Furthering the understanding of interstitial glucose on ECG metrics in people with type 1 diabetes.

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I. Abstract.

Cardiovascular autonomic neuropathy is a common complication of type 1 diabetes and can be considered the leading cause of mortality. It is increasingly important to detect early ECG alterations in healthy individuals with type 1 diabetes to help prevent the future onset of cardiovascular autonomic neuropathy and reduce mortality. This thesis aims to further understand the effect interstitial glucose has on ECG parameters during an eight-hour resting period, a novel low-intensity twenty-watt exercise test and a six-hour nocturnal period in individuals with type 1 diabetes. This thesis is a secondary analysis study including sixteen individuals absent of disease with generally well-controlled type 1 diabetes. Results revealed a reduction in QT (ms), QTc (ms) and HF (ms^2) during the low-intensity exercise test during hyperglycaemia compared to euglycaemia. The increased rate of decline in interstitial glucose reduced heart rate (bpm), rMSSD (ms) and pNN50 (%) during the 6-hour nocturnal period. This thesis concludes that interstitial glucose results in some ECG alterations during parasympathetic withdrawal and the nocturnal period in a healthy disease-free cohort of individuals with type 1 diabetes. There was no effect of relatively similar interstitial glucose levels on ECG parameters during the 8-hour resting period.

II. Declarations.

I declare that the work within this thesis has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

I declare that this thesis is the result of my own investigations, except where otherwise stated and that other sources are acknowledged by footnotes giving explicit references, and that a bibliography is appended.

I give my consent for this thesis to be made available in the University repository and an e-thesis to be made available to Swansea University library.

I declare that the University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

Signature: 

Date: September 2023

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VI. List of abbreviations.

ANOVA - Analysis of variance

ANS - Autonomic nervous system

ATP - Adenosine triphosphate

BG - Blood glucose

BPM - Beats per minute

CAN- Cardiac autonomic neuropathy

CGM - Continuous glucose monitoring

DN- Diabetic neuropathy

ECG- Electrocardiogram

[Gi] -Interstitial glucose concentration

GV - Glycaemic variability

HbA1c (mmol/mol) - Glycated haemoglobin

HRV- Heart rate variability

iG- Interstitial glucose

LF (ms^2)- ECG metric showing absolute power of the low-frequency band (0.04-0.15 Hz)

HF ($ms^{2)}$- ECG metric showing absolute power of the high frequency band (0.15-0.4 Hz)

LF/HF Ratio- ECG metric showing ratio of LF-to-HF power

PCR - Phosphocreatine

pNN50- Heart rate variability metric percentage of successive RR intervals that differ by more than 50 ms (%)

QT Interval - Start of the Q wave to the end of the T wave of ECG waveform

SA Node - Sinoatrial node

SDNN- Heart rate variability metric showing standard deviation of NN intervals (ms)

rMSSD- Heart rate variability metric root mean square of successive RR interval differences (ms)

T1DM – Type 1 diabetes mellitus

VO_2 max- Maximum volume of oxygen

1. Introduction and review of the literature

1.1 Brief introduction

Type 1 diabetes mellitus (T1DM) is characterised as an autoimmune disease caused by the destruction of insulin-producing beta cells in the pancreas, resulting in absolute or relative insulin deficiency (Atkinson et al, 2014; Maah et al, 2010). Insulin deficiency results in an inability for an individual to sufficiently self-regulate blood glucose levels and therefore can result in the acute complications of 'hypoglycaemia' or 'hyperglycaemia'. Hypoglycaemia can be defined as a blood glucose concentration of <3.9 mmol.L^{-1} and hyperglycaemia can be defined as a blood glucose concentration of >10 mmol.L^{-1} (Battelino, 2019). Glycaemic ranges are more specifically defined as level 2 hyperglycaemia (>13.9 mmol.L^{-1}), level 1 hyperglycaemia (10.1-13.9 mmol.L^{-1}), in range (3.9-10.0 mmol.L^{-1}), level 1 hypoglycaemia (3.0-3.8 mmol.L^{-1}) and level 2 hypoglycaemia (<3.0 mmol.L^{-1}) (Battelino, 2019). The inability to self-regulate euglycemia glucose levels in the bloodstream results in T1DM individuals being reliant on exogenous insulin therapy.

1.2 Costs

Insulin-dependent diabetes mellitus causes great personal costs for the individual and also great financial costs for the NHS (Simell, Sintonen, Hahl & Simell, 1996). T1DM affects 400,000 individuals in the UK (JDRF, 2021) and costs the NHS an estimated £1.8 billion per year (Kanavos, van den Aardweg & Schurer, 2012). It has also been reported that 80% of the cost of diabetes is spent on complications rather than direct care (Diabetes UK, 2014).

1.3 Long-term complications

Glycaemic control describes the optimal euglycaemic range for individuals with T1DM (3.9-10.0 mmol.L^{-1}), aiming to avoid hypoglycaemia (<3.9 mmol.L^{-1}) and hyperglycemia (>10 mmol.L^{-1}) (Battelino, 2019). Healthcare professionals will test individuals with T1DM glycated haemoglobin (HbA1c) which measures average blood glucose levels over the past 3 months. The National Institute for Health and Care Excellence (NICE) advises a guideline for individuals with T1DM with a target HbA1c level of 48mmol/mol. Individuals with T1DM with poor glycaemic control can lead to long-term complications such as neuropathy, retinopathy, nephropathy, and cardiovascular disease (Hahl et al, 2002).

1.3.1 Retinopathy

Prolonged high blood glucose levels (hyperglycaemia) and events of lack of glucose supply (hypoglycaemia) can also result in diabetic retinopathy. Retinopathy is caused by damage to the retina of the eye and can lead to severe visual impairment and blindness. Diabetic retinopathy is the leading cause of visual impairment among people of working age (Porta & Bandello, 2002). Diabetes duration also has a direct effect on diabetic retinopathy with a study suggesting that within 20 years of diagnosis, virtually all patients had developed a degree of diabetic retinopathy and almost half of the patients had developed proliferative diabetic retinopathy which is defined as the growth of new and abnormal blood vessels in the retina of the eye (Porta & Bandello, 2002).

1.3.2 Nephropathy

Nephropathy is the deterioration of the normal functioning of the kidneys. Abnormalities in the renal tissue occur during hyperglycaemia as it promotes increased blood flow, intracapillary pressure and endothelial cell dysfunction and also during hypoglycaemia as a lack of glucose supply causes a reduced blood flow leading to damage to the cells. Consequently, there is increased permeability in the glomerulus (Papadopoulou-Marketou, Chrousos & Kanaka Gantenbein, 2017). Early stages of increased permeability of the glomerulus can be reversible however maintained and frequent states of hyperglycaemia can lead to irreversible damage. Studies suggest that 25-40% of patients with T1DM develop a level of diabetic kidney disease (Papadopoulou- Marketou, Chrousos & Kanaka Gantenbein, 2017). Good glycaemic control and favourable glycated haemoglobin (HbA1c) levels have been found to help prevent the onset of diabetic nephropathy however research has also uncovered that using renal biopsies as well as family history of kidney disease are indicators for diabetic nephropathy. Studies show specific genes which are associated with increased risk of developing diabetic nephropathy (Papadopoulou-Marketou, Chrousos & Kanaka Gantenbein, 2017). Management of diabetic nephropathy includes yearly screening for the presence of increased albuminuria (a condition in which albumin a protein found in the blood is detected in urine), measurements of glomerular filtration rate should be performed, improved glycaemic control by intensive insulin treatment, improved lipid control, favourable blood pressure measurements and reducing salt intake can all be used as primary methods however during end stage renal disease dialysis and transplantation may be required (Papadopoulou- Marketou, Chrousos & Kanaka Gantenbein, 2017).

1.3.3 Neuropathy

Neuropathy is the most common long-term complication of T1DM and is defined as damage to the sensory, motor and autonomic nervous systems caused by prolonged hyperglycaemia and hypoglycaemic events (Yagihashi, Mizukami & Sugimoto, 2011). Hyperglycaemia causes overactivity in pathways such as superoxide production and therefore overactive membrane bound receptors, and hypoglycaemia reduces blood flow leading to neural hypoxia both resulting in nerve cell damage (Giacco & Brownlee, 2010). Damage to the autonomic nervous system increases mortality in T1DM patients and sensory and motor neuropathic damage can result in an increased risk of the need for amputation of limbs, especially lower limbs. Nerve injury occur due to an increased flux of the polyol pathway, enhanced glycation end-product formation, excessive release of cytokines, activation of protein kinase C, and enhanced oxidative stress as a consequence of high hyperglycaemic prevalence (Yagihashi, Mizukami & Sugimoto, 2011).

1.3.4 Cardiovascular Disease

A major long-term complication of T1DM is cardiovascular disease. It is known that premature atherosclerosis is one of the main factors that contribute to the statistic that mortality among individuals with T1DM is three times more prevalent than in the general population (Schofield, Ho & Soran, 2019). Cardiovascular risk factors in the T1DM population include age, sex, diabetes duration, HbA1c, systolic blood pressure, low-density lipoprotein (LDL) cholesterol, glomerular filtration rate and lifestyle choices such as smoking and exercise, as recorded by Steno Type 1 Risk Engine (Schofield, Ho & Soran, 2019). Hyperglycaemia is a well-

established factor that contributes to an increased risk of developing cardiovascular disease. Therefore, good glycaemic control is vital in preventing the onset of cardiovascular implications. Other methods of reducing cardiovascular risk include blood pressure control, statin use and lifestyle interventions such as smoking cessation and regular exercise. In addition, research has shown that the use of insulin pump therapy compared to multiple daily insulin injections has been associated with a decrease in cardiovascular mortality since insulin pumps result in lower glucose variability (Schofield, Ho & Soran, 2019).

In conclusion, the long-term complications of T1DM highlight the importance of individuals maintaining a high level of glycaemic control as a primary controllable method to help prevent irreversible and life-shortening damage.

1.4 Cardiovascular autonomic neuropathy (CAN)

Cardiovascular autonomic neuropathy (CAN) is a common complication of T1DM and can be considered the main cause of mortality (Anaruma et al, 2016; Vinik et al, 2013). CAN is diagnosed when cardiovascular implications are already established and it is considered that these complications are generally irreversible (Anaruma et al, 2016). Therefore, it is increasingly important to detect early electrocardiogram (ECG) changes within individuals with T1DM to prevent the onset of CAN and in turn, reduce mortality. The main established risk factor for the onset of CAN in T1DM is poor glycaemic control, other factors include hypertension, retinopathy, smoking, age, and gender (Serhiyenko & Serhiyenko, 2018; Vinkik & Ziegler, 2007).

