboratory for a further 2 hour post-exercise recovery period. During this time, further capillary glucose measurements, along with blood and urine sampling respectively, were undertaken. The participants were required to be at minimal risk of a hypoglycaemic episode immediately following the trial (>5mmol/L), as determined via capillary blood glucose measurement, before they were authorized to leave the laboratory.

Visit 3-4- Experimental Trials

Participants reported to the laboratory at least 5 days following the familiarisation session to complete the first of two main experimental trials. Ahead of each experimental trial, participants were encouraged to maintain a euhydrated state pre-trial by consuming only water overnight where possible, and upon awakening up to 1 hour prior to arrival at the laboratory. Participants were required to bring a first-morning urine sample to the laboratory, from which an aliquot was retained for subsequent analysis.

Following the initial near-nude body mass measurement having voided their bladder and bowels, participants were cannulated to allow for efficient blood sampling to occur throughout the exercise and post-exercise periods respectively. They were asked to lie in a supine position on the bed within the laboratory, whereby a 20-gauge venous cannula was then inserted into an antecubital vein of their chosen arm. A baseline (6ml)

blood sample was drawn following insertion of the cannula. The patency of the cannula was maintained by using pre-prepared saline solution (0.9% BD PosiFlush™ 3ml Saline Syringe). The flushing procedure was repeated after every blood sample drawn throughout the experimental trial. A total of 11 blood samples were obtained across each trial, including one obtained upon initial arrival to the laboratory (6ml) and immediately pre-exercise (2ml) respectively. Further 2ml blood samples were obtained at 15 minute intervals during exercise, and at 15 and 45 minutes post-exercise respectively. A large blood sample (6ml) was drawn immediately upon the completion of the exercise protocol (0hrs post-exercise), with further large blood samples drawn at 30 minute intervals during the initial hour of the post-exercise recovery period, and at 2 hours post-exercise.

Participants were provided with a standardized breakfast prior to beginning exercise, which consisted of a portion of instant porridge (Original Instant Porridge Pot, Quaker Oats™) containing a fixed amount of carbohydrates and with a fixed volume of fluid added (175ml). In circumstances where a participant was intolerant to lactose or gluten respectively, we provided a quantity of Gluten-Free Oats which matched the carbohydrate content of the porridge pots, with the same volume of fluid also used in preparation. The participants were encouraged to individually manage the dose of short-acting insulin administered to compensate for the carbohydrates consumed during the breakfast, provided they replicated this during all subsequent trials. Participants were then asked to remain seated for a 15-minute period post-breakfast consumption prior to commencing the exercise protocol.

Once the participant was seated on the ergometer, a small (2ml) blood sample was drawn from the cannula, and a heart rate monitor was attached to the participant to collect heart rate data throughout the exercise period. A finger prick glucose measurement was also completed to ensure that participants were within the guideline glucose values (5-11.9mmol/L) prior to commencing exercise and to identify any participants who may have required consumption of further carbohydrates and/or to adjust their rate of insulin administration based on the finger prick glucose concentration. Based on the results of the immediate pre-exercise finger prick glucose measurement, participants were asked to provide details of any adjustments to their basal insulin regimen ahead of commencing the exercise period. The participants were requested to

replicate the selected basal/bolus exercise regimen as closely as possible during subsequent experimental trials.

The exercise protocol completed during both experimental trials was identical to the familiarisation session, with increased venous blood and interstitial glucose sampling frequency. The participant's interstitial glucose concentration was measured using the FreeStyle Libre™ flash interstitial glucose monitor every 5 minutes. Furthermore, online expired gas analysis was completed for 2 minutes at 15 minute intervals, while small (2ml) blood samples were drawn from the cannula every 15 minutes. Throughout the exercise period, a carbohydrate-rich energy bar (Go-Energy Bar™; Science in Sport) was available to be consumed by any participant who was trending towards hypoglycaemic glucose concentrations. During the control experimental trial, participant's estimated fluid losses induced through exercise were offset with water provided in three equal aliquots at 15 minute intervals during the 60 minute exercise period. During the progressive dehydration trial, participants were fully fluid-restricted throughout the exercise period.

In accordance with research previously conducted by Bussau et al (2009) to prevent an immediate decline in post-exercise glucose concentrations, participants were asked to complete a 10 second maximal effort sprint during the final 10 seconds of the exercise protocol. All blood samples and interstitial glucose measurements, coupled with final expired gas analysis, were completed prior to beginning the sprint during the final seconds of the exercise period. The participants also provided details of any additional short-acting insulin bolus administered during the exercise period to counteract hyperglycaemia where relevant. Furthermore, any carbohydrates consumed during exercise to prevent hypoglycaemia were also noted by the investigators. Once the participants had completed the exercise, their heart rate monitor was removed and they fully voided their bladder into the pot provided, from which an aliquot was retained for subsequent analysis. Participants were then asked to remove all saturated clothing and towel dry, before a post-exercise near-nude body mass was obtained.

Participants remained within the laboratory for a further 2 hour post-exercise recovery period after the completion of the exercise protocol, where short term post-exercise glycaemic control was assessed. During this time, participants were encouraged to remain seated/supine throughout due to postural changes altering fluid composition

in the body. During the control trial alone, participants were provided with a bolus of fluids (380ml still water) to be consumed within 5 minutes of commencing the post-exercise recovery period, with no other fluids provided thereafter for the remainder of the trial. In contrast, participants remained fully fluid-restricted throughout the post-exercise recovery period of the dehydration trial. Venous blood samples were collected at 15 minute intervals during the first hour of the post-exercise recovery period, with a final blood sample obtained at 120 minutes post-exercise. Interstitial glucose concentrations were monitored throughout the post-exercise period using the FreeStyle Libre™ flash interstitial glucose monitor at 5 minute intervals across the entire 120 minutes. Participants were also required to void their bladder at 1 hour and 2 hours post-exercise for subsequent analysis of the urine aliquots retained from each sample provided. As with the exercise period, information was recorded for any insulin administered or carbohydrates consumed during this post-exercise recovery period based on the correction of the interstitial glucose responses to each intervention/experimental trial.

Following the collection of the final venous blood sample and interstitial glucose measurement, the cannula was removed from the participant's arm and a final finger prick capillary glucose measurement was obtained. Based on the DVLA's regulations regarding necessary blood glucose concentration prior to operating a vehicle (DVLA, 2019), coupled with the aim to prevent any incidences of hypoglycaemia immediately following the culmination of the trial, participants were not authorized to immediately leave the laboratory. A final capillary blood glucose sample was obtained via finger prick, where participants were only cleared to leave the laboratory if they exceeded a blood glucose concentration of 5mmol/L, did not present any symptoms of hypoglycaemia and were not trending towards hypoglycaemic concentrations based on the direction of the FreeStyle Libre™ display arrow. Participants voided their bladder into the pot provided before a final near-nude post-trial body mass measurement was obtained. All participants were then provided with a morning urine sample collection pot and 48 hour post-trial insulin dosing and carbohydrate diary to be completed and returned to the investigator prior to the remaining experimental trial.

An estimation of the effect of varying hydration status on longer-term glycaemic control following each experimental trial was completed using the aforementioned

insulin and carbohydrate diary, coupled with the continuous collation of the interstitial glucose concentration data, during the initial 48 hours post-trial. The participants were required to complete finger prick capillary glucose tests at each significant mealtime at the minimum (breakfast, lunch, dinner), along with recording any insulin administered or estimated quantity of carbohydrate consumed at any point between meals during the post-trial period. Having completed one experimental trial, participants were then asked to return 2 days prior to their next experimental trial to have a new flash interstitial glucose monitor fitted to the opposite arm. Removal of the final interstitial glucose monitor up to 5 days following the final experimental trial signaled the culmination of the individual's participation in the study. A schematic of the full trial day protocol is shown in Figure 3.

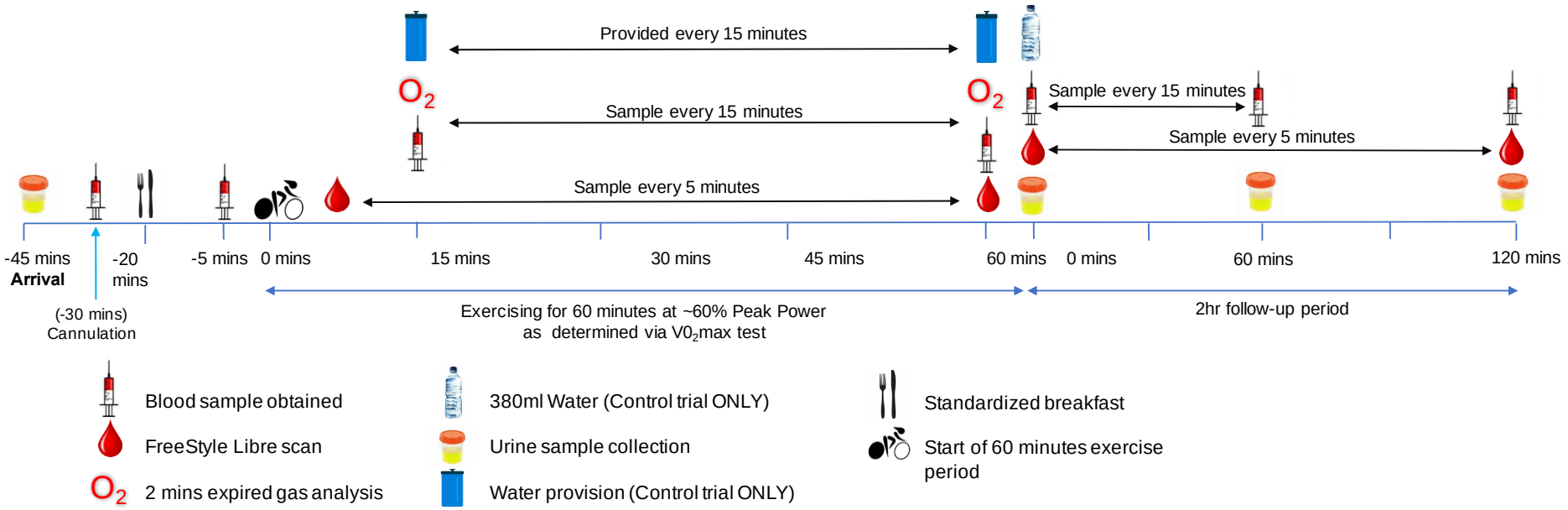

Figure 3: Example experimental trial and sampling timeline during the progressive dehydration and euhydration (control) trials.

Sample Analysis

The total sample volume for each urine sample was measured by weighing imminently upon provision using electronic scales (to the nearest 1g), before a 2ml aliquot was retained within individually-allocated storage tubes. The urine mass was calculated by subtracting the weight of the individual urine pots (calculated pre-trial) from the total urine volume excreted, with each pot rinsed and dried thoroughly to ensure there was no cross-contamination of urine samples, thus affecting urine osmolality values. Urine osmolality was assessed using the freezing-point depression method (Löser Osmometer, Germany) and was completed in duplicate measurements. The aliquots of all urine samples collected throughout each experimental trial were retained and stored at 3°C for subsequent analysis of urine osmolality within 24 hours of sample provision.

During the exercise and post-exercise periods, all venous blood samples collected were also used for the determination of venous (whole blood) glucose concentrations. Once the blood sample was stored within a hemo-repellent serum collection tube following withdrawal from the cannula, immediately a micropipette was utilized to withdraw duplicate 100µl aliquots from the blood sample prior to clotting. The aliquots of venous whole blood were added to an eppendorff containing 1ml of 0.4M perchloric acid solution (1:10 dilution), which itself was partially submerged in an ice bath. The eppendorff was mixed vigorously before being placed within the ice bath, where the perchloric acid negated any ongoing chemical reactions in the blood sample drawn. At the culmination of each experimental trial, each sample was placed into the micro-centrifuge at 12,500 x g for 5 mins at 4°C to allow separation of the whole blood pellet from the PCA solution, before the samples were stored at -80°C for future analysis. Once thawed, a quantity of each sample was dispensed into an eppendorff in equal measures, before the solutions were vortexed and aliquoted into allocated sample tubes (200µl samples). Glucose Oxidase reagent kits were utilized in conjunction with the inbuilt iLab Aries biochemical (IL, USA) analysis software to determine estimated whole blood glucose concentration using a diagnostic assay.

With each venous whole blood sample drawn from the cannula, the blood sample collected into the serum collection tube was allowed to clot at room temperature for 2

hours prior to centrifugation. Each blood sample collected was centrifuged at 3500rpm for 15 minutes at 4°C. Following centrifugation and separation of each whole blood component, the maximal portion of serum from each sample was dispensed into a separate eppendorff for subsequent analysis of the various hormone concentrations. The samples were stored at -80°C until analysis was undertaken. Serum cortisol and glucagon concentrations were each analyzed using a competitive immunoassay ELISA (Enzo Life Sciences, UK), with samples thawed and then centrifuged at 12.5 x g for 3 minutes at 5°C. Each serum sample was measured in duplicate, with the standard curves produced for each plate allowing for the determination of the respective absolute hormone concentrations using graphical software (GraphPad Prism Version 7; GraphPad Software, California, USA). The determination of copeptin concentrations was completed using a specific sandwich immunoluminometric assay on a BRAHMS Kryptor analyzer (Thermo Scientific, Germany) as previously described by Fenske et al (2009). The copeptin analysis was conducted in the laboratory of Prof. Olle Melander (Lund University, Sweden) by their technical support team.

All online expired breath-by-breath analysis was completed using the OxyCon Pro software for the collection of both oxygen consumption (VO_2) and carbon dioxide production (CO_2) data, coupled with estimated respiratory exchange ratio (RER) data. Following each experimental trial, it was possible to determine estimated rates of carbohydrate and fat oxidation during the exercise period using the equations of Jeukendrup and Wallis (2004) denoted for moderate-high intensity (50-75% VO_2max) exercise:

Carbohydrate oxidation (g/min): (4.21 x VCO2) – (2.962 x VO2) – (0.4 x n)

Fat oxidation (g/min): (1.695 x VO2) – (1.701 x VCO2) – (1.77 x n)

where **n** is estimated nitrogen content, which is considered to be negligible.

The insulin analogues administered by each participant during the study were either humilin, insulin glargine, insulin aspart or determir. Research has identified that commercially-available ELISA kits for the determination of insulin concentrations within whole blood are not sensitive to a variety of different insulin analogues commonly prescribed to diabetic patients (Parfitt et al, 2015). This includes the analogues insulin determir and insulin aspart administered by selected participants in this study. As a

result, insulin data were expressed as the estimated total units administered using the individual basal rates provided. Details of any additional insulin administered during each experimental trial and the 48 hour post-trial insulin diary notes were included. Insulin data are calculated based on the total units administered up to 8 hours prior to each experimental trial, total insulin units administered within-trial, and total post-trial insulin administration over a 24 and 48 hour period. Total carbohydrate intake over the 48 hour period following each trial was calculated using the relevant nutritional information provided and the quantity consumed in the diet, and was detailed in the post-trial diary. Additional insulin and carbohydrate requirements for the correction of hypoglycaemia or hyperglycaemia were also included in any total insulin and carbohydrate counts.

Statistical Analysis

FreeStyle Libre™ data has been expressed as both absolute interstitial glucose concentrations, and as the mean change from baseline (either ~30 mins pre-exercise or 60 mins of exercise value) during both the exercise period and the post-exercise recovery period, respectively.

To determine whether the participants were in a similarly hydrated status prior to beginning each of the main experimental trials, there was a paired T-test conducted on pre-trial body mass and morning urine osmolality between trials. Furthermore, the changes in body mass between trials following exercise and at the culmination of each experimental trial, were compared to the previous body mass measurement or initial body mass measurement via paired T-tests. The primary outcome variables measured during exercise and the 2 hour post-exercise recovery period, including interstitial glucose and blood glucose responses, urinary variables, endocrine responses (cortisol, copeptin and glucagon respectively) and physiological variables including heart rate and substrate oxidation, were analyzed using a two-way repeated measures ANOVA. Further post-hoc analysis with Bonferroni corrections was undertaken if a significant main effect (trial, time and time by trial) was observed for any of the outcome variables. A one-way ANOVA was also completed to compare the mean changes in interstitial glucose concentrations from baseline during exercise and at hourly intervals during the post-exercise recovery period between trials. Multiple paired T-tests, with appropriate adjustment of statistical significance based on the number of T-tests undertaken, was

also utilized to assess whether there were any differences in the average hourly interstitial and blood glucose concentrations between trials during exercise and the post-exercise period respectively. Analysis of the collated interstitial glucose data to assess the acute glycaemic response over subsequent days following each experimental trial was completed with a two-tailed T-test for the prevalence of stratified glucose concentration ranges- hypoglycaemia, euglycaemia, mild hyperglycaemia and severe hyperglycaemia. Finally, an assessment of any potential differences between trials regarding total insulin administration and carbohydrate intake over the initial 24 and 48hours following the experimental trials was completed with respective two-sided T tests.

All statistical analysis was completed using IBM SPSS statistics version 26, with statistical significance accepted as $p<0.05$. All data were only considered to be significantly different if $p<0.05$, or lower, with the additional Bonferroni correction adjustment on each variable. Data are presented as Mean ± Standard error of the mean (SEM) within text and in figures, with the data in tables being presented as Mean ± Standard deviation (SD). All graphical content was completed using GraphPad Prism Version 7 software.

Results

Participant Characteristics

Of the 11 participants initially recruited, only 9 participants fully completed all experimental trials and procedures associated with the study. One participant dropped out of the remainder of the study for personal reasons following the completion of the VO_2max test, and as such their results are not included in the overall study findings. Furthermore, one participant was subsequently removed from the analysis at the culmination of the study due to beginning administration of concomitant medication halfway through the trials. The form of concomitant medication is believed to negatively impact upon glycaemic control (data shown in Appendix 1), as previously reported by Sawka et al (2001), Derijks et al (2008) and Knol et al (2008). The n=9 participants mean physical characteristics and results from the diabetes self-management questionnaire (DSMQ) are detailed in Table 1.