During clinical practice methods of assessing the onset of CAN include QT interval prolongation (Pop-Busui et al, 2013). QT interval is the time from the start of the Q wave until the end of the T wave. Short-term ECG monitoring, heart rate variability (HRV), blood pressure (BP) monitoring, muscle sympathetic nerve activity, and cardiovascular sympathetic testing are methods used to assess the onset of CAN (Serhiyenko & Serhiyenko, 2018). During short-term ECG monitoring frequency domain, metrics are used such as VLF (very low frequency), LF (low frequency), and HF (high frequency). "LF represents combined effects of sympathetic and parasympathetic influence, whereas HF represents vagal activity" (Serhiyenko & Serhiyenko, 2018). During clinical monitoring, a decrease in HF suggests a decrease in parasympathetic dysfunction in the early stages of cardiovascular autonomic neuropathy (Serhiyenko & Serhiyenko, 2018). HRV monitoring also assesses parasympathetic activity using time domain measures such as standard deviation of RR intervals (SDNN), root square mean of successive intervals between normal heartbeats (RMSSD), and the percentage of successive RR intervals that differ by more than 50 ms (pNN50) (Serhiyenko & Serhiyenko, 2018). A reduction in HRV metrics is associated with CAN (Vinik et al, 2013). This is important to this research study as it validates the importance of using ECG and HRV monitoring methods in detecting early signs of complications such as CAN. These studies outline how specific ECG alterations may indicate early signs of CAN and therefore help inform which metrics are of interest to this research study.

1.5 Glucose

1.5.1 Glucose variability

It is well established that chronic hyperglycaemia is the primary risk factor for individuals with T1DM as described above however it is also believed that frequent or large fluctuations in

glucose, also described as glycaemic variability (GV), may also independent risk factors contribute to the onset of long-term complications such as cardiovascular disease (Suh & Kim, 2015). Research studies have also shown that intermittent high glucose rather than continuously high glucose has been shown to have detrimental effects (Krishna, Kota & Modi, 2013 & Suh & Kim, 2015). Interestingly a study exploring the role of GV on the development of microvascular complications found that an increased HbA1c explained only 11% of the variation in the risk of retinopathy and therefore suggesting there are other significant factors that could be contributing to complication onset (Lachin et al, 2008). There is growing evidence to suggest that significant GV is a key factor in the progression of diabetic complications due to factors such as absolute insulin deficiency, erratic absorption of exogenous insulin, incomplete suppression of hepatic glucose production, and altered counterregulatory (Suh & Kim, 2015).

1.5.2 Rate of change in glucose

In addition to GV alone, the rate of variability or change in glucose is an important factor to consider when exploring long-term complications. Research has shown that an increased rate of glucose variability is associated with an increased risk of cardiac complications in T1DM (Rodbard, 2018). Increased changes in glucose, particularly during episodes of hypoglycaemia have been thought to create increased stress responses such as oxidative stress and inflammation both of which have been linked to the contribution of cardiovascular disease (Chow et al, 2014). However, it is relatively unknown how the rate of change in GV during hyperglycaemic episodes can impact cardiovascular health in individuals with type 1 diabetes.

1.5.3 Hyperglycaemia

The long-term effects of hyperglycemia are discussed above, however, it is important to acknowledge the short-term physiological impacts of hyperglycemia on cardiovascular health, particularly in relation to sudden cardiac events and the ‘dead in bed’ syndrome which is so prominent in type 1 diabetes. A study found that short-term hyperglycemia caused an increase in oxidative stress in type 1 diabetes by increased production of reactive oxygen species causing increased inflammation and blood vessel damage (Ceriello et al, 2017). Short-term hyperglycemia also contributes to endothelial dysfunction which can increase the onset of atherosclerosis by impairing the inner lining of the blood vessels and impacting the formation of plaque (Esposito et al, 2018). Platelet dysfunction is also a by-product of short-term hyperglycemia which can result in an increased risk of thrombotic events and abnormal blood clotting in individuals with type 1 diabetes (Tscheope et al, 1991).

1.6 Physiology of the Autonomic Nervous System and Cardiac Cycle

1.6.1 The autonomic nervous system

The autonomic nervous system (ANS) is a component of the peripheral nervous system which is responsible for generating involuntary processes such as heart rate as shown in Figure 1. The ANS is divided into two branches: the parasympathetic branch and the sympathetic branch (Robinson et al, 1996). The parasympathetic influence is generally responsible for the reduction in heart rate whereas the sympathetic influence is responsible for an increase in heart rate. As a result, at rest, the parasympathetic influence is dominant however during the influence of stimuli such as exercise the sympathetic effect is dominant (Fornasiero et al, 2018). Other examples of the effects of the sympathetic and parasympathetic influences of the ANS are shown below in Figure 2.

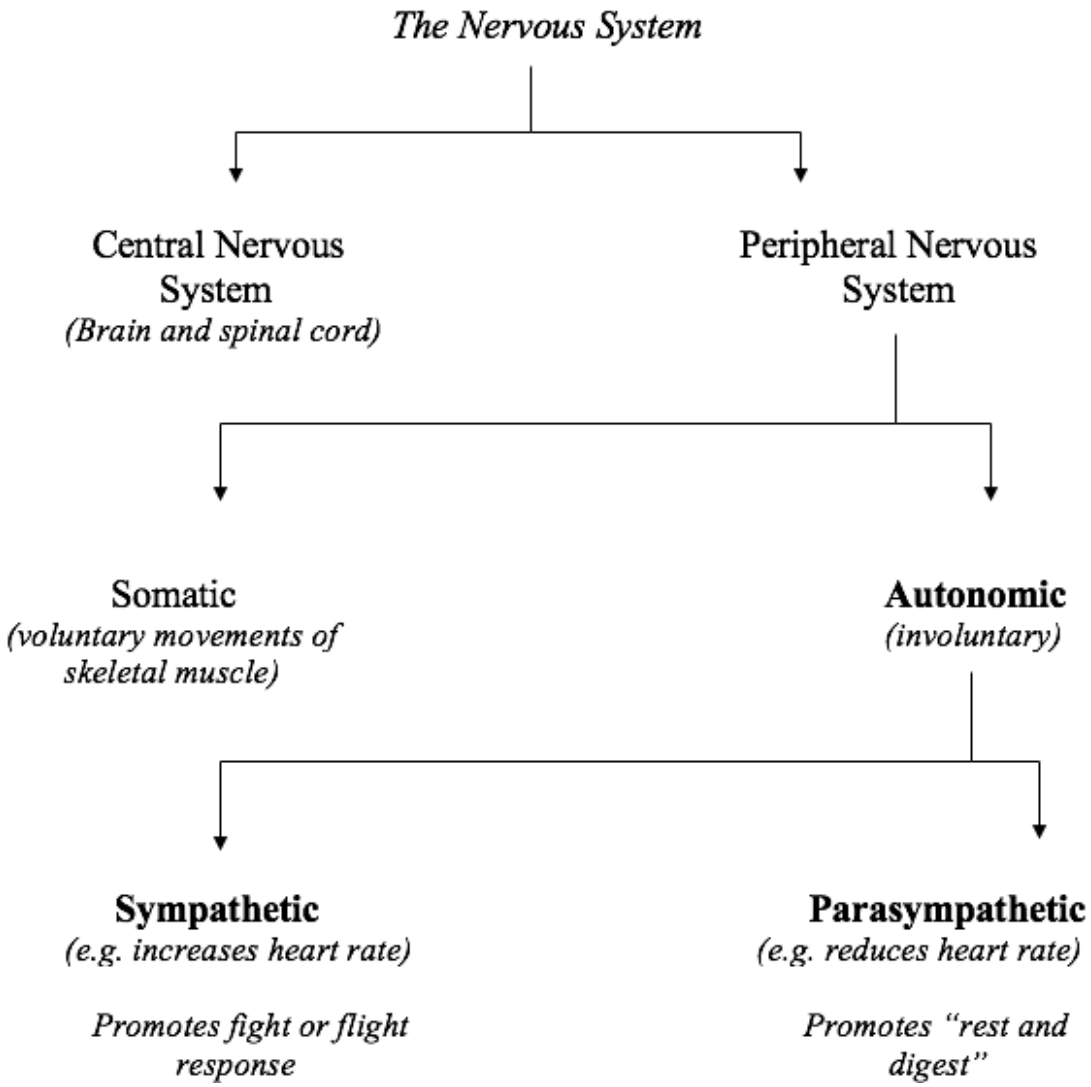


Figure 1. A schematic to show the nervous system.

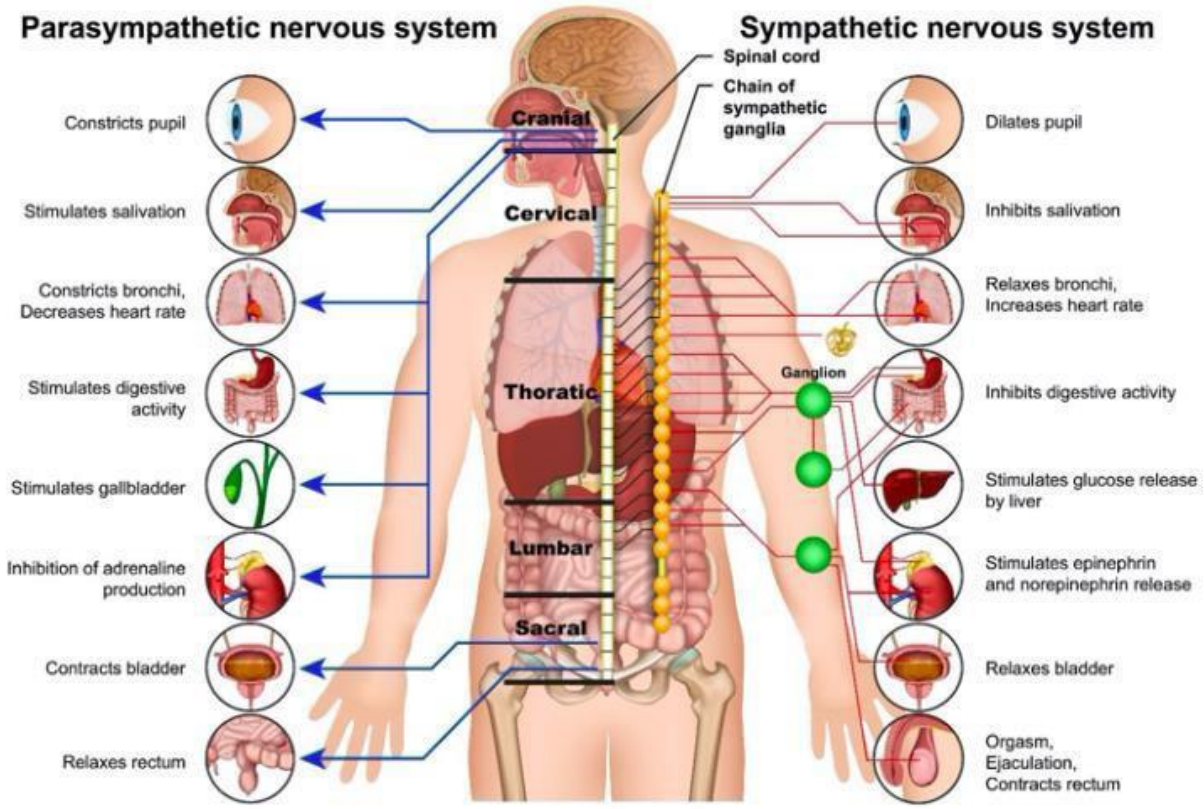


Figure 2. A schematic to show differences between the parasympathetic and sympathetic systems within the autonomic nervous system (*Autonomic Dysreflexia*, 2022).