Table 1: Participant (n=9) physical characteristics and diabetes self-management data obtained prior to, and following the completion of the initial maximal aerobic capacity (VO_2max) test. All data presented as Mean ± SD.

Age (y):	37.9 ± 3.2
Height (m):	1.74 ± 0.29
Body mass (kg):	74.7 ± 3.1
Duration of T1DM diagnosis (y):	15 ± 2
DSMQ score:	34 ± 1
VO_2max (ml/kg/min):	46.2 ± 2.6
60% max exercise workload (W):	142 ± 11

Trial Day Responses

Hydration Status

Upon arrival at the laboratory prior to each experimental trial, the participants mean initial body mass was not different between trials (P=0.16, Table 2). There was also no significant difference in the osmolality of the first morning urine sample aliquots prior to each experimental trial (P=0.13, Figure 4), where the mean urine osmolality was 588 ± 151 mOsm/kg. Taken together, these results indicate that the participants were of a similar hydration status prior to beginning each experimental trial.

Following the completion of the exercise protocol, there was a statistically significant difference in post-exercise body mass between trials (p<0.01, Table 2). During

the dehydration trial, the mean absolute body mass loss induced with exercise was –0.8 ± 0.6kg, leading to a mean post-exercise body mass of 73.8 ± 3.1kg. There was a statistically significant difference between the post-exercise and pre-exercise body mass measurements during the dehydration trial ($p<0.01$). In contrast, there was only a minimal mean body mass loss (–0.1 ± 0.4kg) induced during the exercise period of the control trial, and subsequently there was no significant difference in post-exercise body mass (74.6 ± 3.2kg) compared to the pre-exercise body mass ($p=0.30$, Table 2). During the post-exercise recovery period of the dehydration trial, the sustained fluid restriction protocol led to a further -0.2 ± 0.3kg mean body mass loss from the post-exercise body mass. There was a statistically significant difference between the post-trial body mass (73.6 ± 3.1kg) and both the initial pre-exercise body mass ($p<0.01$) and the post-exercise body mass measurements ($p<0.01$; Table 2) during the dehydration trial. Following the control trial post-exercise recovery period, the participants lost on average -0.2 ± 0.3kg, but there remained no significant difference between the post-trial body mass (74.4 ± 3.2kg) and either of the previous body mass measurements. The mean absolute body mass loss from the respective post-exercise body mass measurements was not significantly different between trials ($p=0.30$). However, there was a statistically significant difference in post-trial body mass between trials ($p<0.01$), while the mean % total body mass loss from the respective pre-exercise body mass measurements was also significantly different between trials ($p<0.01$, Table 2).

There was a statistically significant main effect of trial alone ($p<0.01$) on the urine osmolality responses over time, coupled with a statistically significant time by trial interaction ($p<0.01$, Figure 4). There was a linear increase in urine osmolality over time during the dehydration trial, resulting in a statistically significant difference between the initial urine sample (AM) osmolality and urine sample osmolality obtained at 1 hour ($p=0.35$) and 2 hours post-exercise ($p<0.01$) during the dehydration trial. The linear decrease in urine sample osmolality over time during the post-exercise recovery period of the control trial also resulted in a significant difference between the initial sample osmolality and the 2 hour post-exercise value ($p<0.01$). Following the clear divergence in urine osmolality response between trials throughout the post-exercise recovery period

post-hoc analysis showed that the mean difference between trials at 2 hours post-exercise (309 $\pm$ 63mOsm/kg) was statistically significant ($p<0.01$).

There was a statistically significant effect of both time ($p<0.01$) and trial ($p=0.01$) on post-exercise cumulative urine output, along with a significant time by trial interaction ($p<0.01$, Figure 5). Post-hoc analysis showed that across both the control and dehydration trials, there was a statistically significant difference between the cumulative urine output at the beginning of the post-exercise period (0 hours) and at 2 hours post-exercise (both trials, $p<0.01$). Furthermore, at 2 hours post-exercise, there was a significant difference between the mean cumulative urine output during the control trial (538 $\pm$ 96g) compared to the corresponding value during the dehydration trial (143 $\pm$ 19g, $p<0.01$).

Table 2: Participant body mass measurements obtained prior to (Pre-Trial Body Mass) and during each experimental trial (Post-Exercise, Post-Trial). The % total body mass loss is calculated as the overall change from Pre-Trial to Post-Trial body mass corrected for the total volume of fluid consumed. All body mass measurements recorded in near-nude state and to the nearest 0.1kg. All data presented as Mean $\pm$ SD. Significance accepted at $p<0.05$.

	Control	**Dehydration**
Pre-Trial Body Mass (kg)	74.7 $\pm$ 9.5	74.4 $\pm$ 9.3
Post-Exercise Body Mass (kg)	74.6 $\pm$ 9.5	73.8 $\pm$ 9.2[1,*]
Post-Trial Body Mass (kg)	74.4 $\pm$ 9.7	73.6 $\pm$ 9.2[1,2,*]
% Total Body Mass Loss	-0.4 $\pm$ 0.5	-1.1 $\pm$ 0.4[*]

[1] significant difference from initial body mass measurement
[2] significant difference from previous body mass measurement
[*] significant difference compared to Control trial

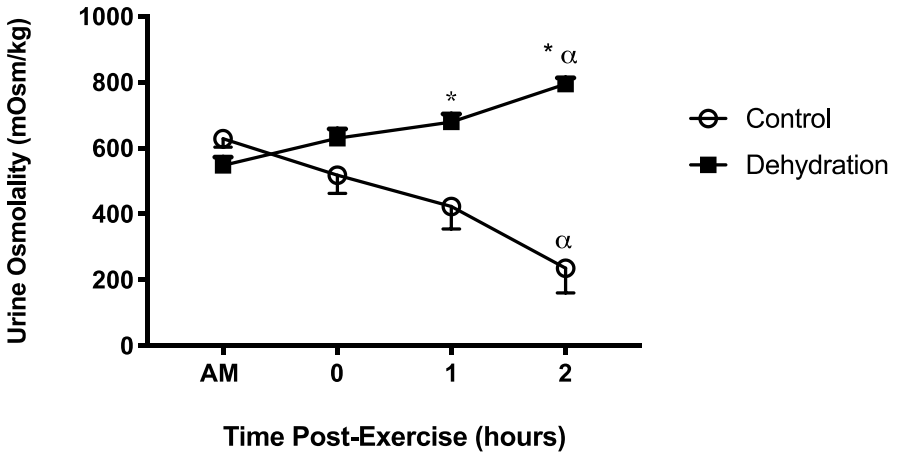

Figure 4:
Urine osmolality of the initial sample provided upon awakening (AM), at the culmination of the 60 minute exercise period (0hrs) and at each hourly interval during the post-exercise recovery period, following either the full replacement of fluid losses (Control) or with total fluid restriction to induce a progressive dehydration (Dehydration). Data presented as Mean ± SEM, with statistical significance accepted at $p<0.05$. Difference between trials indicated as *, difference from initial (AM) value indicated by α.

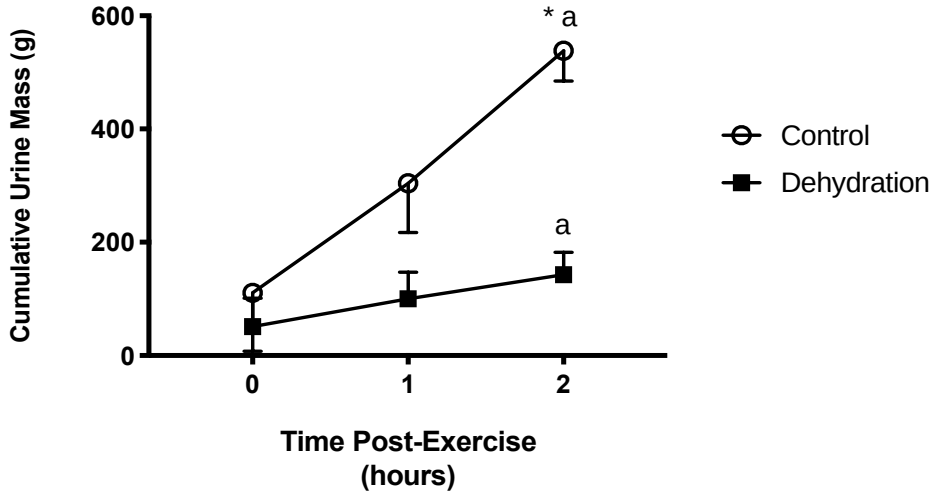

Figure 5: Cumulative Urine Mass over the 60 minute exercise period (0hrs) and each hour over the subsequent 2 hour post-exercise recovery period, following either the full replacement of fluid losses (Control) or with total fluid restriction to induce a progressive dehydration. Data presented as Mean ± SEM, with statistical significance accepted at $p<0.05$. Significant difference between trials indicated as *, with significant differences from initial (immediately post-exercise- 0 hours) value indicated by α.

Within-Trial Glucose Responses

The interstitial glucose data are presented as the change from the baseline interstitial glucose concentrations which, during the exercise period, are calculated from the pre-breakfast (~30 minutes pre-exercise) interstitial glucose concentration. There was a statistically significant main effect of time ($p<0.01$) on the interstitial glucose response during the exercise period (Figure 6A). The interstitial glucose concentration continued to increase from the baseline concentration (Control: 9.8 ± 0.9 mmol/L, Dehydration: 7.0 ± 0.4 mmol/L) as the exercise period proceeded, until the peak increase in interstitial glucose concentration occurred at 25 minutes during both experimental trials (Control: $+2.1 \pm 1$ mmol/L, Dehydration: $+3.7 \pm 0.7$ mmol/L). During the remainder of the exercise period, the interstitial glucose concentrations continued to decline until returning to near-baseline levels at the culmination of exercise during the dehydration trial. In contrast, the decline in interstitial glucose concentrations from the peak response during the control trial continued until the final interstitial glucose measurement (60 minutes) was below the pre-exercise interstitial glucose concentration. The change from the baseline interstitial glucose concentration was significantly different at the 5 and 10 minute time points during exercise compared to the 0 minute (immediately pre-exercise) value during the control trial. During the dehydration trial, there was a statistically significant difference in the change from baseline interstitial glucose concentration between the 0 minute and 10-25 minute timepoints respectively (10, 20 minutes, $p<0.05$; 15, 20 minutes, $p<0.01$), and between the 5 minute and 10-25 minute timepoints respectively (all timepoints $p<0.05$).

There were no significant differences in the interstitial glucose responses throughout the post-exercise recovery period between trials or at any timepoint (Figure 6A). The baseline interstitial glucose concentration during the post-exercise recovery period was denoted as the initial (0 mins) post-exercise interstitial glucose concentrations (Control: 8.8 ± 1.2 mmol/L, Dehydration: 7.8 ± 0.8 mmol/L). During the first hour of the post-exercise period, the mean interstitial glucose concentration continued to increase from the baseline interstitial glucose concentration across both trials, until there appeared to be a separation by trial. By the culmination of the post-exercise recovery period (2 hours post-exercise), the mean difference in the change in interstitial glucose

concentration between trials was 2.3 mmol/L (Control +0.6 ± 1.0mmol/L; Dehydration +2.9 ± 1.4 mmol/L), yet this did not reach statistical significance (p=0.11).

During the exercise period of the control experimental trial, the mean interstitial glucose concentration was 10.8 ± 1.2mmol/L, which was not significantly different from the baseline interstitial glucose concentration (9.7 ± 0.9mmol/L; p=0.47). In contrast, there was a statistically significant difference (p<0.01) between the average interstitial glucose concentration during the exercise period of the dehydration trial (9.4 ± 0.7mmol/L) compared to the baseline interstitial glucose value (7.0 ± 0.4mmol/L).

There was no significant difference between the baseline interstitial glucose concentration during the post-exercise period of the control trial (8.7 ± 1.2mmol/L) and the average interstitial glucose concentration during the entire (2 hour) post-exercise period (9.6 ± 0.9mmol/L; p=0.20). There was also no significant difference when the average interstitial glucose concentration was expressed at 1 hour intervals in comparison to the baseline concentration (1st hour: 9.5 ± 0.9mmol, p=0.71; 2nd hour: 9.7 ± 1.1mmol/L, p=0.79).

During the post-exercise period of the dehydration trial, there was a statistically significant difference between the baseline interstitial glucose concentration (7.8 ± 0.8mmol/L) and the average interstitial glucose concentration during the entire post-exercise period (9.8 ± 0.7mmol/L; p=0.02). When the average post-exercise interstitial glucose data were expressed at hourly intervals, there were no statistically significant differences between the average baseline glucose concentration and both the 1st (8.8 ± 0.7mmol/L, p=0.09) and 2nd hour (10.7 ± 1.0mmol/L, p=0.07) average interstitial glucose concentrations post-exercise.

The blood glucose data are presented as the change from the baseline blood glucose concentrations which, during the exercise period, is denoted as the pre-breakfast (~30 minutes pre-exercise) blood glucose concentration. As with the changes in interstitial glucose concentration during exercise, there was a statistically significant effect of time alone on blood glucose responses during exercise (p<0.05; Figure 6B). The baseline blood glucose concentrations were 8.8 ± 0.8mmol/L for the control trial and 5.7 ± 0.3mmol/L for the dehydration trial respectively. Upon reaching the peak increase in

blood glucose concentrations 15 minutes into the exercise period of both trials (Control +1.7 $\pm$ 0.7 mmol/L, Dehydration +2.2 $\pm$ 0.5 mmol/L), the pattern of change in blood glucose concentrations was concurrent with that of the interstitial glucose concentrations. There was a steady decline in blood glucose concentrations towards the baseline (pre-breakfast) glucose concentrations during both trials, with the final blood glucose concentration declining below the baseline blood glucose concentration following the culmination of exercise in the control trial only. There was a statistically significant difference between the change in blood glucose concentrations during exercise at the 15 minutes and 60 minute timepoints ($p<0.05$), with significant differences also evident between the 30 minute and 45 minute and 60 minute timepoints respectively (both timepoints, $p<0.05$).

There was no significant difference in the mean changes in blood glucose concentrations from baseline concentrations between each trial over the duration of the post-exercise recovery period (Figure 6B). The baseline blood glucose concentration during the post-exercise recovery period was denoted as the initial (0 mins) post-exercise blood glucose concentration (Control: 7.8 $\pm$ 1.0 mmol/L, Dehydration: 6.0 $\pm$ 0.6 mmol/L). In a similar manner to that of interstitial glucose responses, blood glucose concentrations during the dehydration trial continued to increase throughout the recovery period, even with the change in blood glucose from baseline values plateauing at 60 minutes post-exercise during the control trial (+1.7 ± 0.6mmol/L). At the culmination of the 2 hour recovery period, the mean difference in blood glucose responses was consistent with the interstitial glucose pattern previously described (Control: +1.2 $\pm$ 0.8mmol/L, Dehydration: +3.3 $\pm$ 1.2mmol/L), which also did not reach statistical significance ($p=0.11$).

Analysis of the changes in interstitial glucose concentration in comparison to the changes in blood glucose concentration were separated by each experimental trial and by the exercise and post-exercise recovery periods respectively. There was a statistically significant time by type interaction ($p<0.05$) highlighted during the exercise period of the control experimental trial (Figure 7A). There was a significantly different change from the baseline interstitial glucose concentration between the 0 and 15 minute timepoints ($p<0.05$), along with the 45 and 60 minute timepoints ($p<0.05$). In contrast, there was a

statistically significant main effect of both time ($p<0.01$) and type ($p<0.05$), coupled with a significant time by type interaction ($p<0.01$) for the changes in blood and interstitial glucose concentration from the respective baseline concentrations during the exercise period of the dehydration trial (Figure 7B). The mean difference between the change in interstitial glucose concentration and changes in blood glucose concentration was 0.8 ± 0.1 mmol/L over the duration of exercise within the dehydration trial. The change in interstitial glucose concentrations during exercise was significantly different between the 0 and 15 minute timepoints ($p<0.01$) and the 30 minute and 60 minute timepoints ($p<0.05$) respectively. Analysis of the changes in blood glucose concentrations from baseline showed there were statistically significant differences between the 15 and 60 minute timepoints ($p<0.05$) and between the 30 minute and both the 45 and 60 minute timepoints respectively (both timepoints $p<0.05$). Furthermore, during the dehydration trial only, post-hoc analysis highlighted a statistically significant difference between the interstitial glucose and blood glucose concentrations at the 15 minute ($p<0.01$) and 45 minute ($p<0.01$) timepoints during exercise. During the entirety of the post-exercise recovery period, there were no significant differences between the type of glucose measurement (interstitial or blood glucose), or an effect of time on the changes in either interstitial or blood glucose concentrations.

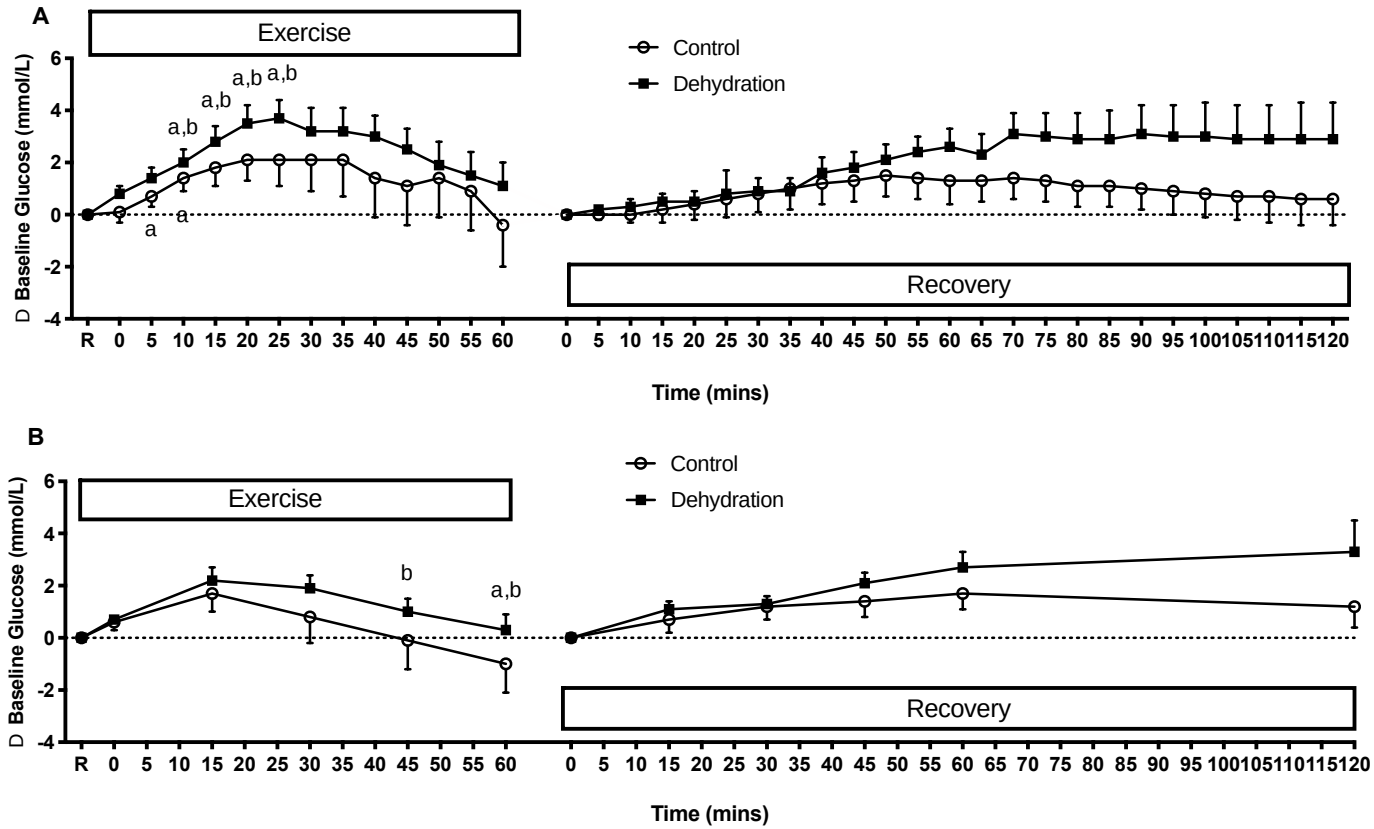

Figure 6: Changes in the (A) interstitial glucose concentrations and (B) blood glucose concentrations during exercise and throughout the post-exercise recovery period of both the control and dehydration trials respectively. Interstitial glucose concentration was measured at 5 minute intervals throughout each experimental trial, with blood glucose measurements obtained every 15 minutes during exercise and the first hour of recovery. During exercise, the baseline (resting) value (R) was taken as the pre-breakfast glucose concentration. Initial post-exercise (0 mins post-exercise) glucose value was then considered to be the baseline concentration for the calculation of changes in glucose concentration during the post-exercise recovery period. All data presented as Mean ± SEM, with statistical significance accepted at $p<0.05$. Significant differences compared to the 0 minute value indicated as α, significantly different compared to the 5 minute value indicated by β in 6(A). α denotes significantly different from 15 minute value, β denotes significantly different from 30 minute value in 6(B).

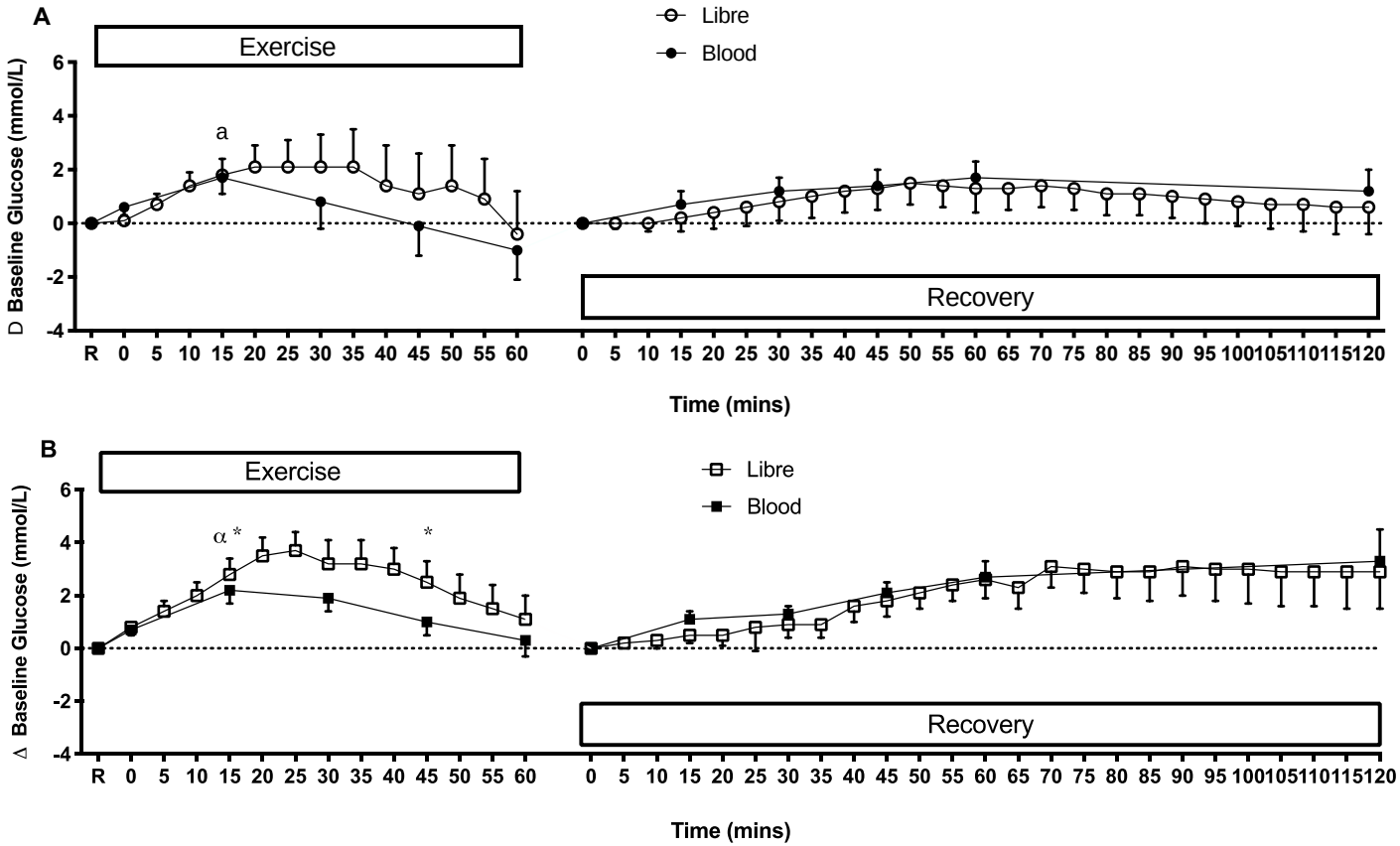

Figure 7: Changes in interstitial glucose concentration and blood glucose concentration during exercise and throughout the post-exercise recovery period of the A) control trial and B) progressive dehydration trial. Interstitial glucose was sampled at 5 minute intervals throughout each experimental trial, with blood glucose measurements obtained every 15 minutes during exercise and the first hour of recovery. During exercise, the baseline (resting) value (R) was taken as the pre-breakfast glucose concentration. Initial post-exercise (0 mins post-exercise) glucose value was then considered to be the baseline concentration for the calculation of changes in glucose concentration during the post-exercise recovery period. All data presented as Mean ± SEM, with significance accepted at $p<0.05$. Significant difference between trials indicated as *, with a significant difference compared to the baseline (R) value indicated by α.

Endocrine Responses

Analysis of serum copeptin concentrations throughout each trial reveal a statistically significant main effect of both trial ($p<0.01$) and time ($p<0.01$), along with a significant time by trial interaction ($p<0.01$, Figure 8). There were no significant differences between either the baseline (pre-breakfast) or immediately pre-exercise copeptin concentrations between trials. However, during the dehydration trial, there was a measurably greater copeptin response following the total fluid restriction protocol employed during the completion of the exercise period. Subsequently, there was a statistically increased copeptin concentration immediately post-exercise (0 hours, $p<0.01$) compared to pre-breakfast (baseline) values during the dehydration trial only, where the mean difference was 25.98 ± 18.53 pmol/L. The copeptin concentration remained elevated above baseline concentrations throughout the post-exercise period of the dehydration trial with continued fluid restriction, with a statistically significant difference between the baseline and 2 hour post-exercise copeptin concentrations ($p<0.01$). There were statistically significant differences in the serum copeptin concentrations immediately post-exercise and at 2 hours post-exercise between trials (both timepoints, $p<0.01$).

The results of the cortisol assay show that there was a statistically significant main effect of trial ($p<0.01$) on serum cortisol concentrations (Figure 9). The mean difference in cortisol concentrations between the trials was 6290 ± 610 pg/ml. From baseline (pre-exercise) values, the mean cortisol concentration increased following the exercise period during the dehydration trial until peaking at 30 minutes post-exercise (40690 ± 8760 pg/ml). In comparison to the control trial, the mean cortisol concentration remained elevated throughout the post-exercise recovery period of the dehydration trial, before returning towards pre-baseline values at the culmination of the experimental trials. There was a statistically significant difference between the mean cortisol concentrations at 1 hour post-exercise($p<0.01$) and 2 hours post-exercise ($p=0.03$) respectively, compared to the 30 minute post-exercise value. The serum cortisol concentrations at 30 minutes post-exercise were significantly different between trials ($p<0.01$) and remained elevated above the baseline concentration during the dehydration trial until the culmination of the post-exercise period (2 hours post-exercise).

There was also a significant main effect of both time ($p<0.01$) and trial ($p<0.01$) on serum glucagon concentrations during and following exercise, coupled with a significant time by trial interaction ($p<0.01$, Figure 10). The baseline glucagon concentrations (pre-breakfast) were significantly different between trials ($p<0.01$), where the mean concentration was 6.75 ± 3.01 pg/ml prior to the control trial, and 4.07 ± 3.10 pg/ml pre-dehydration trial. During exercise and the subsequent post-exercise recovery period, glucagon concentrations did not greatly fluctuate throughout both experimental trials. There was a slight decline in serum glucagon concentration from baseline values until 2 hours post-exercise during the control trial. In contrast, glucagon concentrations remained around the baseline concentrations until the culmination of the dehydration trial.

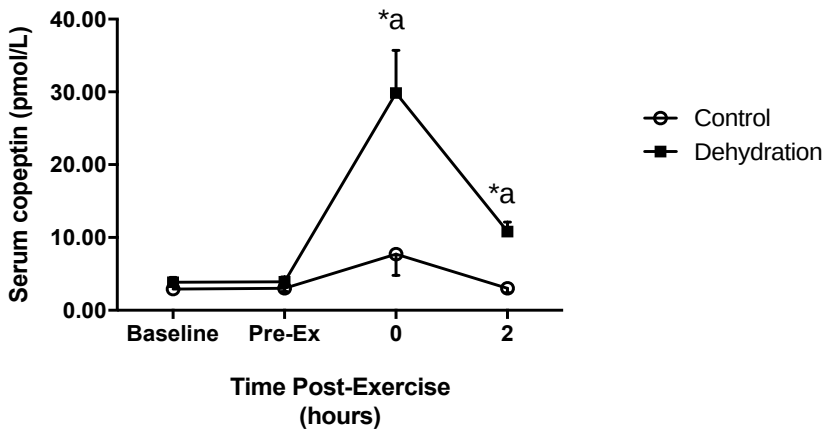

Figure 8: Copeptin concentrations by trial, determined from blood samples obtained prior to commencing exercise and throughout the post-exercise recovery period. Baseline copeptin concentrations taken as the pre-breakfast (approximately 30 minutes pre-exercise) concentration. All data presented as Mean ± SEM, with statistical significance accepted at $p<0.05$. Any significant difference between trials indicated as *, significant difference from baseline value indicated by α.

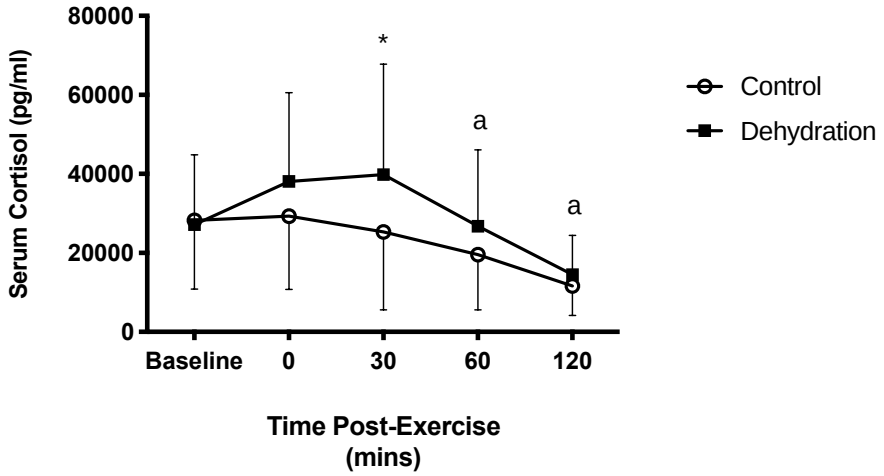

Figure 9: Cortisol concentrations by trial, determined from blood samples obtained throughout the post-exercise recovery period. Baseline cortisol concentration taken as the pre-breakfast concentration. All data presented as Mean ± SEM, with statistical significance accepted at $p<0.05$. Any significant differences between trials indicated as *, significant difference from 30 minute post-exercise value indicated by α.

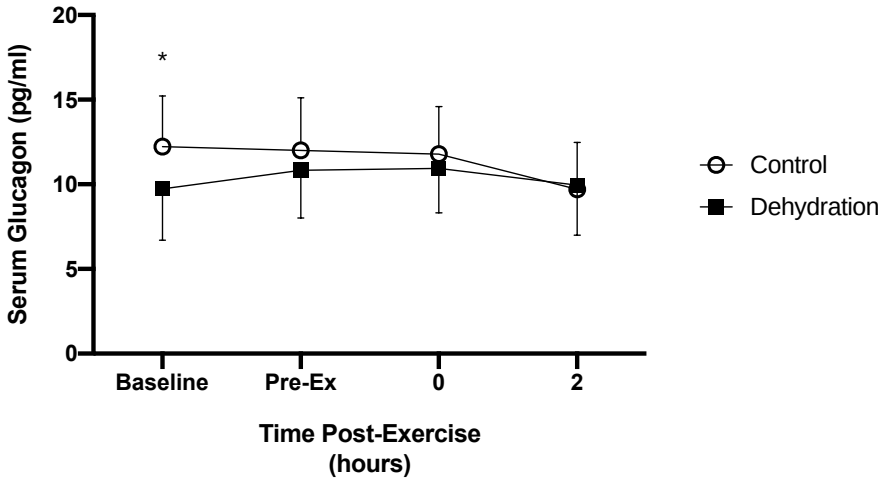

Figure 10: Glucagon concentrations by trial, determined from blood samples obtained prior to commencing exercise and throughout the post-exercise recovery period. Baseline glucagon concentrations taken as the pre-breakfast concentration. All data presented as Mean ± SEM, with statistical significance accepted at $p<0.05$. Any significant difference between trials indicated as *.

Post-Trial Glucose Data

Analysis of all interstitial glucose data collated over the 48 hour period following each experimental trial highlighted statistically significant differences in the % of total interstitial glucose data points within the defined glycaemic ranges. There was a statistically significant increased prevalence of mild hyperglycaemia (7-11mmol/L) in the 24 hour period following the control experimental trial ($p<0.01$, Figure 11A), compared to post-dehydration trial. Although there appeared to be a greatly increased prevalence of euglycaemic interstitial glucose values (4-7mmol/L) during the 24 hours following the dehydration trial, there was no statistically significant difference compared to the 24 hour period post-control trial ($p=0.051$). Analysis of the entire 48 hour period following the respective experimental trials highlighted that there was a continued significantly increased prevalence of mild hyperglycaemia ($p<0.01$) following the control experimental trial (Figure 11B), compared to the 48 hour period post-dehydration trial. Overall, the majority of the total interstitial glucose concentrations sampled during the entire 48 hour period post-trial were within a hyperglycaemic concentration range following both experimental trials (Control: $64 \pm 20\%$; Dehydration: $58 \pm 21\%$), however less than 5% were within a hypoglycaemic range following the respective experimental trials.

Analysis of the 48 hour post-trial average interstitial glucose concentration was completed in four blocks of 12 hours, which included 2 overnight periods (13-24 hours and 37-48 hours respectively; Figure 12). Over the total duration of the 48 hour period, the average interstitial glucose concentration was higher following the control trial (9.5 ± 0.2 mmol/L) in comparison to post-dehydration trial (9.0 ± 0.2 mmol/L), which was significantly different ($p<0.05$). However, there were no statistically significant differences in the hourly average interstitial glucose concentrations between trials across each 12 hour period up to 48 hours post-trial (each 12 hour series, $p>0.05$). The highlighted section of Figure 7 shows a discernible reduction in the average interstitial glucose concentration during the first overnight period (13-24 hours post-trial) following the dehydration trial (mean concentration 8.0 ± 0.2 mmol/L), which contrasts the glycaemic response during the same period following the control trial (mean concentration 10.1 ± 0.1 mmol/L).