1.6.2 Hormonal activity

Hormones released by the endocrine system mediate heart rate by influencing the parasympathetic and sympathetic branches of the autonomic nervous system which as explained above influence cardiac processes such as heart rate. The endocrine system is made up of several glands that produce hormones shown below in Figure 3. The adrenal medulla which is the inner part of the adrenal glands located on top of each kidney releases the hormones epinephrine and norepinephrine in response to external stress which in turn activates

the sympathetic strand of the autonomic nervous system (Sherwood, 2015). Additionally, the hypothalamus produces a corticotropin-releasing hormone in response to stress which stimulates adrenocorticotropic hormone release from the pituitary gland (Sherwood, 2015). As a result, adrenocorticotropic hormones stimulate the release of the stress hormone cortisol from the adrenal glands which can modulate autonomic activity by influencing neurotransmitter release and sensitivity (Sherwood, 2015).

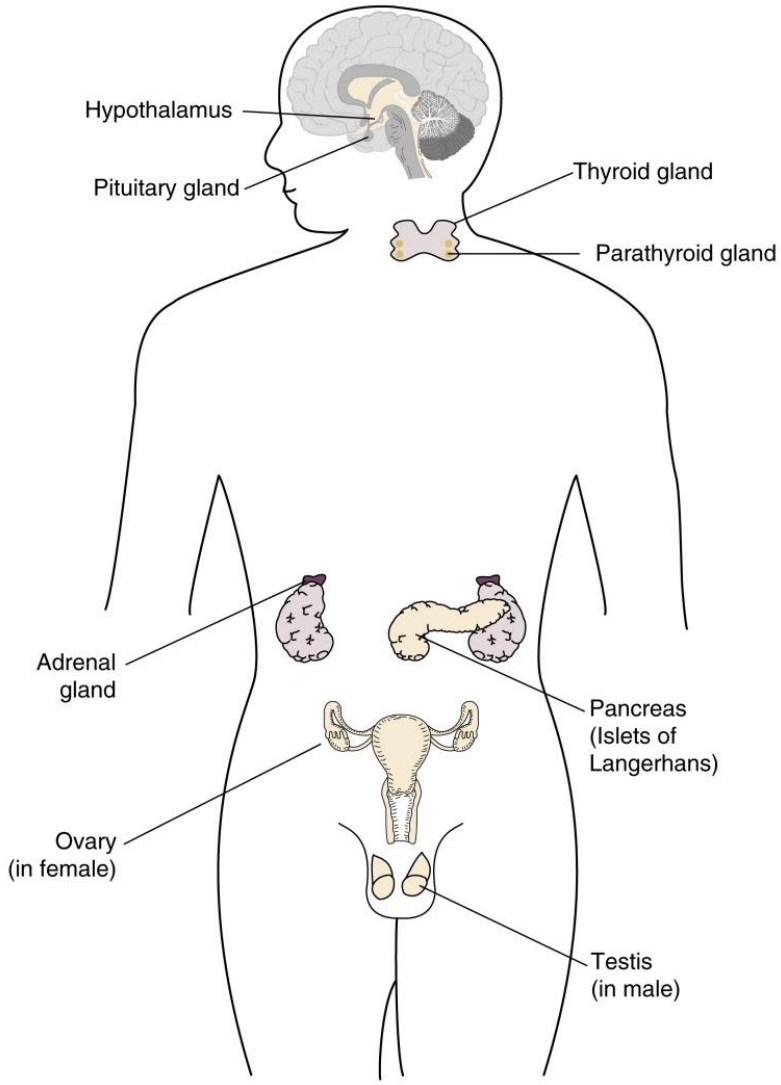


Figure 3. Schematic of the endocrine system (Hiller-Sturmhöfel & Bartke, 1998).

1.6.3 The Cardiac Cycle

The heart is regulated by the cardiac cycle which is divided into two basic phases: diastole and systole. Diastole is a period in which ventricles are relaxed and blood passively fills the left and right atria via the vena cava and pulmonary vein, and then through open atrioventricular mitral and tricuspid valves into the respective ventricles. Systole is a period in which the left and the right ventricles contract, and as a result, blood is ejected into the aorta and pulmonary artery. During systole, atrioventricular valves are closed and therefore no further blood enters the ventricles until the next period of diastole, however blood from the vena cava and pulmonary vein still enters the respective atria (Pagel & Freed, 2018). The rate at which the cardiac cycle occurs (heart rate) is initiated by an electrical impulse stimulated by pacemaker cells called the sinoatrial node in the right atrium of the heart as shown in Figure 4 (Nerbonne & Kass, 2005). The impulse travels to the atrioventricular node and along the atria walls causing articular contraction. The impulse then travels down the bundle of His towards the apex of the heart, up Purkinje fibers, and along the left and right ventricular walls resulting in ventricular contraction (Nerbonne & Kass, 2005). Oxygenated blood is pumped from the ventricles via the aorta and pulmonary artery to the body and lungs respectively (Pelech, 2004).

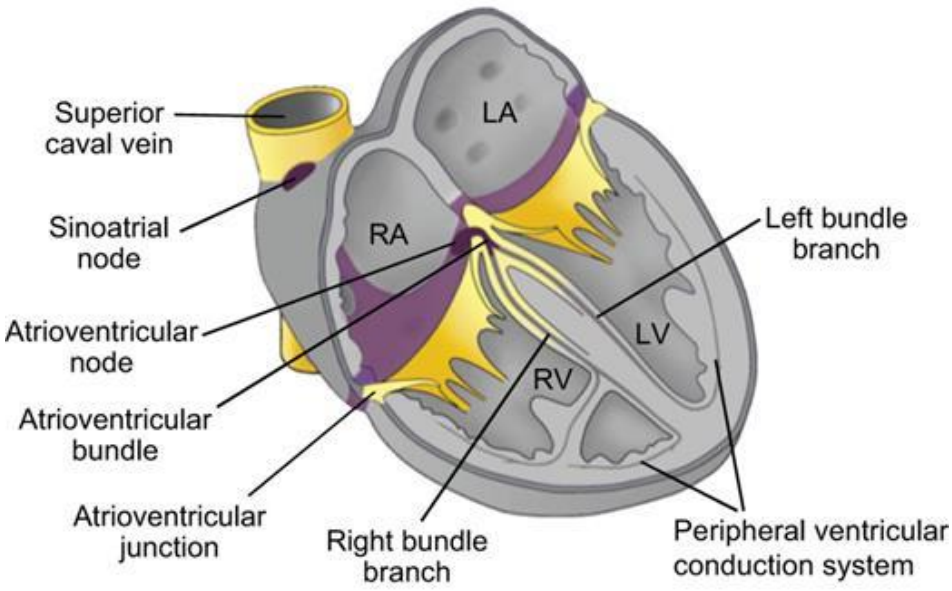


Figure 4. A diagram to show the elements of the cardiac conduction system (Van Weerd & Christoffels, 2016).

1.6.4 Cardiac Action Potential

Cardiac pacemaker cells have unstable resting potentials, and an electrical impulse will be initiated at the sinoatrial (SA) node by spontaneous depolarisation. Depolarization occurs due to the activation of sodium (Na^+) channels and the influx of Na^+ ions. If the increased voltage surpasses the threshold potential (-40mV) further Na^+ channels open and the action potential increases from -90mV to ~+10mV (Pinnell, Turner, & Howell, 2007). If the threshold potential is not reached an action potential will not be activated which is referred to as the 'all-or-nothing' law. There is then partial repolarization due to a decrease in Na+ permeability. The next phase involves a 'plateau phase' where depolarization is maintained by membrane permeability to Na+ ions and therefore prolonging the action potential. Calcium channels become inactive towards the end of the 'plateau

phase' and there is an influx of potassium ions which results in repolarization (Pinnell, Turner, & Howell, 2007).

1.7 ECG and HRV

1.7.1 ECG introduction

An electrocardiogram (ECG) is a device that records the electrical activity of the heart by providing insight into heart rate and rhythm (NHS, 2018). An ECG recording is able to give insight into the electrical activity of the heart by measuring several ECG waveform metrics such as heart rate (HR), QT interval, QTc interval, and heart rate variability metrics such as standard deviation of NN intervals (SDNN), root mean square of successive RR intervals (RMSSD), percentage of successive RR intervals that differ by more than 50 ms (pNN50%), the absolute power of the low-frequency band (LF), the absolute power of the high-frequency band (HF) and the ratio of LF-to-HF power (LF/HF ratio). Additional information regarding ECG metrics can be found in sections 2.2.4, 2.2.5, and 2.2.6. ECG devices can have several leads, generally ranging from 3-12 leads, which describes the number of electrodes that are used. Electrodes are placed on specific areas of the torso (and limbs when using a 12-lead) ECG to gain optimum insight into the electrical activity of the heart. A 12-lead ECG is generally considered the gold standard; however, research has confirmed that 3-lead ECGs are equally as accurate in identifying significant cardiac abnormalities (Antonicelli et al, 2012).

1.7.2 Heart Rate Variability

Heart rate variability (HRV) can be defined as the fluctuations in the time intervals between

adjacent heartbeats (McCraty & Shaffer, 2015). A higher HRV is generally desirable as it shows the physiological ability to react and adapt to external stressors in the environment. HRV is incredibly sensitive to external stressors and is the most usable method of exploring vagal nerve and nervous system activity as it is non-invasive, and devices are easily accessible (Shaffer & Ginsberg, 2017).

Table 1. Time domain heart rate variability metrics definitions (Shaffer & Ginsberg, 2017).

Time Domain HRV Metric	**Definition**
SDNN (ms)	Standard deviation of NN intervals
rMSSD (ms)	Root mean square of successive RR interval differences
pNN50 (%)	Percentage of successive RR intervals that differ by more than 50 ms

Table 2. Frequency domain heart rate variability metrics definitions (Shaffer & Ginsberg, 2017).

Frequency Domain HRV Metric	**Definition**
LF (ms^2)	Absolute power of the low-frequency band (0.04-0.15 Hz)
HF (ms^2)	Absolute power of the high-frequency band (0.15-0.4 Hz)
LF/HF Ratio (ms^2)	Ratio of LF-to-HF power

1.7.3 Normal HRV Values

Table 3. Normal short-term HRV values (Shaffer & Ginsberg, 2017).

ECG Measure	Mean (SD)	Range
SDNN (ms)	50 (16)	32-93
RMSSD (ms)	42 (15)	19-93
LF (ms^2)	519 (291)	193-1,009
HF (ms^2)	657 (777)	83-3,630
LF/HF (ms^2)	2.8 (2.6)	1.1-11.6

1.8 Benefits of Physical Activity

1.8.1 General population

It is well-established that physical activity is hugely beneficial to health in the general population. Physical activity helps prevent the onset of chronic diseases such as cardiovascular disease, cancer, obesity, hypertension, osteoporosis, osteoarthritis, and depression (Chimen et al, 2012; Warburton, Nicol & Bredin, 2006).

1.8.2 T1DM

There are also many health benefits of physical activity specifically for individuals with T1DM. Individuals with T1DM generally have a lower $V0_2$ max than healthy age-, BMI- and activity-matched participants with the absence of diabetes (Chimen et al, 2012). $V0_2$ max reflects the maximal capacity of the body to transport and utilise oxygen and is commonly used to determine fitness and can be used to predict mortality (Regensteiner, 2004). Numerous studies have shown that physical activity programmes improve $V0_2$ max and fitness in individuals with T1DM with $V0_2$ max increasing by up to 27% (Fuchsjager- Mayrl, Pleiner, Wiesinger et al, 2002; Landt et al, 1985; Mosher et al, 1998).