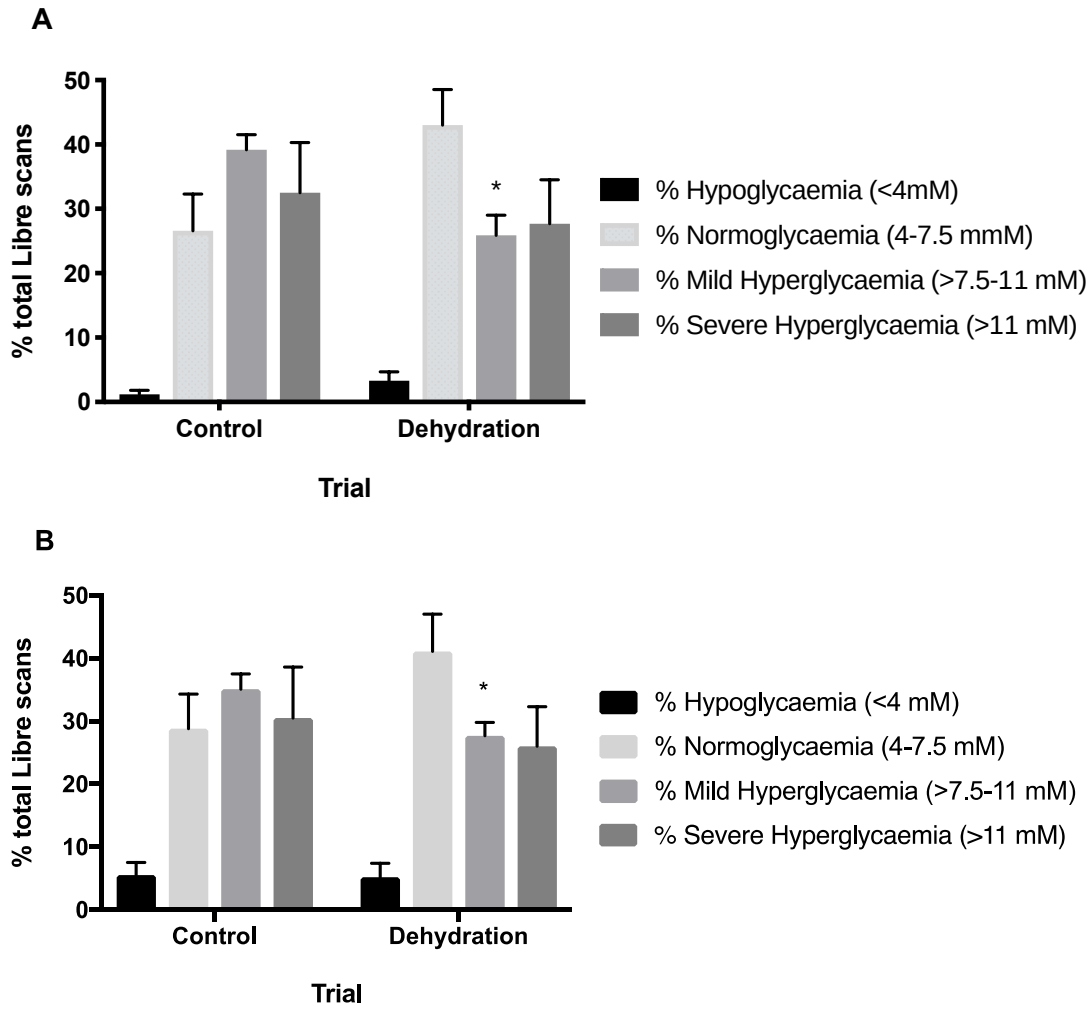

Figure 11: Prevalence of hypoglycaemic, normoglycaemic, mild hyperglycaemic and severe hyperglycaemic interstitial glucose concentrations in the A) initial 24 hours following each experimental trial and B) up to the initial 48 hours following each experimental trial. Each value is expressed as a percentage of the total number of interstitial glucose measurements obtained within each glycaemic range during the respective timeframes following the experimental trials. All data presented as Mean ± SEM. Significance accepted at $p<0.05$, with significant differences in prevalence of respective interstitial glycaemic ranges between trials indicated as *

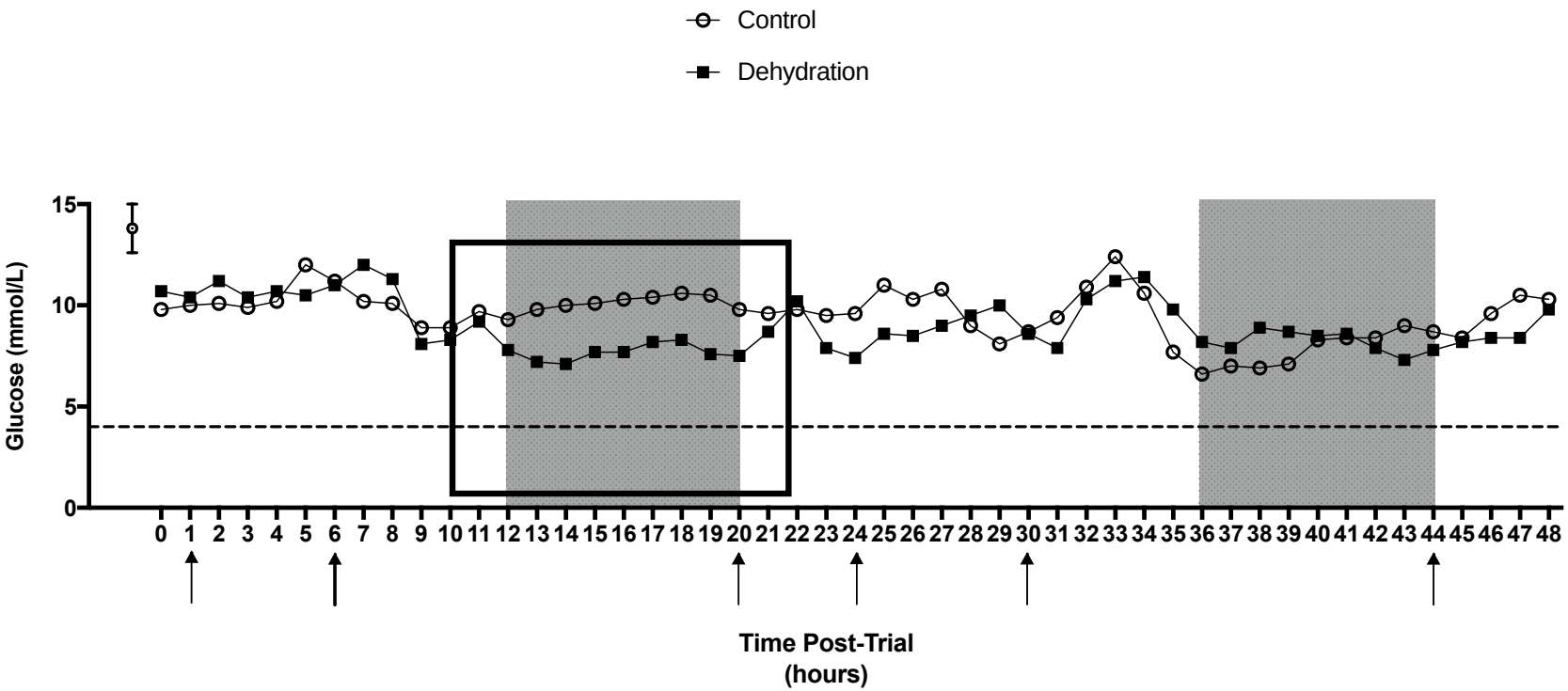

Figure 12: Mean interstitial glucose concentration collated over 1 hour intervals during the 48 hours immediately following each experimental trial. The shaded area of the graph denotes each overnight period, and the section of the graph highlighted by the black outlined box shows the period where the greatest difference between trials was evident (overnight period 12-20 hours post-trial). Each arrow indicates the mean timepoint at which a main meal was consumed during the 48 hour period following each experimental trial. Data presented as mean values at each timepoint for each experimental trial, with a pooled SEM shown in the top left of the figure.

Insulin / Carbohydrate data

There was no significant difference in the total insulin units administered up to 8 hours prior to arrival at the laboratory (pre-trial) for each experimental trial (p=0.06, Table 3). Furthermore, carbohydrate consumption in the same overnight period pre-trial was also not significantly different between the control and dehydration trial (p=0.79). Throughout each experimental trial, the mean units of insulin administered was 3.91 $\pm$ 1.6 during the control trial and 3.65 $\pm$ 1.21 during the dehydration trial, with no significant difference between trials (p=0.23, Table 3). When the data were expressed by individual time periods, there was no significant difference between trials for the insulin administered with the consumption of the standardized breakfast pre-trial (1.65 $\pm$ 0.92 vs 1.56 $\pm$ 0.73; p<0.05), during exercise (0.92 $\pm$ 0.38 vs 0.72 $\pm$ 0.30; p=0.17) or post-exercise (2.26 $\pm$ 0.89 vs 2.17 $\pm$ 0.74; p=0.63). Although there was no statistically significant difference in the total units of insulin administered during exercise, n=2 participants required an additional insulin bolus of 0.5 units on average during the control trial only. In contrast, n=3 participants required an additional mean insulin bolus of 0.87 units during the post-exercise period of the control trial, with n=2 participants requiring an additional mean insulin bolus of 0.75 units during the dehydration trial. There was no statistically significant difference in the mean amount of carbohydrate consumed during the control (47 $\pm$ 34) or dehydration trial (30 $\pm$ 0; p=0.17). All participants consumed 26g of carbohydrate pre-exercise via the standardized breakfast, however participants only required further carbohydrate supplementation during the exercise and post-exercise periods respectively of the control trial. N=2 participants required an average of 56 $\pm$ 29g of carbohydrate to combat hypoglycaemia during exercise, while a further n=2 participants consumed 21 $\pm$ 7g of carbohydrate during the post-exercise recovery period of the control trial.

The total units of insulin administered and amount of carbohydrate consumed (g) over the duration of each trial (intra-trial) is the sum of insulin administration and carbohydrate consumption during the pre-trial, pre-exercise, exercise and post-exercise time periods respectively. The mean units of insulin administered by participants was not significantly different between trials (p=0.23; Table 3), nor the amount of carbohydrate consumed (p=0.24). Participants administered an average of 8.9 $\pm$ 1.2 units in total over

the entirety of each experimental trial, while participants consumed an average of 49 ± 7g of carbohydrate per trial.

In conjunction with the average interstitial glucose concentration data, there were no significant differences between trials for the total insulin units administered over the first 24 hours post-trial (p=0.78) or between 24-48 hours post-trial (p=0.51) respectively (Table 3). Furthermore, there were no significant differences between trials in the estimated total carbohydrate consumption in the first 24 hours post-trial (p=0.37) or between 24-48 hours post-trial (p=0.55). Participants administered an average of 80.55 ± 4.31 total units of insulin and consumed an estimated 440 ± 27g of carbohydrate in the 48 hours immediately following each experimental trial.

Table 3: Total insulin administration and carbohydrate consumption pre-trial (the overnight period up to 8 hours prior to arrival at the laboratory), within trial (intra-trial), 0-24 hours post-trial and 24-48 hours post-trial, respectively. All insulin data includes both basal and bolus dose administration. Intra-trial data were calculated as the sum of pre-exercise, during exercise and over the 2 hour post-exercise recovery period. All data presented as Mean ± SD.

	Insulin (units)		**Carbohydrate (g)**	
	Control	**Dehydration**	**Control**	**Dehydration**
Pre-Trial	6.35 ± 3.46	6.79 ± 3.40	23 ± 6	28 ± 15
Intra-Trial	3.91 ± 1.60	3.65 ± 1.21	47 ± 34	30 ± 0
0-24 hours	44.6 ± 12.46	43.61 ± 8.06	252 ± 21	247 ± 30
24-48 hours	36.28 ± 8.62	36.70 ± 5.83	189 ± 23	191 ± 16

Within-Trial Variables

During the exercise protocol, there appeared to be a progressively elevated heart rate over the duration of the progressive dehydration trial compared to the control trial, whereby the mean difference in heart rate at the culmination of exercise was 13 ± 10 bpm. However, a statistically significant main effect of time only was evident for heart rate during exercise ($p<0.01$, Figure 13). The repeated measures analysis showed that the mean heart rate values at 15, 20, 30, 40, 45 and 50 minutes respectively were all significantly different from the heart rate value at 5 minutes (all timepoints, $p<0.05$) during the control trial. During the dehydration trial, there were significant differences between heart rate values at 50 and 60 minutes compared to the resting pre-exercise (0 minutes) value (both timepoints, $p<0.01$), coupled with significant differences between mean heart rates at 15-60 minutes compared to the 5 minute value (all timepoints, $p<0.05$).

There was a statistically significant main effect of time on the participants' respiratory exchange ratio during the exercise protocol ($p<0.01$, Table 4). In comparison to the respective values at the 15 minute timepoint, there was a statistically significant decrease in respiratory ratios at 45 minutes during the dehydration trial ($p<0.01$) and at 60 minutes during the control trial ($p<0.05$). In accordance with the respiratory exchange ratio analysis, there was a statistically significant main effect of time alone on both carbohydrate ($p<0.01$) and fat oxidation rates ($p<0.01$) respectively across the duration of the exercise protocol (Table 4). As expected with the mode and duration of exercise undertaken, there was an overall progressive decline in the rate of carbohydrate oxidation over time, coupled with an increased rate of fat oxidation over time. The repeated measures analysis highlighted a statistically significant difference between the mean rate of carbohydrate oxidation at 15 and 60 minutes during the control trial ($p<0.05$), where there was also a significantly different rate of fat oxidation evident ($p<0.01$). During the dehydration trial, there was a significant difference between the respective 15 minute and 45 minute carbohydrate oxidation rates ($p<0.01$) and fat oxidation rates ($p<0.05$).

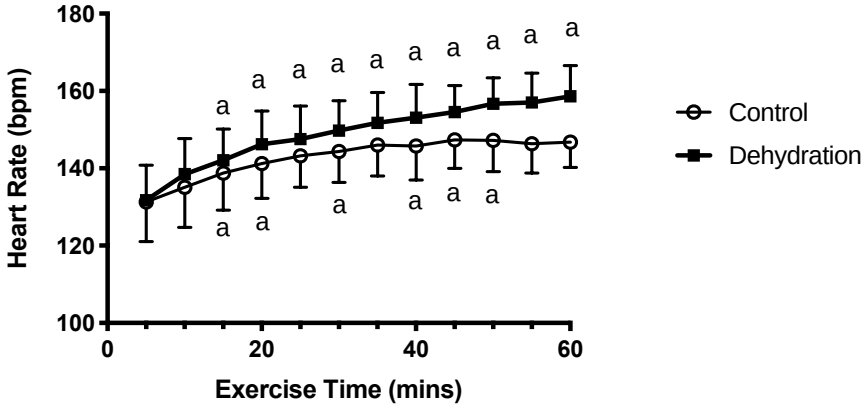

Figure 13: Heart rate data by trial, obtained at 5 minute intervals over the duration of exercise, where participants were euhydrated (control), or were wholly fluid restricted (dehydration) throughout. All data presented as Mean ± SD, with statistical significance accepted as $p<0.05$. α denotes significant difference from the respective 5 minute values during the experimental trials.

Table 4: Respiratory Exchange Ratios (RER), Carbohydrate (CHO) oxidation rate and Fat oxidation rates calculated at 15 minute intervals during the exercise period via online expired gas analysis. All data presented as Mean ± SD.

Time	**RER**		**CHO (g/min^{-1})**		**FAT (g/min^{-1})**	
	Control	**Dehydration**	**Control**	**Dehydration**	**Control**	**Dehydration**
15 mins	0.92 ± 0.04	0.92 ± 0.05	2.07 ± 0.73	2.02 ± 0.53	0.26 ± 0.19	0.29 ± 0.18
30 mins	0.91 ± 0.05	0.89 ± 0.05	2.01 ± 0.72	1.86 ± 0.63	0.32 ± 0.21	0.38 ± 0.18
45 mins	0.89 ± 0.03	0.88 ± 0.05 [1]	1.86 ± 0.58	1.73 ± 0.65 [1]	0.37 ± 0.15	0.43 ± 0.23 [1]
60 mins	0.89 ± 0.05 [1]	0.89 ± 0.05	1.83 ± 0.66 [1]	1.76 ± 0.77	0.39 ± 0.19 [1]	0.45 ± 0.25

[1] significant difference from 15 minute value

Discussion

The aim of the present study was to investigate whether an alteration in hydration status, mediated by a restriction of fluid intake during and following a period of exercise, affected glycaemic control in patients with Type 1 Diabetes Mellitus (T1DM). In contrast to the control trial, where participants consumed a sufficient volume of fluid to offset exercise-induced fluid losses, the fluid restriction protocol successfully induced ~1% body mass loss across the duration of the progressive dehydration trial. This degree of body mass reduction induced an increase in circulating vasopressin concentration, as assessed indirectly by changes in serum copeptin concentration, compared to a euhydrated (control) trial. Serum copeptin is considered a valid biological marker of vasopressin release (Szinnai et al, 2007).

This study is the first to our knowledge to assess whether an alteration in hydration status could impact upon resting, within-exercise, or post-exercise glycaemic responses of patients with T1DM. It is well established that under conditions of reduced fluid availability, including the inadequate replacement of extracellular fluid losses induced during exercise, serum osmolality is elevated and subsequently stimulates the secretion of vasopressin from the posterior pituitary (McConnell et al, 1997; Rotondo et al, 2016; Carroll and James, 2019). The primary osmoregulatory function of vasopressin has been comprehensively investigated, however only a paucity of research to date has investigated the effects of vasopressin on whole-body substrate metabolism and glucose control. *In vitro* research has identified several potential cellular signaling cascades stimulated by the release of vasopressin, culminating in an upregulated endocrine response- namely elevated serum cortisol and glucagon concentrations (Yibchok-anun et al, 2004; Mavani et al, 2015; Carroll and James, 2019). It is unclear whether glycaemic control, an indicator of whole-body glucose utilization and a key factor in the successful management of T1DM (Kennedy et al, 2012), may be affected by these alterations in substrate metabolism mediated via the elevated vasopressin concentration.

Prior to each experimental trial, there were no significant differences in initial body mass measurements, first-morning urine osmolality or serum copeptin concentration between trials, highlighting that participants were of a similar baseline hydration status. Changes in body mass are consistently utilized to inform guidelines on the volume of fluid to be consumed daily and around exercise to offset the negative

effects of dehydration on health and performance respectively (Buoite-Stella et al, 2018). McConnell et al (1997) previously justified the successful induction of a range of hydrated states via the changes in body mass elicited by varied fluid provision protocols during a similar moderate intensity continuous exercise protocol. Furthermore, Carroll and James (2019) highlight that changes in total body mass are an effective indicator of acute alterations in body water content, provided pre-trial conditions are similar between trials. Increased pre-exercise and/or exercise-induced extracellular fluid (sweat) losses are associated with a decrease in plasma volume and concurrent increase in serum osmolality, leading to the secretion of vasopressin (McConnell et al, 1997; Montain et al, 1997; Melin et al, 2001; Maresh et al, 2004). In the research conducted to date with non-diabetic subjects only, the greatest change in serum vasopressin concentrations following fluid restricted exercise was consistently shown to be directly correlated with the greatest body mass loss and subsequently the greatest increase in serum osmolality, independent of baseline hydration status (Montain et al, 1997; Maresh et al, 2004).

The successful induction of a mildly dehydrated state can also be observed via the significantly increased urine osmolality and significantly lower cumulative urine mass following exercise in the dehydration trial, compared to the control trial. At rest, T1DM patients often display a markedly increased urine flow rate and a concomitantly decreased urine osmolality compared to healthy subjects (Ahloulay et al, 1999; Bankir et al, 2001). Excess plasma glucose stimulates urinary glucose excretion (glycosuria) and may lead to prolonged fluid losses, which is symptomatic of untreated T1DM (Bankir et al, 2001). However, T1DM patients have been shown to display an upregulated renal concentrating ability, where there is increased reabsorption of fluids that would otherwise have been excreted with any excess glucose in the urine (Ahloulay et al, 1999). This is the only study to date to have assessed urinary responses of T1DM subjects during exercise, where the alterations in urine concentrating ability are likely to be influenced by fluctuations in vasopressin concentration. These findings compliment previous research with non-diabetic subjects, which have shown that the urine flow rate is decreased with various degrees of dehydration (increase in plasma osmolality) due to an increased rate of renal fluid reabsorption stimulated by increased vasopressin secretion (McConnell et al, 1997; Ahloulay et al, 1999; Bankir et al, 2001). Increased fluid conservation in response to hypohydration would also be expected to lead to a linear increase in urine osmolality,

as observed during the progressive dehydration trial in the current study. Melin et al (2001) highlighted that following exercise-induced dehydration, non-diabetic subjects displayed an increased urine osmolality in line with an increased vasopressin concentration.

Overall, the data indicate that the fluid restriction protocol employed throughout the progressive dehydration trial elicited a significant, negative change in the participants' hydration status, compared to the control experimental trial. Analysis of the changes in the selected markers of hydration status (body mass, urine osmolality and cumulative urine output) post-exercise and post-trial respectively showed statistically significant differences in comparison to pre-trial values during the progressive dehydration trial. At the culmination of the post-exercise recovery period, participants in the progressive dehydration trial also had a significantly increased body mass loss, elevated urine osmolality and reduced cumulative urine output compared to the control trial. During the post-exercise recovery period, the serum copeptin concentration was also significantly elevated above the time-aligned values for euhydrated subjects. Taken together, these results confirm that a mild state of dehydration was successfully induced and subsequently stimulated the secretion of vasopressin to promote an increased rate of renal fluid reabsorption, aiding the maintenance of intracellular fluid composition despite the continued extracellular fluid losses.

Vasopressin Physiology and Glycaemic Responses

Although the relationship between vasopressin, fluid intake and whole-body osmoregulation is well established, the increased vasopressin concentration stimulated by the progressive dehydration protocol during the current study is also thought to affect glucose metabolism. A milieu of endocrine signalling cascades may be stimulated by the binding of vasopressin to peripheral sub-receptors, which promote an upregulated rate of endogenous glucose production (Koshimizu et al, 2012; Mavani et al, 2015, Muscogiuri et al, 2016). Vasopressin has been shown to directly stimulate the release of glucagon from the pancreatic islet alpha cells, and both directly and indirectly stimulate the release of cortisol from the adrenal gland *in vitro* (Perraudin et al, 1993; Yibchok-anun et al, 2004; Koshimizu et al, 2012). The increase in hepatic glucose production stimulated by the secretion of both glucagon and cortisol, combined with the absence of endogenous insulin production that characterizes T1DM, may lead to an unimpaired continued rise in circulating glucose concentrations (Mavani et al, 2015). To date, there has been a paucity of research which has investigated the cellular signalling pathways linking increased vasopressin release in response to fluid restriction or deprivation, and the proposed effect on glucose metabolism. Observational studies and longitudinal research with non-diabetic subjects have shown that differences in habitual fluid intake or chronic manipulations of daily fluid intake lead to alterations in long-term glycaemic control and affect circulating concentrations of a variety of metabolic hormones (Burge et al, 2001; Johnson et al, 2017; Enhorning et al, 2019; Carroll and James, 2019). While no cellular markers of endogenous glucose production were measured during the current study, the balance of glucose production and disposal was assessed by tracking interstitial and blood glucose concentrations during and following a bout of submaximal exercise. There was a tendency for an elevated interstitial and blood glucose response during the progressive dehydration trial, which is consistent with an upregulated hepatic glucose output following vasopressin-mediated stimulation of increased glucagon and/or cortisol secretion.

The results of the current study show that although there was a trend for a greater glycaemic response during the dehydration trial, there were no statistically significant differences in the change in glucose concentrations between trials during exercise or the subsequent post-exercise recovery period. There was a greater peak

increase in both blood and interstitial glucose concentrations during the exercise period of the progressive dehydration trial, compared to the control trial. Furthermore, blood and interstitial glucose concentrations declined from the maximal glycaemic response to below the respective baseline glucose concentrations during the control trial only. In contrast, mildly dehydrated subjects' interstitial and blood glucose concentrations remained above the respective baseline glucose concentrations throughout exercise. During the post-exercise recovery period, there was a continued increase in blood and interstitial glucose concentrations from baseline values until the culmination of the dehydration trial. The peak interstitial and blood glucose responses during the control trial occurred at 60mins post-exercise, before gradually declining towards the respective baseline concentrations. The discrepancies in glycaemic control during and following exercise may be the result of a vasopressin-mediated increase in endogenous glucose production (Cryer, 2012; Mavani et al, 2015; Yostens, 2018). However, despite the hypothesized vasopressin-mediated increase in glucagon concentrations and potentially upregulated hepatic glucose production, the progressive dehydration protocol did not lead to any significant differences in serum glucagon concentrations during or following exercise, compared to euhydrated subject values.

It was anticipated that the increased serum copeptin (vasopressin) concentration mediated by the progressive dehydration protocol would stimulate a further increase in glucagon secretion via V1b receptor binding and subsequent stimulation of the pancreatic islet alpha cells (Koshimizu et al, 2012; Mavani et al, 2015; Muscogiuri et al, 2016). A series of *in vitro* studies conducted by Abu-Basha et al (2002) and Yibchok-anun et al (2004) previously highlighted a dose-dependent increase in alpha cell glucagon secretion with administration of increased physiological doses of vasopressin. Any vasopressin-mediated increase in glucagon secretion was thought to transiently impair T1DM patients' glucoregulation due to the absence of endogenous, counterbalancing insulin production combined with the glucagon-mediated rise in hepatic glycogenolysis and gluconeogenesis (Bankir et al, 2001; Mavani et al, 2015). There was a statistically significant difference in baseline (pre-breakfast) serum glucagon concentrations between trials. However, thereafter there was no significant difference in pre-exercise or any post-exercise glucagon concentrations between euhydrated and mildly dehydrated subjects, despite the significantly elevated post-exercise copeptin concentrations during the

dehydration trial versus euhydrated subject values. The differing baseline glucagon concentration between trials did not translate to any significant difference in baseline blood or interstitial glucose concentrations between trials. Furthermore, baseline glucagon concentrations in each trial did not reach the elevated basal levels previously reported by Farhy et al (2012) for T1DM patients under resting, euglycaemic conditions.

There are several physiological mechanisms that may affect the vasopressin-mediated glucagon response, and therefore account for the absence of any significant differences in glucagon concentrations between euhydrated and mildly dehydrated subjects in the current study, independent of the significant changes in vasopressin concentrations between trials. Firstly, the limited *in vitro* research that has investigated the changes in vasopressin-mediated glucagon secretion to date have studied the effects of administering a wide vasopressin concentration range (3-300pmol/L) in isolated pancreatic alpha cells. The vasopressin concentration threshold that is required to stimulate an increase in glucagon secretion from the alpha cells, beyond the reported elevated basal vasopressin concentrations associated with T1DM patients, therefore remains unclear (Abu-Basha et al, 2002; Yibchok-anun et al, 2004; Koshimizu et al, 2012).

This is the first study to date to assess changes in vasopressin-mediated glucagon secretion *in vivo* following differing fluid intake regimens. Salehi et al (2006) previously postulated that the relationship between glucagon secretion and circulating glucose concentrations may be U-shaped in nature, whereby only hypoglycaemia and severely hyperglycaemic glucose concentrations can stimulate the maximal glucagon response. The underlying intracellular mechanisms that explain this extreme glucose-dependent response are unclear, and no research to date has elucidated whether the circulating glucose concentrations affect vasopressin-mediated glucagon secretion. However, this concept contrasts the impaired glucagon counter-regulatory response to hypoglycaemia associated with T1DM (Diedrich et al, 2002; McCrimmon and Sherwin, 2010; Younk et al, 2011). Glucagon is the primary counter-regulatory hormone, whereby a combination of the impaired counter-regulatory neuro-endocrine responses with administration of an excessive insulin dose and an impaired awareness of hypoglycaemia symptoms leads to an increased risk of hypoglycaemia arising in T1DM patients (Diedrich et al, 2002; McCrimmon and Sherwin, 2010; Cryer, 2012; Yosten, 2018). Furthermore, the average blood and interstitial glucose responses during exercise and the post-exercise recovery

period of both trials of the current study were sufficient to prevent a decline to within a hypoglycaemic concentration range (<3.9mmol/L) at any time. The dysfunction of the pancreatic alpha cells, and subsequently dysregulated glucagon secretion in response to hypoglycaemia, is therefore unlikely to be responsible for the impaired vasopressin-mediated glucagon response during the dehydration trial. Although there was a trend for a greater glycaemic response during the dehydration trial, neither the blood or interstitial glucose concentrations rose to severely hyperglycaemic concentrations during either experimental trial. Furthermore, the hypothesized increase in glucagon secretion associated with severe hyperglycaemia is only applicable to patients who are newly diagnosed with T1DM, or T1DM patients with poor glycaemic control, when severe hyperglycaemia regularly arises (Yosten , 2018). Further research is required to fully elucidate the physiology linking glucagon release and glucose concentrations in T1DM patients, including whether the vasopressin-mediated glucagon response may be affected. The results of the current study instead align with research conducted by Zander et al (1985), which highlighted that the glucagon response during exercise-provided there was no immediate threat of hypoglycaemia arising, was maintained throughout continuous, moderate intensity exercise. Blood and interstitial glucose concentrations tended to remain close to or were elevated beyond the respective baseline glucose concentrations during the exercise and post-exercise periods of each experimental trial, with no incidences of hypoglycaemia reported throughout the study. There was therefore no required glucagon counter-regulatory response during or following exercise.

In contrast to the insignificant differences in vasopressin-mediated glucagon concentrations between trials, the results show that there was a significantly elevated serum cortisol concentration at 30 minutes of the post-exercise recovery period of the dehydration trial, compared to the control trial. Furthermore, serum cortisol concentrations during the progressive dehydration trial remained elevated above the corresponding values for the euhydrated trial throughout the post-exercise recovery period. The current study is one of very few to have investigated the association between variable hydration status and either salivary or serum cortisol responses during and following exercise. Independent of the mode of exercise completed or method of inducing body mass losses (dehydration), cortisol concentrations have generally been

shown to be elevated in mildly dehydrated subjects around exercise (Maresh et al, 2006). However, Svensden et al (2014) highlighted no significant differences in plasma cortisol concentrations between euhydrated and acutely dehydrated (-3.9% body mass loss) non-diabetic participants following prolonged, low intensity exercise, although there was a significant increase in cortisol concentration evident from pre-exercise levels across both trials. No research to date has directly investigated how the elevated vasopressin concentrations associated with T1DM may affect the cortisol response around exercise.

Despite the clear physiological link between hydration status (vasopressin concentration), whole-body osmoregulation and changes in metabolic hormone concentrations, including cortisol, only a few observational or longitudinal studies have shown an association between fluid intake, changes in basal cortisol concentration and subsequently basal glucose responses (Burge et al, 2001; Johnson et al, 2017; Carroll and James, 2019). Separate review papers on the effect of fluid intake on glucoregulation have postulated that as ACTH and cortisol secretion via direct stimulation by vasopressin is not regulated by the same intrinsic negative feedback mechanism as CRH-induced cortisol release, a continuous cycle of increased blood glucose concentrations due to persistent vasopressin-mediated cortisol release may be evident with chronic stress, which would subsequently increase serum osmolality and impair whole-body osmoregulation in T1DM patients (Mavani et al, 2015; Muscogiuri et al, 2016)

The cellular signalling cascades stimulated by an increase in vasopressin concentration that ultimately lead to an increase in adrenal cortisol secretion have been comprehensively studied *in vitro* (Goncharova, 2013*)*. The endogenous secretion and/or exogenous administration of graded doses of vasopressin leads to the direct stimulation of the V1a receptors located on the adrenal gland, resulting in the dose-dependent secretion of cortisol (Perraudin et al, 1993; Goncharova, 2013; Mavani et al, 2015). Furthermore, vasopressin binds to the anterior pituitary V1b receptors and augments the CRH-mediated release of cortisol via ACTH stimulation, or may directly stimulate pituitary ACTH release and subsequently increase adrenal cortisol secretion (Koshimizu et al, 2012; Goncharova, 2013; Mavani et al, 2015). Cortisol exerts several short-term and long-term effects on whole-body glucose metabolism, which is particularly relevant to T1DM patients in the absence of counter-regulatory endogenous insulin production to oppose the cortisol-mediated increased rate of glucose appearance. Both transient and

chronically elevated cortisol levels have been shown to affect hepatic glucose production via gluconeogenesis, epinephrine-mediated skeletal muscle glycogenolysis and peripheral insulin sensitivity (Andrews and Walker, 1999; Kuo et al, 2013). Vasopressin is generally considered to have a permissive role in the cellular response to acute stress via the potentiation of the CRH-mediated increase in ACTH secretion, which leads to an increase in cortisol concentration (Goncharova, 2013; Rotondo et al, 2016). In contrast, vasopressin-mediated increase in pituitary ACTH secretion, and subsequent adrenal cortisol secretion, is solely responsible for the chronic stress response (Kuo et al, 2015; Mavani et al, 2015; Rotondo et al, 2016).

The acute stress response is composed of both non-genomic and transcription-dependent effects, and may account for the transiently greater glycaemic response evident during the dehydration trial of the current study. During the dehydration trial, there was a progressive rise in serum cortisol concentrations from baseline until peak serum cortisol concentrations arose at 30 minutes post-exercise, before declining to baseline (pre-exercise) levels. In contrast, serum cortisol concentrations progressively declined from baseline values over the duration of the control experimental trial. The progressive increase in both vasopressin (copeptin) concentration and serum cortisol concentrations throughout much of the dehydration trial may be indicative of a vasopressin-mediated acute stress response to the mild level of dehydration induced around exercise. Wiesli et al (2005) previously reported a short-term increase in post-prandial glucose concentrations due to an increase in salivary cortisol concentration in response to acute psychosocial stress. Acute cellular stress responses- independent of vasopressin or CRH-mediated ACTH/cortisol secretion, act through non-genomic mechanisms to rapidly preserve and increase the circulating glucose concentration in conjunction with other counter-regulatory hormones (e.g. epinephrine) in response to a novel stress (Kuo et al, 2013). The transient vasopressin-mediated cortisol response to the acute osmoregulatory stress during the current study is also supported by the gradual increase in copeptin (vasopressin) degradation over time from the peak copeptin response, coupled with a short yet statistically significant increase in cortisol secretion during the post-exercise period of the dehydration trial. Bussau et al (2006) also reported a peak cortisol response up to 30 minutes following the completion of a similar maximal sprint activity at the end of continuous, moderate intensity exercise under

hyperinsulinemic conditions. It is unclear whether the maximal sprint at the end of exercise and mild dehydration may synergistically upregulate the response to acute osmoregulatory stress and affect whole-body glucose responses.