Additionally, evidence has shown that physical activity results in reduced insulin requirements within individuals with T1DM with a reduction variation of 6% to over 15% (Fuchsjager- Mayrl et al, 2002; Ramalho et al, 2006; Yki-Jarvinen, DeFronzo, & Koivisto, 1984) and has been shown

to improve glycaemic control by reducing HbA1c (Salvatoni et al, 2005). This is due to increased insulin sensitivity allowing the uptake of glucose to be more efficient (Collerg et al, 2015).

Exercise has benefits on lipids in individuals with T1DM by, increasing HDL by 8-30% decreasing LDL cholesterol by 8-14%, and triacylglycerols by 13-15%. This is due to physical activity acutely increasing lipid peroxide transfer to HDL and increasing reverse transport which is a mechanism that delivers excess cholesterol to the liver for clearance (Ruiz-Ramie, Barber & Sarzynski, 2019). Exercise also helps improve vascular function in response to patients with T1DM experiencing endothelial dysfunction (Chimen et al, 2012). Insulin resistance is a major factor in developing diabetic complications (Chaturvedi et al, 2001), exercise has been found to improve insulin resistance by up to 23% (Chimen et al, 2012). There is limited research to suggest that physical activity has a beneficial effect on blood pressure in individuals with T1DM however there is the suggestion exercise improves blood pressure by a modest 2-3% (Lehmann et al, 1997; Salem et al, 2010).

1.9 Glucose disturbances with exercise in T1DM

1.9.1 Barriers to exercise

Fear of hypoglycaemia during exercise is presented as a main barrier in preventing individuals with type 1 diabetes from undertaking regular exercise, which is problematic as it is well-established physical activity will help prevent the onset of diabetic complications (Greener, 2017). Therefore, it is vital that individuals are given appropriate information and advice regarding insulin and carbohydrate (CHO) alterations they should implement in preparation for exercising. Different types, intensities, and durations of exercise will impact glucose outcomes and therefore require specific insulin and CHO alterations (Houlder & Yardley, 2018).

1.9.2 Aerobic

Aerobic exercise is considered prolonged activity (more than 10 minutes) that requires energy to be produced aerobically (using oxygen) with the "rhythmic and repetitive use of large muscle groups" (Houlder & Yardley, 2018). It is accepted that aerobic exercise leads to a reduction in blood glucose values and might pose a risk of hypoglycaemia, therefore it is important to make appropriate insulin and CHO adjustments accordingly (Houlder & Yardley, 2018). Although, time of day may affect the alteration in blood glucose concentrations with research showing that aerobic exercise performed in a fasted state in the morning could increase blood glucose concentrations (Yamanouchi et al, 2002).

1.9.3 High-intensity intermittent exercise

High-intensity intermittent exercise is a form of aerobic continuous exercise that involves short bouts of high-intensity activity and has shown extremely variable blood glucose responses (Houlder & Yardley, 2018). The main concern regarding high-intensity intermittent exercise is post-exercise nocturnal hypoglycaemia (Iscoe, & Riddell, 2011). In some cases, a hypoglycaemic event occurred 11 hours post-exercise and therefore it is important to acknowledge that different intensity exercise has different risk factors compared to standard continuous, low-intensity aerobic exercise (Houlder & Yardley, 2018).

1.9.4 Anaerobic

Anaerobic exercise is a short, high-intensity exercise that uses energy systems that do not require oxygen (phosphocreatine hydrolysis and glycolysis). In T1DM anaerobic exercise is generally accepted to increase blood glucose concentrations due to an increase in catecholamine levels which are not reduced by the increased availability of insulin (Riddell & Perkins, 2009). This may result in exercise-induced hyperglycemia which may last for several hours post-exercise. Therefore, it may be relevant to increase insulin levels, however, this should be done with precaution as an increase too high could result in post-exercise hypoglycaemia (Riddell & Perkins, 2009).

1.9.5 Resistance

Resistance exercise involves muscular work using weights or any activity working against a form of resistance and improves muscle strength, power, and endurance (Houlder & Yardley, 2018). Resistance exercise is fairly complex as it uses both anaerobic (PCR hydrolysis and glycolysis) and aerobic (oxidative phosphorylation) energy systems (McCarthy, 2019). Research has found that glycaemic responses to one or two sets of resistance training result in significant increases in BG concentrations, however, these were not found during three sets (McCarthy et al, 2019). Therefore, resistance exercise is likely to cause an increase in BG at the beginning of resistance exercise, however as activity is sustained and aerobic energy systems prevail BG levels are likely to reduce post-exercise (McCarthy, 2019). As a result, research has suggested increasing meal intake and after-dinner snacking 24-hour post-resistance exercise to avoid hypoglycaemia (McCarthy, 2019).

1.10 Literature Review

1.10.1 ECG metrics

This section will explore research around each ECG parameter used in this thesis.

HR

Heart rate is the number of times the heart completes a two-part pumping action measured in beats per minute (bpm). A research study found that the rate of rise in glucose altered heart rate at rest (Moser et al, 2020). It was concluded that this could be an increased stress response to a faster change in glucose, therefore causing a rise in heart rate (Moser et al, 2020). Cardiac metabolism is influenced by circadian rhythm by levels of metabolites, metabolic flux, and response to nutrients due to feeding, fasting, waking, and sleep (Zhang & Jain, 2021). Cardiac metabolism could explain some differences between day and night response to interstitial glucose, however, this complex area needs additional extensive research.

QT

The QT interval is the period of time the heart takes to depolarize and then repolarize, measured in milliseconds. It is relatively well established that hyperglycaemia can change QT interval in individuals with type 1 diabetes, however, it is unknown how hyperglycaemia can affect QT interval during parasympathetic withdrawal. Hyperglycaemia could affect cardiac metabolism due to an increased prevalence of glucose to produce adenosine triphosphate (ATP) however further research is required in this area to conclude the reasoning for this change (Bing, 1965).

Research has shown that there is an increased prevalence of sudden nocturnal death in individuals with type 1 diabetes which has been referred to as ‘dead in bed’ syndrome. This makes investigating ECG alterations under various changes in interstitial glucose during the nocturnal period in people with type 1 diabetes important (Gill et al, 2008). It has been found that there is a correlation between sudden nocturnal death and prolonged QT interval (Gill et al, 2008 & Marques et al, 1997). Many individuals of these sudden deaths had also had recent hypoglycaemic attacks and it was widely accepted that prolonged QT had been initiated by hypoglycaemia causing cardiac arrhythmia and sudden death, however, the effect of the rate of the change of glucose during the nocturnal period had not been investigated (Gill et al, 2008).

QTc

QTc is the QT interval (depolarization and repolarization) corrected for heart rate using Bezett’s formula and is measured in milliseconds. It is established that hyperglycaemia altered QTc at rest (Christensen et al, 2010; Mezquita-Raya et al, 2018 & Nguyen & Nguyen et al, 2012). Research has also shown that nocturnal hypoglycaemia can cause prolonged QTc intervals (Gill et al, 2008; Robinson et al, 2004 & Murphy et al, 2004). There is research to suggest that QTc prolongation could be caused by oxidative stress and inflammation within the cardiovascular system which can result in disruption to ion channel function and repolarization abnormalities (Ceriello & Motz, 2004). However, further research is required to establish a definitive link.

SDNN

SDNN is a time-domain heart rate variability metric and represents the standard deviation of the NN intervals measured in milliseconds. The NN interval is the normalised time between two detected heartbeats calculated for every QRS event. Interestingly research by Moser et al (2020) who looked at the effect of the rate of change in interstitial glucose found SDNN to be unchanged whilst other HRV metrics RMSSD and pNN50 (%) were altered. Research has suggested that SDNN is more accurate during longer 24-hour ECG recordings, and therefore RMSSD and pNN50% may be considered more reliable metrics over shorter recordings used in this study (Shaffer & Ginsberg, 2017). Nonetheless, SDNN is still an important metric to consider and is used in most research when investigating heart rate variability.

RMSSD

RMSSD is a time domain heart rate variability metric that represents the root mean square of successive RR interval differences measured in milliseconds. The RR interval represents the time between two successive R waves of the QRS interval. Research by Moser et al (2020) explored the rate of glucose change on HRV metrics at rest and found that rapid increases in glucose resulted in a decrease in RMSSD. Rapid changes in glucose have been suggested to cause a greater stress response which could be resulting in altered HRV in individuals with type 1 diabetes (Moser et al, 2020). During an increased short-term stress response many physiological systems are affected for example musculoskeletal, neuroendocrine, and importantly the cardiovascular system (Dhabhar, 2018). Therefore, it is of interest to further investigate the effect of these short-term changes on long-term cardiovascular health in individuals with type 1 diabetes.

pNN50

PNN50 is a time domain heart rate variability metric that represents the percentage of successive RR intervals that differ by more than 50 milliseconds. Moser et al (2020) found that PNN50 (%) was reduced during the rapid rise in glucose at rest but found no difference at different glycaemic ranges. Therefore, further suggests that it is the change in glucose that is altering HRV metrics. It has been suggested that HRV metrics are being altered due to an increased stress response of the body during faster changes in glucose as described above in the RMSSD section (Moser et al, 2020).

LF

LF is a frequency domain heart rate variability metric that represents the absolute power of the low-frequency band (0.04-0.15Hz) measured in milliseconds squared. LF represents the combined effect of both sympathetic and parasympathetic influence. A study that investigated the effect of a bout of aerobic exercise on heart rate variability in individuals with type 1 diabetes found no change in LF (ms) (Anaruma et al, 2015). However, it is important to note that the exercise bout used in this study was 30 minutes of 40- 60% intensity aerobic exercise, which is different from the exercise test used in this research study, as the sympathetic nervous system will be dominant during aerobic exercise (Anaruma et al, 2015 & Fornasiero et al, 2018).

HF

HF is a frequency domain heart rate variability metric that represents the absolute power of the high-frequency band (0.15-0.4Hz) measured in milliseconds squared. HF represents the extent of vagal activity alone, whereas as stated above LF represents the combined effects of the sympathetic and parasympathetic influence (Serhiyenko & Serhiyenko, 2018). Clinical monitoring has revealed that a decrease in HF suggests a decrease in parasympathetic dysfunction (Serhiyenko & Serhiyenko, 2018).

1.10.2 Glucose Variability and Implementation

Glucose variability (GV) refers to the highs and lows of glucose throughout a day, between days or as a long-term control measuring changes in HbA1c (Wilmot et al, 2019). Recent research has strongly suggested that GV may be a more meaningful measure of glycaemic control compared to, or in addition to HbA1c due to a variety of different factors. For example, GV is said to be a more clinically relevant marker of daily glucose control and risk of hypoglycaemia than HbA1c. Additionally, GV can also be more readily assessed in clinical practise as a result of the increasing availability and uptake of continuous and intermittent glucose monitoring. Research is suggesting there should be a greater focus on time in range with studies suggesting a recommended target of 70% of time in range and aiming for under 4% of time below 70mg/dL (Battelino, 2019). However, it is recognised that further access to CGM devices is made available to individuals with T1DM, standardized reporting of GV across reporting systems and further research investigating the relationship between CGM-derived GV and short- and long-term health outcomes is required to achieve widespread recognition of GV (Wilmot et al, 2019).

Research has shown increased GV, resulting in hypoglycaemic stress, reduced HRV and has been associated with autonomic cardiovascular function, especially during the nocturnal period (Iwasaki et al 2015). Another study has shown that CGM defined GV has resulted in cardiovascular autonomic neuropathy (Jun et al, 2019). Interestingly in this study level 2 hypoglycaemia was the most significant contributor to the onset of CAN (Jun et al, 2019). The studies assessed the onset of CAN using HRV metrics such as low-frequency (LF) power, high-frequency (HF) and the LF/HF ratio. Both of these studies primarily focussed on increased GV resulting in hypoglycaemia, however there was less focus on the effect of GV from hyperglycaemia. Therefore, GV is an increasingly important area to investigate in relation to T1DM complications during all glycaemic ranges.

1.11 Study Rationale

Studies have shown that hypoglycaemia at rest in individuals with type 1 diabetes causes an increase in the QTc interval (Christensen et al, 2010; Mezquita-Raya et al, 2018; Nguyen & Nguyen, 2012). Hyperglycaemia has been found to decrease QTc at rest (Nguyen & Nguyen, 2012) however there is also research to suggest that hyperglycaemia can prolong QTc intervals (Taubel et al, 2022). An objective of this study is to explore the variance in continuous glucose monitoring (CGM) data and how it influences ECG metrics during an 8-hour resting period over 4 trial visits in individuals with type 1 diabetes.

The effect of exercise on ECG metrics in individuals with type 1 diabetes is relatively unknown. Some research suggests there was no difference in ECG parameters in response to

a 30-minute bout of aerobic exercise in individuals with type 1 diabetes compared to a control group, however, HRV metrics were reduced during a recovery period in individuals with T1DM (Anaruma et al, 2016).

Research has shown that nocturnal hypoglycaemia causes prolonged QTc intervals (Gill et al, 2008; Robinson et al, 2004 & Murphy et al, 2004). There is also research to suggest that heart rate variability metrics are reduced during rapid changes in glucose in individuals with type 1 diabetes (Eckstein et al, 2020) and a systematic review study suggests that GV negatively affects heart rate variability metrics in individuals with type 1 diabetes at rest (Helleputte et al, 2020). As described above in section 1.10.2, GV is emerging to become a critical measure of glycaemic control and more research is required to understand it's influence on ECG and HRV metrics in order to better understand complications such as CAN. Detecting early signs of CAN using ECG and HRV metrics is important as it is generally accepted that the complications of CAN are irreversible and therefore supports the needs for further research in this area in individuals with type 1 diabetes (Anaruma et al, 2016, Serhiyenko & Serhiyenko, 2018).

1.11.1 Aim, Objectives, and Hypotheses

Aim

The aim of this study is to further the understanding of interstitial glucose on ECG metrics in people with type 1 diabetes.

Objectives

1. Assess the variance in daytime resting CGM data and how it influences ECG metrics in individuals with type 1 diabetes.
2. Determine the affect of parasympathetic withdrawal using a novel exercise test on ECG waveforms affected by glucose changes (concentrations or thresholds) in individuals with type 1 diabetes.
3. Further the understanding of glycaemic information for the effect of rate of change of CGM on ECG metrics during the nocturnal period in individuals with type 1 diabetes.

Hypotheses

The null hypothesis states there is no clear relationship between interstitial glucose concentration or rate of change of interstitial glucose concentrations in resting or dynamic scenarios on ECG metrics in people with type 1 diabetes.

2. Methods and materials

2.1 Original parent study

The data from this thesis was originally collected for the study "Extent and prevalence of post-exercise and nocturnal hypoglycaemia following peri-exercise bolus insulin adjustments in individuals with type 1 diabetes" McCarthy et al (2021). This thesis re-analyses the incredibly valuable unused secondary objective 23-hour ECG data over 4 trial visits collected from this study with a new set of aims and objectives stated later in the method section which formulate this secondary analysis thesis. The following section describes the design of the parent study. Section 2.2 onwards describes how the ECG data from this study are reanalysed with a set of different objectives stated above to create this secondary analysis thesis.

2.1.1 Study ethics of parent study

The parent study was performed in accordance with good clinical practice and the Declaration of Helsinki. Approval was received from both the national research ethics committee (16/WA0394) and the local health authority (EudraCT number: 2017-004774-34; UTN: U1111-1174-6676) with the following clinical trial registration number; DRKS.de; DRKS00013509. The results of this study have been published in the study "Extent and prevalence of post-exercise and nocturnal hypoglycaemia following peri-exercise bolus insulin adjustments in individuals with type 1 diabetes" McCarthy et al (2021).

2.1.2 Screening visit design of parent study

Prior to the screening visit of the parent study participants were asked to avoid caffeine, alcohol, physical activity, and capillary blood confirmed hypoglycaemia ($\geq$3.9 mmol.L^{-1}) in the 12 hours prior to the visit. The physician discussed the trial design in detail with the participant, gained fully informed consent, and made the participant fully aware of their right to withdraw at any point of the trial and their data withheld. The screening visit involved completing anthropometric measures of body mass index (BMI) and bioelectric impedance analysis (BIA), HbA1c measurement, resting blood, and pulse pressure, a 12-lead resting ECG, and finally a cardiopulmonary exercise test (CPX) to determine VO_2 max.

2.1.3 Trial visit design of parent study

The design of the original parent study involved each participant completing four trial visits. It was a random crossover design study where the participant would either be given a full (100%) or reduced (50%) dose of pre-exercise and post-exercise NovoRapid bolus insulin dose as shown below in Figure 5. Basal insulin was Tresiba for all participants and if participants were switching from an alternative basal insulin, they were given a 7-14 day period to switch to Tresiba. Participants were given breakfast, brunch, lunch, pre-exercise snack, post-exercise snack, and pre-bed snack which were standardised between all four trials. The trial day involved participants visiting a clinical laboratory for a 23-hour day and night time period and completing a 45-minute bout of evening exercise at 60±6% VO_2 max.

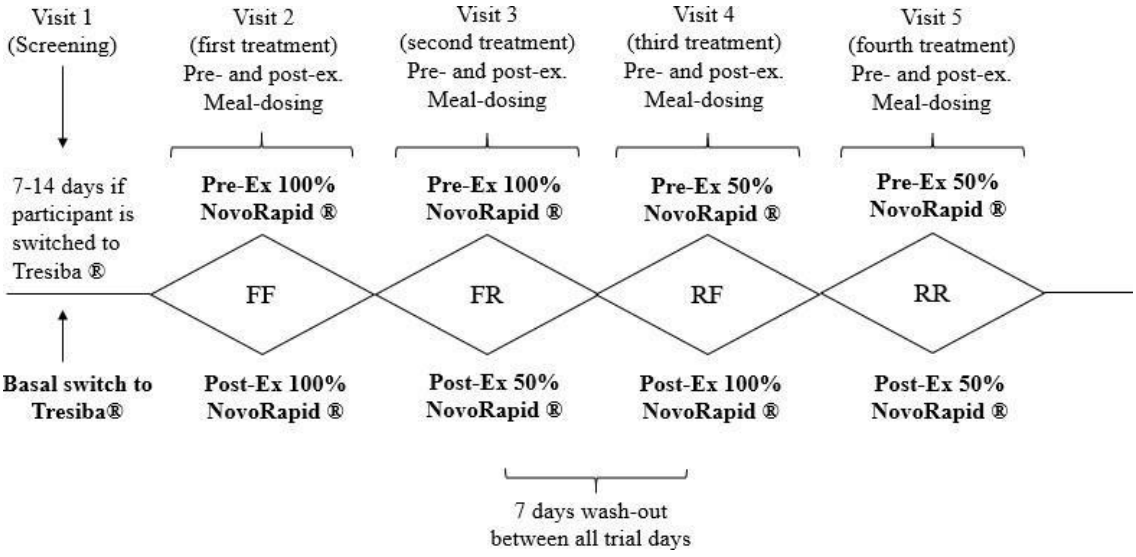


Figure 5. Schematic overview of the parent study trial design.

2.1.4 Eligibility criteria of parent study

The next section states the eligibility, inclusion, and exclusion criteria used in the original parent study, and therefore participants used in this secondary analysis thesis also met these criteria.

To be deemed eligible to be involved in the parent study where the data was originally collected, the following inclusion and exclusion criteria were assessed and documented in a standardised case report form (CRF) by the study physician.

2.1.5 Inclusion criteria of parent study

In order to be considered eligible, all of the following inclusion criteria had to be answered "yes".

- Informed consent was obtained before any trial-related activities. Trial-related activities are any procedures that are carried out as part of the trial, including activities to determine suitability for the trial.
- Male or female aged 18-65 years (both inclusive).
- T1D mellitus (as diagnosed clinically) ≥12 months.
- Treated with multiple daily insulin injections ≥12 months
- Body mass index 18.0-29.4 $kg.m^{-2}$ (both inclusive).
- If patients' BMI is above 29.4 $kg.m^{-2}$ due to muscular hypertrophy, the bioelectrical impedance analysis (BIA) will be used as a further assessment criterion. If patients' fat-free mass (%) is within ± 5% of normal reference values, the investigators can include the patient.
- Mass-specific $\dot{V}$ O2peak >20 $ml.O2.kg^{-1}.min^{-1}$
- Participants performing regular physical cardiorespiratory activity (physically active for at least 30 min at a time, three times per week) (short International Physical Activity Questionnaire [IPAQ] assessed).
- HbA1c ≤9.5%

2.1.6 Exclusion criteria of parent study

Patients were ineligible to take part in the study if they met any of the following exclusion criteria.