In contrast, despite vasopressin-mediated ACTH (and subsequently cortisol) secretion primarily affecting the physiological responses to chronic cellular stress, it is unlikely that this type of response is stimulated by a mild osmoregulatory response, such as the level of dehydration induced during the current study. Perraudin et al (1993) demonstrated that chronically elevated vasopressin concentrations- indicative of a chronic stress response, responded in a biphasic manner and led to the eventual desensitization of adrenal V1a vasopressin receptors. Although there was a gradual decline from the peak cortisol response at 30 minutes post-exercise during the dehydration trial of the current study, there appeared to be no plateau in cortisol concentrations. It is unclear whether this biphasic response is exclusive to V1a receptor mediated increases in cortisol secretion. Furthermore, Aguilera and Rabadan-Diehl (2000) have highlighted that only 2% water deprivation from baseline body composition led to a decrease in vasopressin-mediated ACTH levels due to decreased pituitary V1b receptor expression. It is unclear why there may be discrepancies in the vasopressin-mediated response to different levels of osmoregulatory stress, and future research should aim to assess pituitary receptor function/expression with varying levels of dehydration. Chronic hypercortisolemia arising via repeated vasopressin stimulation often leads to the development of chronic hyperglycaemia and insulin resistance. Kuo et al (2015) highlighted that prolonged elevations in cortisol concentration stimulated an upregulated expression of the key regulatory enzymes in the various tissue-specific pathways that regulate glucose metabolism. However, there is an inevitable delay in the prolonged metabolic actions of cortisol due to the transcription-dependent effects of the elevated cortisol concentrations on the target genes and subsequent protein expression within the nucleus. It is therefore reasonable to conclude that the small, transient elevated post-exercise glycaemic response of mildly dehydrated T1DM patients during the current study is an acute response to the mild osmoregulatory stress and is evidence of the short-term effects of cortisol on glucose metabolism. Although there was continued fluid restriction throughout the post-exercise observation period, both vasopressin (copeptin) and cortisol concentrations declined from their respective peak responses. The results of

the current study contrast with selected review papers that have attempted to assess the effect of fluid intake on glucoregulation. Mavani et al (2015) and Moscogiuri et al (2016) postulated that as ACTH and cortisol secretion via direct stimulation by vasopressin is not regulated by the same intrinsic negative feedback mechanism as CRH-induced cortisol release, a continuous cycle of increased blood glucose concentrations due to persistent vasopressin-mediated cortisol release may be evident with chronic stress, which would subsequently increase serum osmolality and impair whole-body osmoregulation in T1DM patients.

Substrate Utilization

The current study is one of very few to detail the fluctuations in substrate utilization during exercise completed by T1DM patients. Previous research has established that amongst other factors, dysregulated glucose control around exercise (Jenni et al, 2008) and the type of exercise completed (Iscoe and Riddell, 2011; Bally, 2016) are primarily responsible for the effects on whole-body and skeletal muscle metabolism of T1DM patients during and following exercise. To date, there has been limited research conducted that has evaluated substrate metabolism under variable hydrated states, but only in healthy (disease-free) subjects.

The results of the current study showed no significant difference in substrate oxidation between trials as assessed by indirect calorimetry and Respiratory Exchange Ratio (RER). The average RER values during the dehydration trial were never higher than during the control trial, while the estimated rates of carbohydrate oxidation and fat oxidation were not significantly different between trials at any timepoint. Walsh et al (1994) and Logan-Sprenger et al (2013) previously highlighted no significant differences in RER values at any timepoint between euhydrated subjects and those who were dehydrated by up to 3% of pre-exercise body mass during continuous, submaximal exercise. Taken together, the results of these studies are in stark contrast with almost all previous research, which have consistently shown that male and female participants who were partially or wholly fluid restricted during prolonged, submaximal exercise had significantly elevated RER values throughout exercise, compared to euhydrated subjects (Hargreaves et al, 1996; Gonzalez-Alonso et al, 1999; Logan-Sprenger et al, 2012, 2015). Furthermore, research has tended to highlight an increased rate of carbohydrate oxidation and concurrently decreased rate of fat oxidation for dehydrated subjects throughout continuous exercise, compared to euhydrated subjects. During the current study, there was a main effect of time only on the respective substrate oxidation rates during both trials, with an increased rate of fat oxidation and concurrent decline in the rate of carbohydrate oxidation from the initial 15 minute value, which is consistently observed during submaximal, continuous exercise (Fallowfield et al, 1996; Logan-Sprenger et al, 2012).

There was a gradual decline in RER values evident during both experimental trials of the current study, which is indicative of slight, progressive suppression of carbohydrate

oxidation throughout exercise associated with muscle glycogen depletion. However, there were no significant differences in the respective substrate oxidation rates or for the total amount of carbohydrate or fat oxidised during exercise between trials. The results of the current study align with those of Logan-Sprenger et al (2013), who reported no difference in RER values, rate of carbohydrate and fat oxidation, or total oxidation of the respective substrates between euhydrated and mildly dehydrated males. However, in their study they reported a 24% increased rate of intramuscular glycogen utilisation across the duration of exercise during the dehydration trial, compared to euhydrated subjects. Although an increased rate of skeletal muscle glycogenolysis has been consistently reported with varying levels of dehydration across several other studies previously, estimated whole-body carbohydrate oxidation has also been shown to be concurrently increased under hypohydrated conditions (Hargreaves et al, 1996; Gonzalez-Alonso et al, 1999; Logan-Sprenger et al, 2012, 2015).

Although the submaximal exercise protocol during the current study was considerably shorter compared to previous research, this itself does not explain the discrepancies in substrate utilisation responses. A series of studies by Logan-Sprenger et al (2013, 2015) highlighted that up to 60 minutes into an extended submaximal exercise protocol, there was an evident trend for increased intramuscular glycogen utilisation during the respective dehydration trials. These results indicate that even at a similarly mild level of dehydration induced during the current study (~1% pre-exercise body mass), there were significant alterations in substrate metabolism evident at a tissue level. Research to date assessing the physiological mechanisms that stimulate the elevated skeletal muscle glycogenolysis associated with dehydration has proven equivocal, but it is thought that epinephrine secretion or local intramuscular temperature may be responsible (Logan-Sprenger et al, 2013).

It is important to recognise that the assessment of whole-body substrate utilisation during exercise; indicated by RER values, does not allow for the measurement of tissue-specific differences in substrate utilisation. Wallis and Jeukendrup (2005) stated that skeletal muscle metabolism may be accurately reflected in the composition of the expired breaths collected during lower intensity exercise, where there is a reduced blood/muscle lactate accumulation. However, it is possible that selected modes of exercise may lead to systemic RER values inaccurately reflecting skeletal muscle

metabolism, due to discrepancies in skeletal muscle recruitment and movement efficiency patterns (Hargreaves and Spriet, 2008; Cheneviere et al, 2010). A further consideration are the fluctuations in substrate utilisation of non-contracting tissues during exercise, which may lead to alterations in RER values (Febbraio et al, 1996). However, the active muscle mass during cycle-ergometer based exercise, for example, is predominantly lower-body and therefore the whole-body estimates of substrate utilization are likely to correlate with skeletal muscle metabolism (Cheneviere et al, 2010). Jansson (1982) initially showed that the rates of energy consumption at a whole-body (RER) and isolated skeletal muscle level (RQ) were closely matched during low intensity exercise, regardless of the composition of the diet consumed prior to exercise. Furthermore, Gonzalez-Alonso et al (1999) showed that estimates of substrate utilisation from isolated exercising legs were not significantly different compared to the time-aligned RER values during prolonged exercise. The whole-body and tissue-level values also remained significantly elevated above euhydrated RER and RQ values during exercise. RER can therefore be considered as an accurate measurement of substrate oxidation rates during cycle-ergometer based exercise, where oxygen cost and substrate utilisation is predominantly regulated by the metabolically active tissues of the lower body.

Post-Trial Hypoglycaemia, Hyperglycaemia Prevalence

It is unclear whether a similar increase in skeletal muscle glycogen utilisation compared to Logan-Sprenger et al (2013) study occurred during the exercise period of the dehydration trial. The level of dehydration was not as severe during the current study, while there was also no measurement of the rate of skeletal muscle (tissue-specific) substrate utilization during the current study. No study to date has assessed the potentially differing rates of skeletal muscle glycogen resynthesis in T1DM patients compared to non-DM patients. There are a number of factors which influence the rate of glycogen replenishment, including the increased insulin sensitivity of skeletal muscle that arises, particularly following glycogen-depleting exercise (Jensen et al, 2011). The data from the current study show a clear divergence in the average interstitial glucose concentrations between trials in the hours immediately prior to the initial overnight period. However, the mechanisms which may explain the delayed replenishment of intramuscular and hepatic glycogen stores up to 12 hours following the culmination of the dehydration trial are unclear. It is expected that with sufficient ingestion of carbohydrates, glycogen replenishment would occur within 4-6 hours following the proposed increased rate of skeletal muscle glycogenolysis during the dehydration trial. Despite the short-term elevation in cortisol concentration evident during the post-exercise recovery period of the dehydration trial compared to euhydrated subjects, there is evidence from *in vitro* research that an increase in cortisol may induce a transient skeletal muscle insulin resistance.

In the only study to date to assess glycaemic responses of T1DM patients to alterations in cortisol release, a post-prandial increase in salivary cortisol concentration subsequently led to a transient decrease in insulin sensitivity, therefore preventing a sudden decline in glucose concentrations (Wiesli et al, 2005). Glucocorticoid-induced insulin resistance in peripheral tissues including skeletal muscle leads to impaired insulin-mediated glucose uptake (Andrews and Walker, 1999). It was previously thought that chronically elevated glucocorticoid concentrations indicative of a variety of metabolic disorders were required to stimulate changes in glucose utilization (Rizza et al, 1982). However, a short-term increase in glucocorticoid concentration, such as those observed during the dehydration trial of the current study, has been shown to suppress peripheral tissues' insulin sensitivity, rather than lead to the development of a chronically insulin

resistant state (Andrews and Walker, 1999; Geer et al, 2014). Studies to date have not assessed the effect of changes in glucocorticoid concentrations on the intracellular glucose metabolism of Diabetes Mellitus patients, which is surprising given the impaired insulin sensitivity commonly associated with poorly controlled T1DM (Kennedy et al, 2013; Riddell et al, 2017). No investigation of potential alterations in insulin signalling cascades with fluctuations in serum cortisol concentrations were undertaken during the current study. However, elevated glucocorticoid (cortisol) concentrations have been shown to impair GLUT4 vesicle translocation to the cell membrane via the Akt/Protein Kinase B (PKB) signalling pathway (Figure 14) (Morgan et al, 2009). Overall, the impaired translocation of GLUT4 molecules is the result of altered phosphorylation patterns rather than changes in signalling molecule expression, as the expression of GLUT4 itself is upregulated in the presence of increased local glucocorticoid concentrations (Ruzzin et al, 2005). However, the impaired GLUT4 translocation is likely to be overwhelmed by the exercise-induced increase in GLUT4 translocation following skeletal muscle contraction mediated upregulation of AMPK activity (Younk et al, 2011; Riddell et al, 2017). Furthermore, research to date has tended to only assess the effects of synthetic glucocorticoid administration on insulin sensitivity.

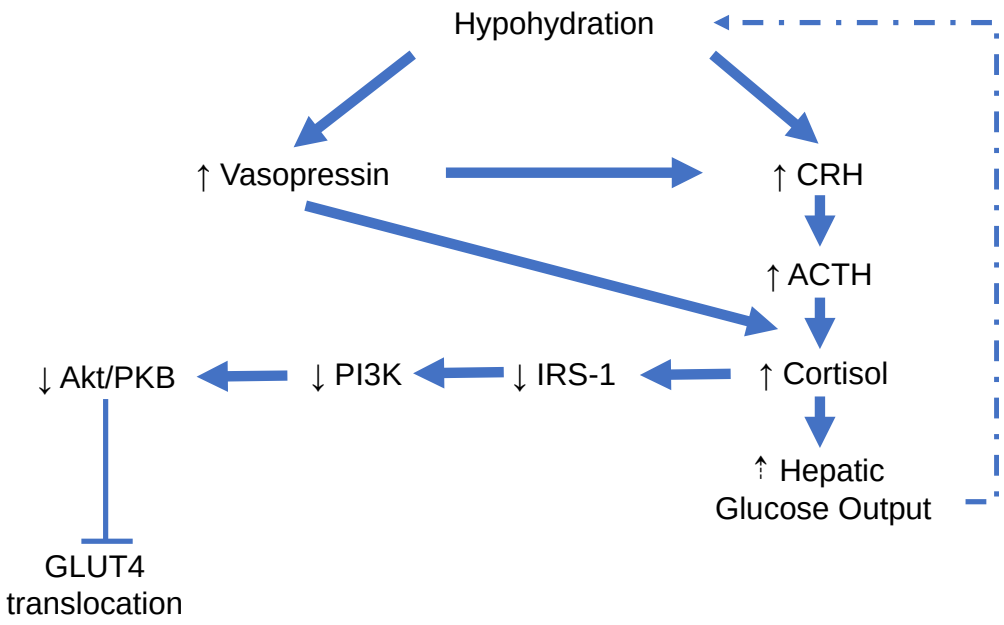

Figure 14: Proposed cellular signalling cascades that initially result in an increased secretion of adrenal cortisol following direct stimulation by vasopressin binding with adrenal V1a receptors, or indirect stimulation of ACTH release by vasopressin binding with anterior pituitary V1b receptors. The vasopressin-mediated increase in cortisol concentrations is hypothesized to lead to alterations in insulin signalling, leading to insulin resistance in peripheral tissues (skeletal muscle).

To date, there has been limited research which has investigated acute post-exercise/post-trial glycaemic responses and glycaemic excursions in the hours immediately following an exercise-based intervention. The focus of such research is often directed towards the overnight responses of Type 1 Diabetes Mellitus patients (up to 12 hours post-exercise), following a manipulation of nutritional or insulin regimens, and where the exercise itself is often completed in the evening (Rhabasa-Lloret et al, 2001; West et al, 2011; Campbell et al, 2013, Gomez et al, 2015). Specifically, there is a desire to avoid late-night (nocturnal) post-exercise hypoglycaemia, where the aim of previous exercise-based strategies has been to stimulate an upregulated neuro-endocrine response and preserve or immediately increase blood glucose concentrations. Analysis of the collated interstitial glucose concentrations for all participants up to 48 hours post-trial highlighted an increased prevalence of euglycaemic interstitial glucose concentration range (4-7mmol/L) up to 24 hours following the dehydration trial, compared to the control trial. Furthermore, there was a significantly decreased prevalence of mild

hyperglycaemic (7-11mmol/L) interstitial glucose concentrations up to 24 hours and 48 hours following the dehydration trial. Based on the average number of total interstitial glucose data points ('scans') obtained over the 48 hour period following the culmination of each experimental trial, discrepancies in glycaemic prevalence can be evaluated objectively. For example, the statistically significant 6.7% reduction in the prevalence of mild hyperglycaemia up to 48 hours post-dehydration trial equates to a total of 3 hours where there is 'tighter' glycaemic control, compared to the same timeframe following the control trial. Furthermore, although not statistically significant, there was a discernible increase in the prevalence of euglycaemic glucose concentrations in the 48 hours immediately following the culmination of the dehydration trial (15.5% increase on average). It is likely that the large standard deviations associated with this individual variable do not equate to statistically significant results. However, due to the decreased cumulative prevalence of hyperglycaemic values ('mild' and 'severe') compared to the 48 hours post-control trial, this equates to approximately 7 hours of additional time within a euglycaemic concentration range. There was no increased threat of hypoglycaemia associated with an improved acute glucose profile during the 48 hour period following the dehydration trial. Furthermore, the differences in acute glycaemic responses following each trial were not the result of any significant differences in the total units of insulin administered or total carbohydrate consumed between trials up to 24 hours and 48 hours following each experimental trial. Although there was no record of daily fluid intake for any participant following each trial during the current study, hypohydration has been shown to have no effect on skeletal muscle glycogen resynthesis (Neufer et al, 1991). In the event that T1DM patients remained hypohydrated following the culmination of the dehydration trial, it is unlikely that hydration status will account for the discrepancies in post-trial hypoglycemia and hyperglycaemia prevalence between trials.

The significantly reduced prevalence of mild hyperglycaemia following the successful exercise-induced mild dehydration is contradictory to the study hypothesis, yet there are clear physiological advantages associated with reductions in hyperglycaemia prevalence. In the short-term, hyperglycaemia has been shown to negatively affect cognitive function and mood (Cryer, 2002; Younk et al, 2011), while an increase in the time spent within a hyperglycaemic concentration range is associated with an increased

likelihood of a more insulin resistant phenotype and impaired glucose handling. Chronic hyperglycaemia or regular glycaemic 'excursions' from a euglycaemic concentration range is indicative of impaired glycaemic control, and will lead to a progressively increased risk of developing micro- and macro-vascular complications including retinopathy, nephropathy, neuropathy, cardiovascular disease, along with impairments in endothelial function and blood flow (Chimen et al, 2007; Kennedy et al, 2013; Riddell et al, 2017). Furthermore, the reduced prevalence of mild hyperglycaemia with no concomitant increased risk of hypoglycaemia arising during dehydration trial of the current study, for example, is a key factor in the successful management of T1DM. Regular or acute hypoglycaemic episodes may affect the patient's ability to adhere to a strict glycaemic management regimen and obtain their desired level of glycaemic control (McCrimmon and Sherwin, 2010).

Blood vs Libre

In an additional, novel component of the current study, a tertiary aim was to assess any potential differences in compartmental glucose responses during exercise and the post-exercise recovery period, via simultaneous measurement of both interstitial glucose and venous whole blood glucose concentrations. During the exercise period of the dehydration trial, there was a statistically significant difference between the change in interstitial glucose concentration and the change in blood glucose concentration at 15 minutes and 45 minutes from the respective baseline concentrations. There was also a significant difference in the change in interstitial glucose concentrations between the initial (0 mins) exercise value and 15 minutes into exercise during both experimental trials. The peak increase in interstitial glucose concentration not only exceeds, but also appears up to 15 minutes after the peak blood glucose response during exercise in both trials. However, there were no significant differences in the change from the respective baseline glucose concentrations during the post-exercise recovery period in either trial.

The current study is one of very few to assess fluctuations in interstitial glucose concentrations during exercise. Most studies to date have assessed interstitial glucose monitor accuracy, including FreeStyle Libre™, in terms of Mean Absolute Relative Difference (MARD) against reference venous or capillary glucose concentrations, but only under resting, euhydrated conditions (Moser et al, 2018). Aberer et al (2017) previously showed that during short, low intensity (2 x 15 minutes) exercise, FreeStyle Libre displayed the greatest accuracy against reference venous blood glucose values in comparison to selected continuous interstitial glucose monitors (CGM) that were worn simultaneously.

During the current study, the measurement of whole blood glucose concentrations may underestimate venous plasma glucose concentrations, potentially accounting for the greater difference between blood and interstitial glucose concentrations compared to previous research. Although the relatively small number of time-aligned paired interstitial and blood glucose concentrations was considered to be a negative outcome of the Aberer et al (2017) study, there was an identical number of paired glucose data points during the current study, based on an interstitial glucose sampling frequency of 5 minute intervals during 60 minutes of exercise.

During the initial stages of exercise, the interstitial glucose response has tended to be blunted in comparison to the rapidly increasing blood glucose concentration in previous studies (Moser et al, 2016). The increase in local blood flow to metabolically active skeletal muscles, coupled with the redistribution of extracellular fluids, leads to an initial increase in interstitial fluid volume. Furthermore, the rate of glucose transport from the capillaries into the interstitial fluid is often insufficient to match the increased blood glucose concentration resulting from increased utilization of endogenous glucose sources or ingested carbohydrates (Moser et al, 2018). Throughout the exercise period of both experimental trials, a physiological lag was evident whereby there was a trend for interstitial glucose concentrations to match, or even exceed, the increases in blood glucose concentrations during exercise in both trials. The lag in interstitial glucose response has been consistently proven due to the time required for glucose to diffuse from the capillaries into the interstitial fluid, which itself is determined by alterations in local blood glucose concentration and the rate of peripheral cellular glucose uptake by metabolically active skeletal muscle (Moser et al, 2018; Ajjan et al, 2018). It is possible that the increased cardiac output stimulated by the onset of exercise may affect the interstitial glucose response in the upper arm, although upper arm activity is minimal during cycle ergometer-based exercise.

During the current study, it is unclear what physiological mechanisms may be responsible for the significant differences in interstitial and blood glucose responses particularly evident during the dehydration trial. Moser et al (2016) highlighted that prolonged exercise or exercise-induced dehydration may decrease interstitial glucose supply compared to venous or capillary glucose levels, although it is unclear the level of dehydration necessary to induce alterations in glucose delivery. Furthermore, exercise-induced dehydration via cycling has been shown to primarily impair extracellular fluid regulation, including interstitial fluid volume, to supplement and maintain intramuscular and intracellular fluid volumes (Yardley et al, 2013; Siegmund et al, 2017). It is unclear how the redistribution of extracellular fluids to maintain critical blood flow to exercising muscles may affect any potential changes in interstitial glucose concentration (Yardley et al, 2013).

The majority of research investigating the interstitial glucose response during exercise has otherwise assessed the reliability of various CGMs in direct comparison to

capillary or venous blood glucose concentrations. Yardley et al (2013) highlighted that the changes in interstitial glucose concentration generally underestimated the changes in blood glucose concentration during exercise and the subsequent post-exercise resting period. Siegmund et al (2017) and Moser et al (2018) completed separate reviews of interstitial glucose responses at rest and during exercise along with different sampling techniques. Overall, both reviews concluded that if the rate of change in blood glucose concentrations exceeded 0.1-0.2mmol/L per minute there were significant differences compared to the rate of change in interstitial glucose concentration. Several exercise-related factors including carbohydrate mobilization/utilization, redistribution of extracellular fluids, prandial state and insulin concentration may elicit the magnitude of change in interstitial glucose concentration to significantly affect accuracy in comparison to aligned blood glucose concentration (Yardley et al, 2013; Siegmund et al, 2017, Moser et al, 2018). To date, research is in its infancy regarding the differing mechanisms that may affect FreeStyle Libre performance/accuracy during exercise, with all studies taking place under euhydrated conditions. Further research is required to establish the exercise-related factors which may have led to the observed differences in glycaemic response and which contrast previous research. For example, the application of the FreeStyle Libre to a site on the upper body compared to the lower body would allow for a direct comparison in interstitial glucose response based on differences in metabolically-active skeletal muscle during exercise, and subsequently increased local blood flow.

Applications / Limitations of Research

Currently, there are no adequate guidelines on hydration requirements during and following exercise available for T1DM patients as there is no consideration of any potential alterations in glucose concentrations mediated through changes in vasopressin concentration. Despite the current evidence which suggests that acute dehydration (i.e. increased vasopressin concentration) may have stimulated several metabolic pathways which affect glucose metabolism, the current guidelines focus instead on performance outcomes, rather than consideration of glucoregulation and T1DM patient's health. The fluid intake guidelines for exercise relate to healthy, non-DM subjects. Hibbert-Jones and Regan (2012), Horton and Subauste (2017) and Riddell et al (2017) detail that an adequate volume of fluid should be consumed prior to, during and post-exercise to prevent dehydration and avoid any negative effects on exercise performance. T1DM patients must however ensure stable glycaemic control around exercise for safe, effective participation (Riddell et al, 2017). A recent study by Buiote-Stella et al (2018) showed that on average, T1DM patients consumed a greater volume of fluid during exercise than the guideline intake, and compared with non-DM patients. However, the elevated rate of fluid intake did not equate to significant alterations in post-exercise average glucose concentrations. Riddell et al (2017) highlight that carbohydrate-electrolyte sports drinks may be used to prevent late-onset hypoglycaemia following exercise coupled with preventing exercise-induced dehydration, but do not recommend a specific volume of fluid to be consumed to optimize glycaemic responses during and following exercise. However, there is evidence to suggest that while dehydration may result in an elevated glycaemic response during and immediately following exercise compared to euhydrated subjects, there may be a prolonged positive effect on glycaemic management observed up to 48 hours following mild exercise-induced dehydration.

Patients with Type 1 Diabetes Mellitus must strike the balance between the postulated beneficial effects of acute exercise-induced dehydration on overall glycaemic control and the detrimental effect of chronic dehydration on overall health outcomes. While dehydration to 1-2% will not affect exercise performance and has been shown in the current study to reduce the prevalence of mild hyperglycaemia with a concurrent increased in time spent in euglycaemia and avoid hypoglycaemia, prolonged or regular dehydration may have negative long-term health consequences. For example, regular

dehydration may lead to the development of renal complications, including renal nephropathy and perhaps renal failure in extreme circumstances, due to the excessive urinary concentrating function associated with chronically elevated vasopressin concentrations (Bankir, 2001). Although our study is unable to confirm whether the vasopressin-mediated alterations in substrate metabolism are solely responsible for the positive effect on glycaemic control, chronically elevated vasopressin concentrations and concurrent hyperosmolality are clear risk factors for organ failure and all-cause mortality (Bouby et al, 1999; Bankir et al, 2001). Prolonged hyperglycaemia may also impair whole body osmoregulation due to the glucose-induced osmotic diuresis stimulated by the necessary excretion of excess glucose in the urine (Thompson et al, 1989). Furthermore, poorly controlled T1DM has been shown to lead to renal resistance to the effects of vasopressin, which may further exacerbate symptomatic polyuria and glycosuria of T1DM (McKenna et al, 2000). Overall, poor glycaemic control may foster the development of renal complications associated with Type 1 Diabetes Mellitus (Chimen et al, 2007; Younk et al, 2011; Riddell et al, 2017).

The limitations of the current study include no assessment of habitual fluid intake prior to beginning the study, or an assessment of total fluid intake during the initial 48 hour period following each experimental trial, where acute glycaemic control was assessed. It is therefore unclear if the degree of dehydration induced via the fluid restriction protocol during the current study is sufficient to account for the discrepancies in glycaemic prevalence due to acute changes in vasopressin concentration and subsequent alterations in glucoregulation. Furthermore, no intramuscular tissue samples were obtained to assess any potential changes in skeletal muscle glycogenolysis purported following the induction of mild dehydration, and which may aid the explanation for increased post-exercise skeletal muscle glycogen resynthesis.

In conclusion, the progressive dehydration protocol stimulated a greater glycaemic response during and following exercise in mildly dehydrated T1DM patients, compared to euhydrated patients, although this was not statistically significant. There was also significantly greater serum copeptin and cortisol concentration during the post-exercise recovery period of the dehydration trial. It is possible that the acutely elevated post-exercise glycaemic response of mildly dehydrated T1DM patients was mediated by the short-term effects of a transient increase in cortisol concentration on peripheral tissues glucose metabolism. Furthermore, although it is likely that the mild levels of dehydration induced during the current study were sufficient to stimulate an increased rate of skeletal muscle glycogenolysis, further research is required to fully elucidate the intramuscular responses to mild levels of hypohydration and whether the rate of glycogen replenishment may affect longer-term glycaemic control. Crucially, mild dehydration aided acute glycaemic control by ensuring there was a reduction in hyperglycaemia prevalence without an additional risk of hypoglycaemia arising. However, T1DM patients must balance the effects of regular or prolonged dehydration and the subsequent deleterious consequences associated with chronically elevated vasopressin concentrations, with the potential for short-term improvements in post-exercise glycaemic control. The results of the current study should be considered as relevant, informative pilot data to inform future research investigating the effects of variable hydration status on T1DM patient glycaemic control. This study was independently funded, with the aim to assess the glycaemic responses of an at-risk population and incorporated flash glucose monitoring technology and a novel nutritional strategy to aid glycaemic control. This is the first study to our knowledge to assess glycaemic responses to hydration status in T1DM patients, and is further complicated by the existence of equivocal evidence regarding the physiological mechanisms stimulated by alterations in hydration status in healthy subjects. There is a clear evidence-based gap in the guidelines for fluid intake around exercise for T1DM patients, which are instead aligned with the population-wide fluid intake recommendations. For T1DM patients, it is vital that safe, effective participation in exercise is maintained via similar nutritional and behavioural strategies to avoid glycaemic disturbances during and particularly the hours following exercise.

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Appendix 1: The Effect of a Concomitant Selective Serotonin Reuptake Inhibitor (SSRI) on T1DM Patient's Glycaemic Control- A Case Study

Background

Depression is twice as prevalent within the diabetes mellitus (DM) population compared to healthy, non-diabetic individuals (Anderson et al, 2001). DM patients diagnosed with co-morbid depression are at a significantly greater risk of an elevated fasting blood glucose concentration and subsequently greater HbA1c concentration, which may result in the development of disease-specific health complications (Lustman et al, 2001; Papelbaum et al, 2011). Antidepressants such as Selective Serotonin Re-Uptake Inhibitors (SSRI) are commonly prescribed to effectively treat depression symptoms, but there is equivocal evidence regarding the concomitant effect of antidepressants on glycaemic control (Sawka et al, 2001; Derijks et al, 2008; Knol et al, 2008; Brieler et al, 2016; Radojkovic et al, 2016). Specifically, treatment with SSRI medication (e.g. fluoxetine) has been shown to have a prolonged positive effect on long-term glycaemic management compared to other classes of antidepressant, including a discernible reduction in T2DM patient HbA1c concentration and upregulated hypoglycaemia counter-regulatory responses with acute SSRI treatment (Briscoe et al, 2008; Brieler et al, 2016; Radojkovic et al, 2016). However, the effects of antidepressant prescription on acute and long-term glycaemic control only have been investigated to date, in the absence of any potential effects of exercise on glycaemic control. Furthermore, there is little evidence available regarding the effect of antidepressant treatment on glycaemic control in T1DM patients, despite the importance of maintaining good glycaemic control for the successful management of T1DM (Kennedy et al, 2011; Fava et al, 2014).

Case Study Outline

One T1DM patient, who only managed to complete 50 minutes of exercise on both trials despite completing the full exercise protocol in the familiarization trial, was withdrawn from the main study. This patient was prescribed a concomitant antidepressant medication within the study timeline. To assess any potential disturbances in glycaemic control around exercise with prescribed SSRI treatment, this patient was selectively examined.

Results

The results of this n=1 case study showed that there was an identical change in glycaemic response during exercise in both trials. Interstitial glucose concentration increased from the respective baseline values (Control: 8.3mmol/L, Dehydration: 12.1mmol/L) until the peak glycaemic response, before declining below baseline concentrations by the culmination of exercise (Figure 15A). A similar response was evident for blood glucose concentration from baseline values (Control: 7.7mmol/L, Dehydration: 10.0mmol/L) until the end of exercise (Figure 15B). However, the exercise session was prematurely stopped following self-reported feelings of nausea and dizziness at the culmination of the initial (dehydration) trial. It is unclear whether the SSRI treatment affected the participant's awareness of hypoglycaemia-related symptoms, as there was no apparent threat of hypoglycaemia based on the participant's interstitial and blood glucose concentrations. Each of these symptoms is a primary side-effect of commencing SSRI treatment (Ferguson, 2001). The participant previously completed the entire familiarisation session safely, including the full duration (60 minutes) of the exercise protocol, prior to beginning the SSRI treatment. During the post-exercise recovery period, there was an elevated glycaemic response evident in the dehydration trial in contrast to the control trial, where both interstitial and blood glucose concentrations tended to remain below the respective baseline concentration (Interstitial: 6.2mmol/L; Blood: 5.5mmol/L). Interstitial and blood glucose concentrations tended to remain elevated above baseline concentrations (Interstitial: 6.5mmol/L; Blood: 7.3mmol/L) during the post-exercise period of the dehydration trial only.

There was an additional unit of insulin required pre-exercise during the dehydration trial, coupled with a further bolus unit of insulin administered during the post-exercise recovery period to prevent severe hyperglycaemia. In contrast, 27g of additional carbohydrate was required to prevent hypoglycaemia during the post-exercise period of the control trial. There was an increased prevalence of hypoglycaemia evident up to 24 hours following both trials in this patient, compared to the rest of the T1DM patient sample who completed both experimental trials (20% vs 5% control trial, 12% vs 5% dehydration trial, respectively). In general, >75% total interstitial glucose concentrations were within hypoglycaemic or euglycaemic concentration ranges for the treated subject following the control trial, compared to ~30% on average for the

untreated T1DM patient sample. The dehydration protocol successfully induced a greater increase in circulating post-exercise copeptin concentrations from baseline values, compared to the control trial (Table 5). Cortisol concentrations decreased over time across both trials, with a tendency for an elevated cortisol concentration during the dehydration trial (Table 5). Finally, glucagon concentrations were evidently greater throughout the control trial, compared to time-aligned values during the dehydration trial. However, there was a significant decline in glucagon concentrations by the culmination of the post-exercise period during both trials (Table 5).

Discussion

To our knowledge, this is the only data available that has assessed glycaemic control specifically around exercise in conjunction with concomitant antidepressant treatment in patients with T1DM. Previous studies by Sanders et al (2008) and Briscoe et al (2008) showed an upregulated counter-regulatory response to individual or repeated episodes of hypoglycaemia in both non-diabetic and T1DM patients respectively with extended SSRI treatment. Although glucagon concentration was elevated at baseline during the control trial, the sampling timeline does not allow for an accurate assessment of glucagon response to the threat of hypoglycaemia arising during the post-exercise recovery period of the control trial. However, there was a significant decline in glucagon concentration evident during the control trial from the immediate post-exercise concentration to the 2 hours post-exercise value. It is therefore likely that the dysregulated alpha cell function, and overall impaired counter-regulatory response to potential hypoglycaemia is unaffected by short-term SSRI prescription. Previous research to date has allowed for a greater duration of SSRI treatment prior to assessing hypoglycaemia counter-regulatory responses under resting conditions. Our interpretation of the current case study is limited by the incomplete dietary intake and insulin administration post-trial diaries following each experimental trial for this T1DM patient only. It is possible that changes in post-exercise glycaemic management, based on the glucose responses during each experimental trial, may explain the discrepancies in prevalence of hypoglycaemia and hyperglycaemia up to 24 hours following each experimental trial, compared to the non-SSRI treated T1DM patient cohort. Overall, there appeared to be a greater risk of hypoglycaemia following both experimental trials

compared to the untreated T1DM cohort, with an evident glucose lowering effect in the 24 hours particularly following the control trial. It is unclear whether the impaired hypoglycaemia counter-regulatory response to exercise associated with T1DM (Diedrich et al, 2002) may impact the proposed SSRI-mediated increase in counter-regulatory hormone secretion.

Further research is required to include an assessment of glycaemic control around exercise with acute and chronic SSRI treatment, as the only exercise-related outcome to have been previously reported is an increased adherence to regular exercise with chronic SSRI treatment (Lustman et al, 2011).

Table 5: Time-aligned endocrine responses of SSRI-treated T1DM patient prior to and post-exercise, where the patient was either euhydrated (control) or were wholly fluid restricted (dehydration trial).