- Known or suspected hypersensitivity to trial product(s) or related products
- Receipt of any investigational medicinal product within 3 months prior to screening for this trial
- Known haemoglobin <80 $g.L^{-1}$ (male) or <64 $g.L^{-1}$ (female), total leukocyte count <3.0 x 10^9.L, thrombocytes <100 x 10^9.L, serum creatinine levels ≥126 $\mu mol.L^{1}$ (male) or ≥111 $\mu mol.L^{-1}$ (female), alanine aminotransferase >2 x the upper limit of normal (ULN), bilirubin > 3 x ULN, alkaline phosphatase >2 x ULN
- Suffer from or history of a life-threatening disease (i.e., cancer judged not to be in full remission except basal cell skin cancer or squamous cell skin cancer), or any clinically significant disease that might influence the participation or the study results, as judged by the investigator
- Participant with a heart rate <40 beats per minute (bpm) at screening (after resting for 5 min in supine position)
- Cardiac problems defined as decompensated heart failure (New York Heart Association (NYHA) class III and IV) at any time and/or angina pectoris within the last 12 months and/or acute myocardial infarction at any time
- Supine blood pressure at screening (after resting for 5 minutes in supine position) outside the range of 90-140 mmHg for systolic or 50-90 mmHg for diastolic (excluding white-coat hypertension; therefore, if a repeated measurement on a second screening visit shows values within the range, the participant can be included in the trial). This exclusion criterion also applied to participants on antihypertensives
- Clinically significant abnormal ECG at screening, as judged by the Investigator
- Proliferative retinopathy or maculopathy and/or severe neuropathy, in particular autonomic neuropathy, as judged by the Investigator
- Any chronic disorder or severe disease which, in the opinion of the Investigator might jeopardise participant's safety or compliance with the protocol
- Participant known to be positive for Hepatitis B surface antigen (HBsAg) or Hepatitis C antibodies (or diagnosed with active hepatitis), for HIV-1 antibodies, HIV-2

antibodies, or HIV-1 antigen

- History of multiple and/or severe allergies to drugs or foods or a history of a severe anaphylactic reaction (except celiac disease – patient must exclude foods that contain gluten from the diet)
- Participant who has donated any blood or plasma in the past month or more than 500 mL within 3 months prior to screening.
- Surgery or trauma with significant blood loss (more than 500 mL) within the last 3 months prior to screening
- Current treatment with systemic (oral or i.v.) corticosteroids, monoamine oxidase (MAO) inhibitors, non-selective or selective beta-blockers, growth hormone. Furthermore, thyroid hormone replacement was not allowed unless use had been stable during the past 3 months
- Significant history of alcoholism or drug/chemical abuse as per Investigator's judgment.
- Smoker (defined as a participant who is smoking more than 5 cigarettes or the equivalent per day)
- Not able or willing to refrain from smoking, or use of nicotine substitute products during the in-patient period
- Recurrent severe hypoglycemia (more than 1 severe hypoglycaemic event during the past 12 months).
- Hypoglycaemia unawareness as judged by the Investigator or hospitalisation for diabetic ketoacidosis during the previous 6 months
- Participants with mental incapacity or language barriers precluding adequate understanding or cooperation or who, in the opinion of their general practitioner or the Investigator, should not participate in the trial
- Potentially non-compliant or uncooperative during the trial, as judged by the Investigator.
- Any condition that would interfere with trial participation or evaluation of results, as judged by the Investigator
- Female of childbearing potential who is pregnant, breast-feeding or intend to become pregnant or is not using adequate contraceptive methods (adequate contraceptive measures include sterilisation, hormonal intrauterine devices, oral contraceptives, sexual abstinence or vasectomised partner). Information pertinent to the stage of

menstruation was not tracked. However, previous work has demonstrated that the phase of the menstrual cycle has no influence on incidence rates of nocturnal hypoglycemia in individuals with T1D.

2.1.7 Participants' characteristics

Participants used in this study were healthy individuals and had generally well-controlled type 1 diabetes with an average HbA1c of 55.70 ± 14.51 mmol/mol and an average diabetes duration of 14.44± 11.11 years as shown below in Table 4. Participants on average had a BMI of 25.98 ± 3.42 kg/m2 and therefore only slightly higher than the recommended healthy BMI of 25 kg/m2. Participants had moderate fitness levels with an average $V0_2$ max of 39.24 ± 10.93 ml.min.kg.bm. The participants were made up of largely males and had an average age of 34.50 ± 13.91 years.

Table 4. The anthropometric and diabetes characteristics of the recruited participants. Data are presented as mean (SD), n=16, HbA1c: Glycated haemoglobin. M; males. F; females. BMI; body mass index.

Characteristic	**Value ± SD**
Age (years)	34.50 ± 13.91
HbA1c (%)	7.24 ± 1.34
HbA1c (mmol/mol)	55.70 ± 14.51
Gender, M, F (n)	13, 3
Diabetes duration (years)	14.44 ± 11.11
Height (m)	1.76 ± 0.09
Body mass (kg)	80.00 ± 9.86
BMI (kg/m2)	25.98 ± 3.42
VO_2 (l.min)	3.14 ± 0.75
VO_2 (ml.min.kg.bm)	39.24 ± 10.93

2.2 Thesis methods and materials

2.2.1 Thesis study design

The data used in this secondary analysis thesis was comprised of three sub-sections: an 8-hour resting period, a 3-minute exercises test, and a 6-hour nocturnal period. Time periods of the three sub-sections were dissected from the total 23-hour period the participants were in the laboratory on four different occasions. The 8-hour resting, and 6-hour nocturnal time periods were selected to avoid feeding times and enable exploration during the most rested periods. The 3-minute exercise test at 20 watts was dissected from the 60±6% VO_2 max test and was the initial warm-up period from each trial visit. This low-intensity time period was selected in order to explore parasympathetic withdrawal before the sympathetic influence becomes dominant during higher-intensity exercise (Yamamoto et al, 1991). This study design enables for comparison of ECG data between the same individuals under extremely similar and well-controlled conditions over four separate trial visits.

The CGM and ECG data for this secondary analysis thesis will be analysed in the following way.

1. Explore reproducibility in CGM and ECG alterations over four separate 8-hour rest time periods in individuals with type 1 diabetes. Nine participants were included in this study that completed all four trial visits and had complete CGM data and sufficient ECG data (M: n=7, F: n=2, HbA_{1c} : 60.2±9.6 mmol/mol [7.7±0.9], age: 39.9±14.6 years, diabetes duration: 18.1±13.5 years). Meals were timed and matched, and physical activity levels were similar for all four trial visits. This analysis is valuable as its own entity, however, it also allows for the following sub-sections to explore CGM variations with the appreciation of this resting control baseline.

2. The objective was to determine ECG alterations during a 3-minute low-intensity (20 watts) exercise test during hyperglycaemia (>10mmol.L^{-1}) compared to euglycaemia (3.9-10mmol.L^{-1}) in T1DM. Ten participants who completed the bout of low-intensity exercise in both hyperglycemia and euglycemia on separate occasions on two of their four trial visits were included in this sub-section (M: n=8, F: n=2, HbA_{1c}: 55.4±17.3 mmol/mol [7.2±1.6%], age: 32.9±10.7 years, diabetes duration: 12.9±6.7 years).

3. The objective was to determine ECG alterations during a 'FAST' rate of change in nocturnal glycemia compared to a 'SLOW' rate of change in nocturnal glycemia in individuals with T1DM. Ten participants who had appropriate nocturnal glycaemic reductions during two of their four trial visits were included in this study (M: n=8 F: n=2, HbA_{1c}: 55.7±14.5 mmol/mol (7.2±1.3%), age: 34.5±13.9 years, diabetes duration: 14.4±11.1 years). Average duration of glucose drop was 40±17 minutes and was time matched for each participant between FAST and SLOW conditions. Participants with insufficient CGM or ECG data or whose glucose rose during the nocturnal period were excluded from this sub-section of analysis. Participants spent the overnight period in a clinical research facility.

2.2.2 Nocturnal rate of change 'FAST' v 'SLOW'

Nocturnal rate of change in glycemia was categorised into either a 'FAST' rate of reduction or a 'SLOW' rate of reduction. Participants were included in this sub-section if over two of their four overnight trial visits included a night with an increased drop in nocturnal glucose and a night with a steadier drop in glucose. The increased drop was labelled 'FAST' and the steadier drop was labelled 'SLOW'. Drops were identified using the starting CGM value and the end CGM value of the specific linear glucose reduction. The duration time taken for each drop was recorded in order to calculate the rate of reduction. The mean duration of glucose drops was 40±17 minutes and was the same for each participant between their 'FAST' and 'SLOW' drop but there was slight variation in drop duration between participants. Repeated-measures T-tests were then conducted on starting glucose and then ending glucose of each drop between the 'FAST' and 'SLOW' conditions. The rate of change in nocturnal glucose was calculated using

the following equation.

Rate of change = Difference in starting and ending glucose / Time taken of drop

A repeated-measures T-test was then conducted between the rate of change for the 'FAST' rate of change category compared to the 'SLOW' rate of change category. ECG data were extracted in 30-second intervals for each drop duration for both 'FAST' and 'SLOW' conditions and compared using a repeated-measure T-test.

2.2.3 Glycaemic ranges

Glycaemic ranges are defined as level 2 hyperglycemia (>13.9 mmol.L^{-1}), level 1 hyperglycemia (10.1-13.9 mmol.L^{-1}), in range (3.9-10.0 mmol.L^{-1}), level 1 hypoglycaemia (3.0-3.8 mmol.L^{-1}) and level 2 hypoglycaemia (<3.0 mmol.L^{-1}) (Battelino, 2019).

2.2.4 ECG Waveform

An ECG waveform records the electrical activity of each heartbeat and is interpreted using waves, intervals, and segments. See Figure 6 for the ECG waveform diagram.

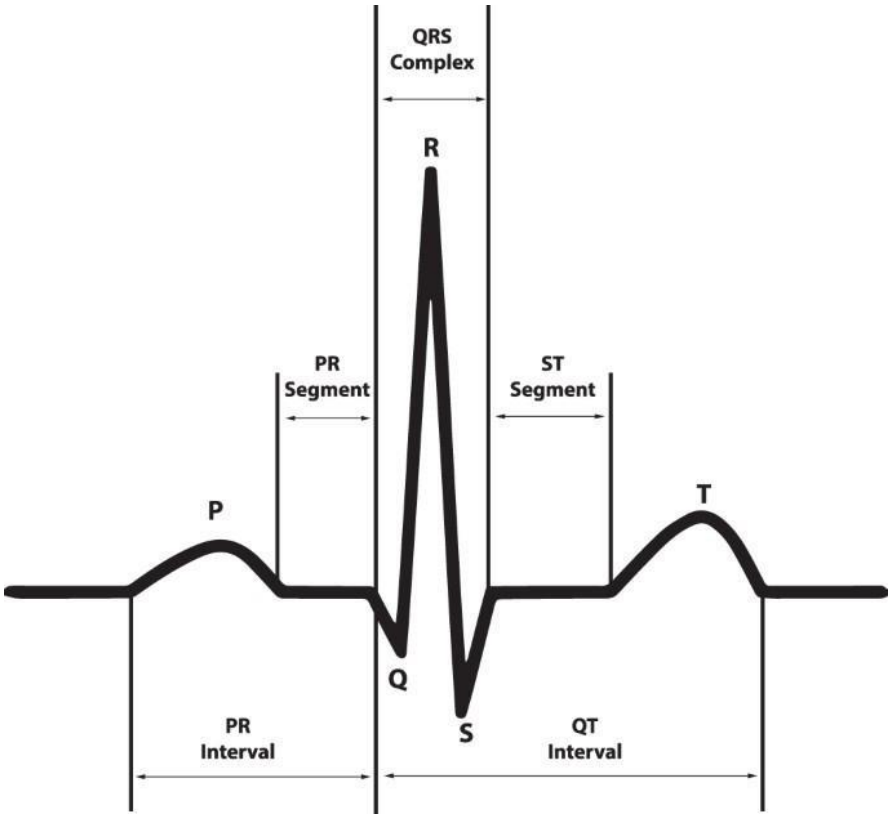


Figure 6. ECG Waveform Diagram (Zheng et al, 2020).

The P wave represents atrial depolarization, and this wave should be identifiable in a healthy individual's ECG waveform. The PR interval, from the start of the P wave until the start of the Q wave represents the time taken for the electrical impulse to travel between the atria and the ventricles of the heart. The QRS complex represents the depolarization of the ventricles and the T wave represents the repolarization of the ventricles. The ST segment starts at the end of the S wave and the beginning of the T wave and represents the time period between ventricular depolarization and repolarization. Additionally, the QT interval represents the time the heart takes to both depolarize and repolarize.

Normal QT intervals can be hard to distinguish due to contributing factors, for example, women generally have a longer QT interval than men and lower heart rates resulting in a longer QT interval. Generally, a normal QT interval is considered below 400-440ms (0.4- 0.44 seconds). A method to validate the QT interval is to use QTc which is corrected using Bezett's Formula (See Fig.7 below). QTc values are considered prolonged for males if they are >450 ms and for females >470 ms. Bezett's formula is accepted as the most popular method of retrieving corrected QT (QTc) values and is used in most academic methodologies in comparison to other methods such as Fridericia's formula, the nomogram method, and the linear subject-specific formula. However, a criticism of Bezett's formula is that there is the suggestion the formula may overcorrect QT values at higher heart rates and under correct at lower heart rates (Christensen et al, 2010). However, Bezett's formula is still the generally accepted method of correcting QT values and the method used in the methodology within this thesis.

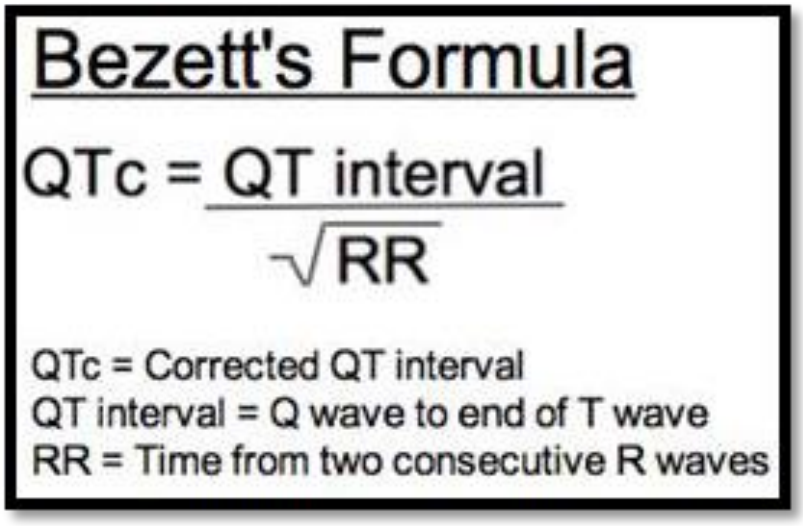


Figure 7. Bezett's Formula of Corrected QT Intervals.

2.2.5 ECG monitoring

ECG data were obtained by participants wearing a 3-lead ECG (Bittium Faros 180) during all trial visits over each 23-hour period. The Bittium Faros 180 device measures ECG sampling up to 1000 Hz including heart rate variability and ECG metrics such as heart rate, QTc, rMSSD, pNN50%, LF, HF, and LF/HF ratio. The Bittium Faros 180 ECG device has been successfully used in many studies to measure ECG metrics (Albert et al 2021; Bent et al, 2020; Lumikari et al 2019 & Nemcova et al, 2020).

Although a 12-lead ECG is considered the gold standard, research suggests a 3-lead ECG is equally as accurate in identifying cardiac abnormalities (Antonicelli et al, 2012). The benefits of using a 3-lead ECG include it being a more wearable device than a 12-lead ECG enabling the ECG to be worn over a long duration as there aren't obstructing limb leads enabling participants to exercise and sleep whilst wearing the ECG device. See Figure 8 below for an image of the Faros 180 device and Figure 9 for lead placement.

ECG data were recorded in 30-second intervals and each metric was retrieved into Excel documentation from the Cardiscope Scientific Software for analysis. Therefore, resulting in a numeric value for each ECG metric every 30 seconds over the 23-hour period. See Figure 10 for the Cardioscope Scientific software interface.

Figure 8. Faros 180 3-lead ECG monitoring device (Bittum, 2022).

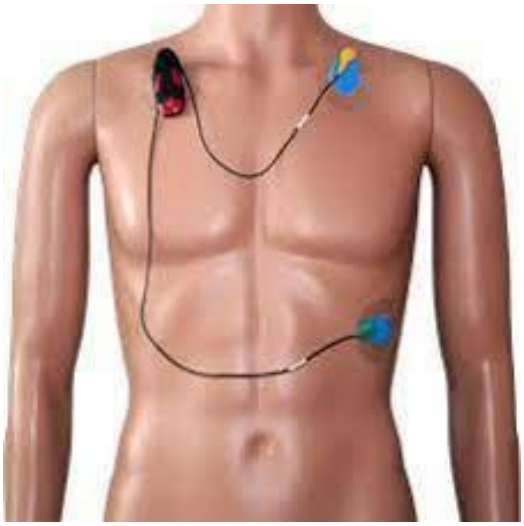
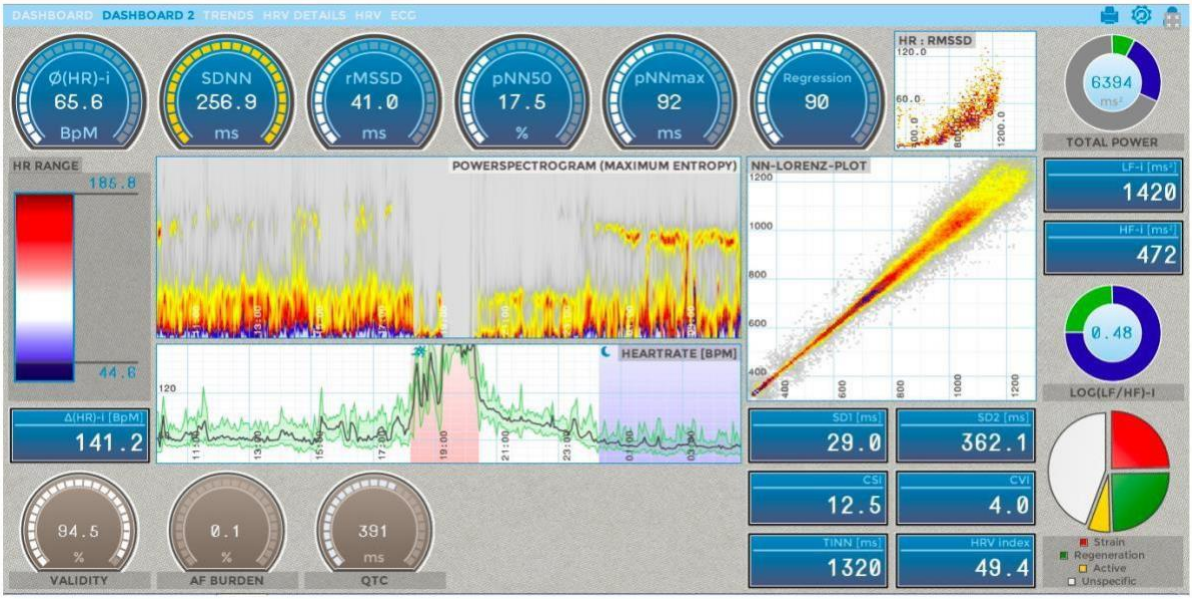
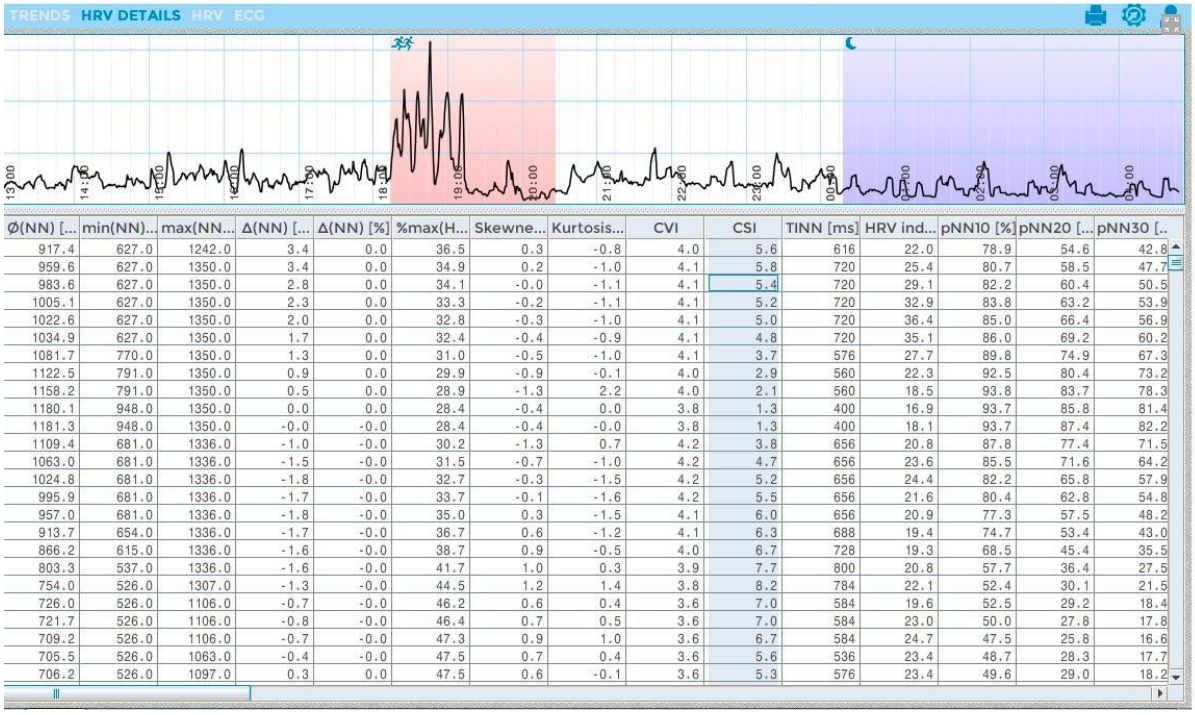


Figure 9. Faros 180 ECG monitoring device electrode and lead placement.