Time	**Cortisol (pg/mL)**		**Glucagon (pg/mL)**		**Copeptin (pmol/L)**	
	Control	**Dehydration**	**Control**	**Dehydration**	**Control**	**Dehydration**
Baseline (Pre-Exercise)	59280	80550	8.93	4.27	3.87	8.60
0hrs Post-Exercise	33930	59280	10.70	3.25	6.50	35.45
2hrs Post-Exercise	19870	14200	1.00	1.00	2.63	12.62

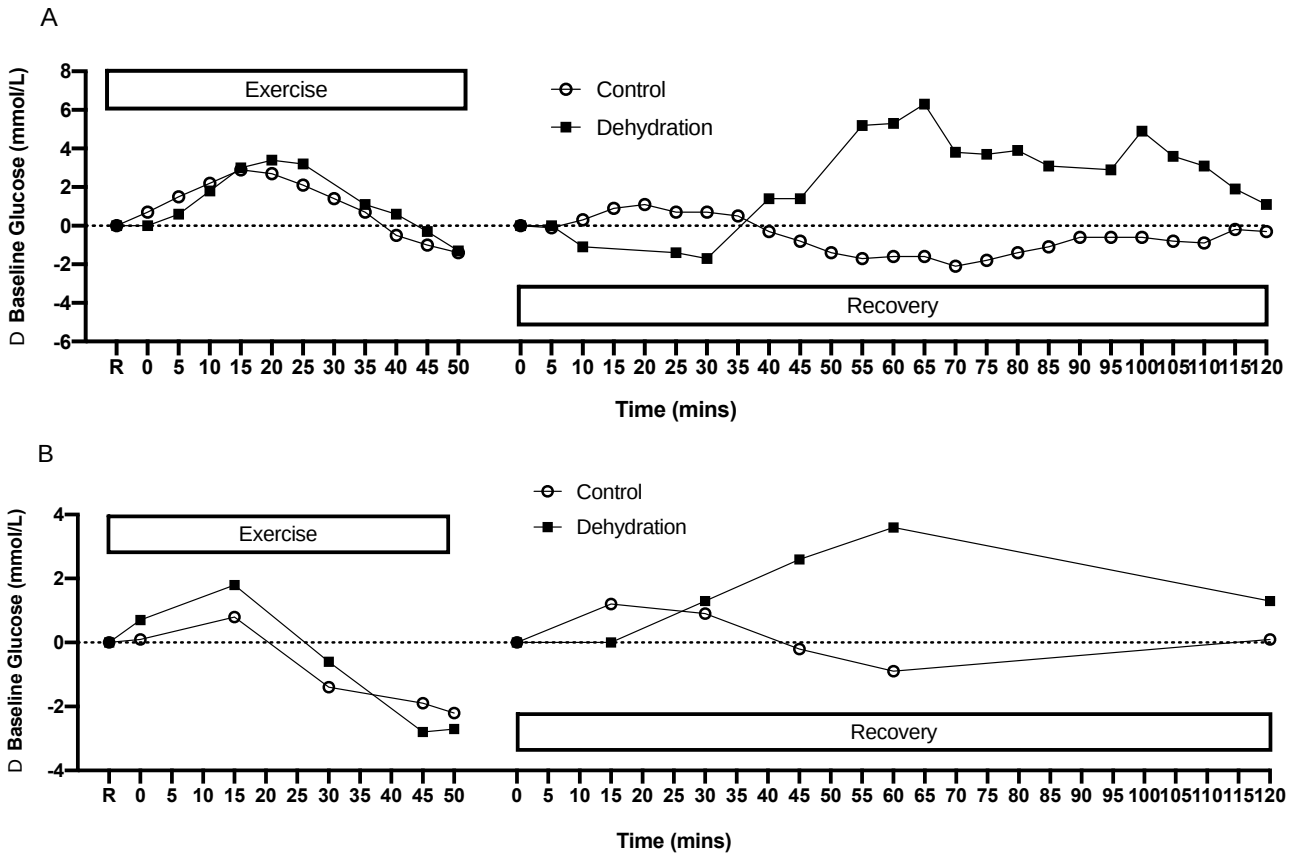

Figure 15: Changes in the (A) interstitial glucose concentration and (B) blood glucose concentration of a T1DM patient prescribed ongoing SSRI treatment, during exercise and throughout the post-exercise recovery period of both the control trial and dehydration trials. Interstitial glucose concentration was measured at 5 minute intervals throughout each experimental trial, with blood glucose measurements obtained every 15 minutes during exercise and the first hour of recovery. During exercise, the baseline (resting) value (R) was taken as the pre-breakfast glucose concentration. Initial post-exercise (0 mins post-exercise) glucose value was then considered to be the baseline concentration for the calculation of changes in glucose concentration during the post-exercise recovery period.

Appendix 2: Intra-Monitor Comparison of FreeStyle Libre Performance

In contrast to the multitude of studies that have investigated interstitial glucose responses against reference blood glucose concentrations (Yardley et al, 2013; Luijf et al, 2013; Moser et al, 2016; Fokkert et al, 2017; Aberer et al, 2017), there is little evidence available regarding the inter-reliability of flash interstitial glucose monitors applied to opposing anatomical sites (left and right upper arm). Participants (n=9) were fitted with an additional flash interstitial glucose monitor (FreeStyle Libre™) on their vacant arm prior to the control trial only to investigate any discrepancies in the glycaemic response of interstitial glucose monitors worn simultaneously during and following exercise under euhydrated conditions.

The results highlighted a trend for an elevated interstitial glucose response throughout exercise with the monitor most recently applied prior to the experimental trial (Monitor 2, Figure 16). However, there was no statistically significant difference in either the pre-exercise baseline interstitial glucose concentrations between trials (Monitor 1: 9.7mmol/L, Monitor 2: 9.8mmol/L; $p>0.05$), nor the glycaemic response of each flash glucose monitor from the respective baseline concentrations during exercise ($p>0.05$). During the post-exercise recovery period, there was also no significant difference in baseline post-exercise interstitial glucose concentration between trials (9.0mmol/L vs 10.2mmol/L; $p>0.05$). Furthermore, there was no statistically significant difference in interstitial glucose response between monitors from the respective post-exercise baseline concentrations ($p>0.05$). Assessment of acute glycaemic control showed no significant difference in the average interstitial glucose concentrations collated for each monitor at hourly intervals up to 48 hours following the respective experimental trials (Figure 17, $p>0.05$).

Bailey et al (2015) conducted the only other study to date which has directly assessed the accuracy of two FreeStyle Libre™ monitors worn simultaneously on the upper left and right arm. The results concur with the current study, whereby there was no significant difference between the accuracy of the flash glucose monitors on opposing sites, where sensor accuracy was expressed as the Mean Absolute Relative Difference (MARD) from capillary and venous whole blood glucose concentrations.

There may be differences in flash interstitial glucose monitor sensor activity/accuracy between initial application and when nearing the end of the 14 day

wear period, but there was no effect on the interstitial glucose responses between monitors evident during the current study. *In vivo* analysis of FreeStyle Libre™ monitor sensitivity to changes in reference capillary blood glucose concentrations across the entire 14 day wear period under free-living conditions confirmed that there was no significant decrement in sensor sensitivity after Day 1 (Hoss and Budiman, 2017). Initial local inflammation around the insertion site has previously been shown to affect interstitial glucose concentrations within the first 24 hours of flash interstitial glucose monitor application (Bailey et al, 2015).

Previous attempts to assess the inter-reliability of the FreeStyle Libre monitor have applied the sensor in locations which may have compromised the interstitial glucose response (Siegmund et al, 2016). A FreeStyle Libre monitor applied to the upper rear of one arm was significantly more accurate against reference capillary blood glucose measurements in comparison to a monitor applied on the abdomen, following the ingestion of a carbohydrate bolus (Fokkert et al, 2017). However, Hoss and Budiman (2014) have shown that under free-living conditions, there was no significant difference in monitor sensitivity between monitors applied to applied to the arm and the abdominal wall.

The current study is the first to our knowledge to assess the accuracy of simultaneous FreeStyle Libre monitors in response to exercise. Previous research has detailed the fluctuations in interstitial fluid volume and composition with exercise, and the subsequent effects on interstitial glucose concentrations (Moser et al, 2017; Siegmund et al, 2016). Despite potential differences in posture when simultaneous venous blood glucose sampling in one arm was undertaken along with interstitial glucose sampling during exercise, there was no significant difference in interstitial glucose responses between monitors during exercise. Further research is required to accurately assess potential variations in flash interstitial glucose monitors worn simultaneously during differing modes of exercise, with variable fluid intake or with short-term, rapid changes in interstitial glucose concentration to fully assess the accuracy of flash interstitial glucose monitors worn simultaneously (Ajjan et al, 2018).

The results of the current case study, coupled with previous research, do indicate that application of the FreeStyle Libre to the preferred site as per the manufacturer's

instructions will result in equally reliable interstitial glucose measurements, independent of the arm selected, during continuous, moderate intensity exercise.

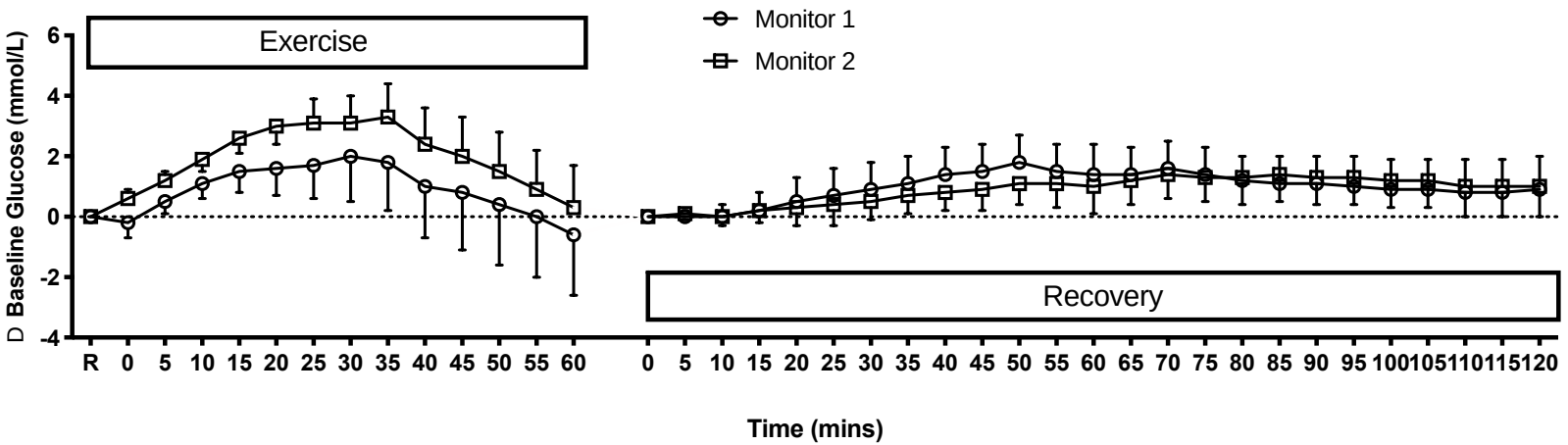

Figure 16: Intra-monitor comparison of the mean interstitial glucose response obtained from individual flash interstitial glucose monitors (FreeStyle Libre™) during the exercise and post-exercise recovery period of the control experimental trial. Each glucose monitor was scanned at 5 minute intervals throughout the experimental trial. During exercise, the baseline (resting) concentration (R) is represented by the pre-breakfast interstitial glucose concentration. Initial post-exercise (0 mins post-exercise) glucose concentration is represented by the baseline interstitial glucose concentration during the post-exercise recovery period. All data presented as Mean ± SEM.

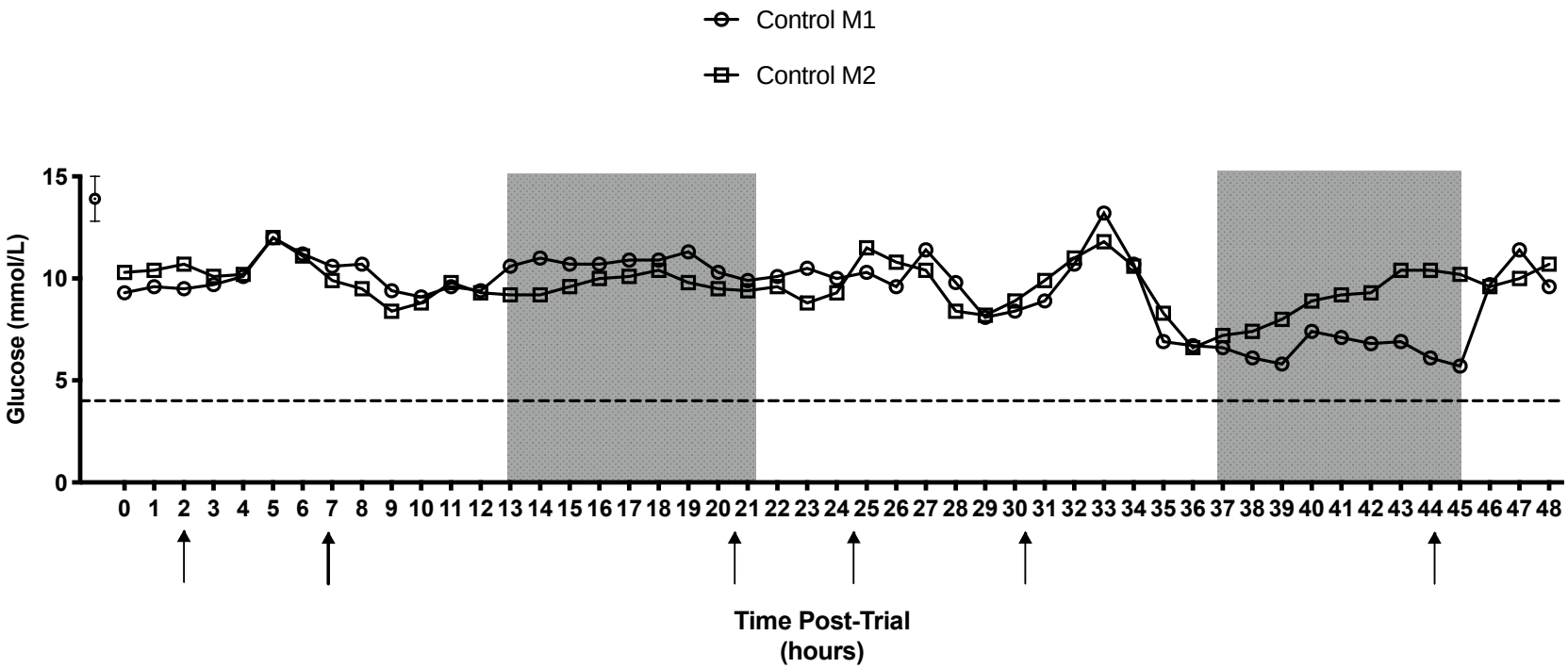

Figure 17: Mean interstitial glucose concentrations collated over 1 hour intervals during the 48 hours immediately following the control experimental trial for individual flash interstitial glucose monitors (FreeStyle Libre™) applied to opposing sites on the upper arm. The shaded area of the graph denotes each overnight period. Each arrow indicates the mean timepoint at which a main meal was consumed during the 48 hour period following each experimental trial. Data presented as the mean glucose concentration at each timepoint for each interstitial glucose monitor, with a pooled SEM shown in the top left of the figure.

Appendix 3: Diabetes Self-Management Questionnaire

The following statements describe self-activities related to your diabetes. Thinking about your self-care over the last 8 weeks, please specify the extent to which each statement applies to you	**Applies to me very much**	**Applies to me to a considerable degree**	**Applies to me to some degree**	**Does not apply to me**
1. I check my blood sugar levels with care and attention	☐ 3	☐ 2	☐ 1	☐ 0
2. The food I choose to eat makes it easy to achieve optimal blood sugar levels	☐ 3	☐ 2	☐ 1	☐ 0
3. I keep all doctors' appointments recommended for my diabetes treatment	☐ 3	☐ 2	☐ 1	☐ 0
4. I take my diabetes medication (e.g. insulin, tablets) as prescribed	☐ 3	☐ 2	☐ 1	☐ 0
5. Occasionally I eat lots of sweets or other carbohydrate-rich foods	☐ 3	☐ 2	☐ 1	☐ 0
6. I record my blood sugar levels regularly (or analyse the value provided with my blood glucose monitor/meter)	☐ 3	☐ 2	☐ 1	☐ 0
7. I tend to avoid diabetes-related doctors' appointments	☐ 3	☐ 2	☐ 1	☐ 0
8. I do regular physical activity to achieve optimal blood sugar levels	☐ 3	☐ 2	☐ 1	☐ 0
9. I strictly follow the dietary recommendations given by my doctor or consultant diabetes specialist	☐ 3	☐ 2	☐ 1	☐ 0
10. I do not check my blood sugar levels frequently enough as would be required for achieving what is deemed good blood glucose control	☐ 3	☐ 2	☐ 1	☐ 0
11. I avoid physical activity, although it would improve my glycaemic control	☐ 3	☐ 2	☐ 1	☐ 0
12. I tend to forget to take or skip my diabetes medication (e.g. insulin, tablets)	☐ 3	☐ 2	☐ 1	☐ 0
13. Sometimes I have real 'food binges' (not triggered by hypoglycaemia)	☐ 3	☐ 2	☐ 1	☐ 0
14. Regarding my diabetes care, I should see my medical practitioner(s) more often	☐ 3	☐ 2	☐ 1	☐ 0
15. I tend to skip planned physical activity	☐ 3	☐ 2	☐ 1	☐ 0
16. My diabetes self-care is poor	☐ 3	☐ 2	☐ 1	☐ 0

Scoring of the DSMQ:
The questionnaire is comprised of 4 **sections**:

- Glucose Management (Qs 1, 4, 6, 10, 12)
- Dietary Control (2, 5, 9, 13)
- Physical Activity (8, 11, 15)
- Health Care Use (3, 7, 14)

For each **section**: (total score / maximum score possible) x 10 = transformed score
(N.B. where negatively worded questions apply, the score is inversed
e.g. score of '0' for Q regarding avoidance of appointments would be considered as a '3')

The greater the total transformed score (sum of total for each section), the more effective the self-management of Diabetes Mellitus.