Ø(NN) [...	min(NN)...	max(NN...	Δ(NN) [...	Δ(NN) [%]	%max(H...	Skewne...	Kurtosis...	CVI	CSI	TINN [ms]	HRV ind...	pNN10 [%]	pNN20 [...	pNN30 [..
917.4	627.0	1242.0	3.4	0.0	36.5	0.3	-0.8	4.0	5.6	616	22.0	78.9	54.6	42.8
959.6	627.0	1350.0	3.4	0.0	34.9	0.2	-1.0	4.1	5.8	720	25.4	80.7	58.5	47.7
983.6	627.0	1350.0	2.8	0.0	34.1	-0.0	-1.1	4.1	5.4	720	29.1	82.2	60.4	50.5
1005.1	627.0	1350.0	2.3	0.0	33.3	-0.2	-1.1	4.1	5.2	720	32.9	83.8	63.2	53.9
1022.6	627.0	1350.0	2.0	0.0	32.8	-0.3	-1.0	4.1	5.0	720	36.4	85.0	66.4	56.9
1034.9	627.0	1350.0	1.7	0.0	32.4	-0.4	-0.9	4.1	4.8	720	35.1	86.0	69.2	60.2
1081.7	770.0	1350.0	1.3	0.0	31.0	-0.5	-1.0	4.1	3.7	576	27.7	89.8	74.9	67.3
1122.5	791.0	1350.0	0.9	0.0	29.9	-0.9	-0.1	4.0	2.9	560	22.3	92.5	80.4	73.2
1158.2	791.0	1350.0	0.5	0.0	28.9	-1.3	2.2	4.0	2.1	560	18.5	93.8	83.7	78.3
1180.1	948.0	1350.0	0.0	0.0	28.4	-0.4	0.0	3.8	1.3	400	16.9	93.7	85.8	81.4
1181.3	948.0	1350.0	-0.0	-0.0	28.4	-0.4	-0.0	3.8	1.3	400	18.1	93.7	87.4	82.2
1109.4	681.0	1336.0	-1.0	-0.0	30.2	-1.3	0.7	4.2	3.8	656	20.8	87.8	77.4	71.5
1063.0	681.0	1336.0	-1.5	-0.0	31.5	-0.7	-1.0	4.2	4.7	656	23.6	85.5	71.6	64.2
1024.8	681.0	1336.0	-1.8	-0.0	32.7	-0.3	-1.5	4.2	5.2	656	24.4	82.2	65.8	57.9
995.9	681.0	1336.0	-1.7	-0.0	33.7	-0.1	-1.6	4.2	5.5	656	21.6	80.4	62.8	54.8
957.0	681.0	1336.0	-1.8	-0.0	35.0	0.3	-1.5	4.1	6.0	656	20.9	77.3	57.5	48.2
913.7	654.0	1336.0	-1.7	-0.0	36.7	0.6	-1.2	4.1	6.3	688	19.4	74.7	53.4	43.0
866.2	615.0	1336.0	-1.6	-0.0	38.7	0.9	-0.5	4.0	6.7	728	19.3	68.5	45.4	35.5
803.3	537.0	1336.0	-1.6	-0.0	41.7	1.0	0.3	3.9	7.7	800	20.8	57.7	36.4	27.5
754.0	526.0	1307.0	-1.3	-0.0	44.5	1.2	1.4	3.8	8.2	784	22.1	52.4	30.1	21.5
726.0	526.0	1106.0	-0.7	-0.0	46.2	0.6	0.4	3.6	7.0	584	19.6	52.5	29.2	18.4
721.7	526.0	1106.0	-0.8	-0.0	46.4	0.7	0.5	3.6	7.0	584	23.0	50.0	27.8	17.8
709.2	526.0	1106.0	-0.7	-0.0	47.3	0.9	1.0	3.6	6.7	584	24.7	47.5	25.8	16.6
705.5	526.0	1063.0	-0.4	-0.0	47.5	0.7	0.4	3.6	5.6	536	23.4	48.7	28.3	17.7
706.2	526.0	1097.0	0.3	0.0	47.5	0.6	-0.1	3.6	5.3	576	23.4	49.6	29.0	18.2

Figure 10. Cardioscope Scientific software interface (Smart Medical, 2022).

2.2.6 Anthropometry

Body Mass Index

Participant height (Holtain Stadiometer, Holtain Ltd, UK) and mass (Seca Digital Scales, Seca Ltd, UK) were taken for the quantification of body mass index (BMI) using the following equation.

BMI = Body mass (kg) / Height (m2)

2.3 Cardiovascular and diabetes information

2.3.1 HbA1c measurement.

Capillary fingertip (4 µl) blood samples were obtained (Accu-Chek Safe-T-Pro Plus Disposable Lancets) and dispensed into pre-filled cartridges (The Quo-test A1c test kit®) for the quantification of glycated haemoglobin (HbA1c). The Quo-test® A1c test cartridges have been calibrated using samples provided by the European Reference Laboratory via the National Glycohaemoglobin Standardisation Program (NGSP) network. Once inserted into the TheQuo-Test® system (EKF Diagnostics, SKU: 312100VS), the test result was produced and subsequently checked against the inclusion/exclusion criteria. TheQuo-Test® HbA1c system is a fully automated HbA1c analyser that uses patented boronate affinity fluorescence quenching technology recognised as interference-free with a measuring range of between 4-15% A1c DCCT and an imprecision CV <3% at 7% A1c DCCT. The system is certified by the National Glycohemoglobin Standardisation Program and the International Federation of Clinical Chemistry (IFCC) and all results are IFCC-traceable. The baseline anthropometrical, cardiovascular, and diabetes characteristics of the 19 recruited participants are outlined in Table 1.

2.3.2 Continuous glucose monitoring

Continuous glucose values were recorded using the Abbott FreeStyle Libre 1 device, and data were recorded in 15-minute intervals. “FreeStyle Libre is a sensor-based glucose monitoring system that comes with a reader and a sensor. The sensor is applied to the back of your upper arm and can be worn for up to 14 days. Simply swipe the reader over the sensor to get a complete picture of your glucose levels” (Abbott, 2022). The FreeStyle Libre 1 also can download CGM data over a selected period of time. The FreeStyle Libre 1 device is a well-established method of measuring interstitial glucose for individuals living with T1DM and has also been effectively used in many research studies (Dunn et al, 2018 & Moser et al, 2020).

Figure 11. FreeStyle Libre 1 continuous glucose monitoring device (Abbott, 2022).

2.3.3 Exercise test

The exercise test was a 3-minute test performed at 20 watts on a Lode Eccentric Corival cycle ergometer (See Figure 12). The Eccentric Corival is an ergometer with an electromagnetic braking principle. The maximal eccentric workload is 250 watts. The range of target rpm (30-100 rpm) can be customized and adjusted during the training (*Corival Eccentric*). Specific exercise test wattage and duration increments can be customized before the test commences. The Lode Eccentric Corival cycle ergometer has been successfully utilised in many research studies for graded exercise tests (Chasland et al 2017; dos Santos et al, 2020; Jemni et al, 2019 & Ward et al, 2021).

The aim of the 3-minute exercise test was to explore the effect of parasympathetic withdrawal. It was the initial warm-up period of the parent study submaximal exercise test. The exercise test was performed at 20 watts and therefore a very low intensity and therefore there is no requirement for an increased cardiovascular output or 'fight or flight' response that the sympathetic influence of the ANS provides (McCorry, 2007). A research study looking at autonomic control during exercise reveals that during a progressive submaximal exercise test, parasympathetic activity gradually decreased as soon as exercise commenced however sympathetic activity only increased when participants exceeded their predetermined ventilatory threshold (Yamamoto, Highson & Peterson (1991). The participants in this study had a moderate fitness level (VO_2 39.24 ± 10.93 ml.min.kg.bm) therefore, during this 3-minute 20-watt exercise test, the parasympathetic influence can be said to be dominant.

Figure 12. Lode Eccentric Corival cycle ergometer (Load, 2022).

2.4 Statistical and Data Analysis

Cardioscope Scientific software by Smart Medical was used to transfer all raw ECG and HRV data into multiple Excel documents which included a column for the time of recording along columns with values for each ECG and HRV metric in 30-second intervals. Resting and nocturnal data time points were extracted using the times of feeding. Feeding time points were avoided within resting data extractions to allow for data analysis during the most rested time periods. Insulin administration, nutritional intake, and continuous glucose concentrations throughout the trial visit days were analysed in order to show control. CGM data were stratified into predetermined glycaemic ranges using the five ranges outlined by Battelino (2019); level 2 hyperglycemia (>13.9 $mmol.L^{-1}$), level 1 hyperglycemia (10.1-13.9 $mmol.L^{-1}$), in range (3.9-10.0 $mmol.L^{-1}$), level 1 hypoglycemia (3.0-3.8 $mmol.L^{-1}$) and level 2 hypoglycemia (<3.0 $mmol.L^{-1}$).

Statistical analyses were conducted using the software IDM SPSS Statistics Version 28. All data were tested for normality using the Shapiro-Wilk normality test. Statistical significance was accepted at $p<0.05$. F values were identified and reported during the analysis of variance (ANOVAs) to determine the significance of group differences.

2.4.1 Daytime 8-hour resting period

Daytime 8-hour insulin administration, nutritional intake, continuous glucose concentrations, continuous glucose coefficient of variation, time in glycaemic range, and ECG between and within trial data were analysed using several repeated measures ANOVAs and post hoc analysis was conducted using Bonferroni correction.

2.4.2 Low-intensity exercise test

Pre-exercise insulin administration and nutritional intake were analysed using repeated measure ANOVAs. Exact start times of the low-intensity exercise tests were identified using spirometry data files stating the exact exercise start time of the 20w exercise test and this value

was matched to ECG data using time and duration, as well as cross-referencing starting heart rate for both spirometry and ECG data. A repeated measure T-test was conducted between euglycemic and hyperglycaemic interstitial glucose values. ECG data for these time periods were extracted and multiple repeated measures T-tests were conducted to explore differences in ECG metrics during parasympathetic withdrawal for euglycemic compared to hyperglycaemic conditions.

2.4.3 6-hour nocturnal period

Data analysis was conducted on the 6-hour nocturnal data by first identifying individuals with a 'FAST' and 'SLOW' drop in iG over the 6-hour night-time period during two of the four trials and these participants were included in this subsection of analysis. The rate of change was calculated using the start and end of drop glucose concentrations and the drop duration. A repeated measure T-test was conducted between the two conditions' rate of change. A t-test was conducted between the two conditions' mean interstitial glucose. The mean and coefficient of variation values of nocturnal glucose across the four trials were reported. ECG data for these exact time periods were extracted, and multiple repeated measures T-tests were conducted to explore ECG differences between 'FAST' and 'SLOW' drops in iG. ECG alterations were reported as medians and interquartile ranges.

3. Results

Results for each sub-section (8-hour resting period, low-intensity exercise test, and 6-hour nocturnal period) data have been presented in the following order: insulin and nutrient intake data exploration, CGM data exploration, and finally ECG data exploration. The aim of this results section is to explore ECG alterations during different time periods and CGM variations with the appreciation of insulin and nutrient intake differences in individuals with type 1 diabetes.

3.1 Daytime 8-hour resting period

3.1.1 Daytime (8 h) insulin administration and nutritional intake

Basal and mealtime bolus insulin units injected during the 8-hour daytime period are shown in the table below (see Table 5). There was a difference in brunch bolus insulin between the four trials [$F_{(3, 24)}$=3.261, p=0.039], post hoc analysis revealed that Trial 1 differed by an average of (1.156 U) from Trial 4 (p=0.014). There was a difference in lunch bolus insulin between the four trials [$F_{(3, 24)}$=4.749, p=0.010], post hoc analysis revealed that Trial 1 differed by an average of (1.389 U) from Trial 4 (p=0.044).

Mealtime energy and macronutrient intake for meals during the 8-h day for participants over four trials can be found in Table 5. There was a difference between mealtime (breakfast, brunch, and lunch) bolus insulin administration for Trial 2 [$F_{(2, 16)}$=14.642, p<0.001], post hoc analyses revealed breakfast bolus administration differed by an average of (2.889 units) compared to brunch bolus administration (p=0.004). There was also a difference in mealtime bolus for Trial 3 [$F_{(2, 16)}$=8.706, p=0.003], post hoc analysis revealed breakfast bolus differed by an average of (2.278 units) compared to brunch bolus administration (p=0.004). Finally, there was a difference in mealtime bolus administration for Trial 4 [$F_{(2, 16)}$=9.369, p=0.002), post hoc analysis revealed that breakfast bolus insulin differed by an average of (2.125 units) compared to brunch (p=0.002).

Table 5. Basal insulin, energy, macronutrient, and bolus insulin intake during breakfast, brunch, and lunch over four trials. Reported as mean ± standard deviation. n=9. * represents statistical significance between trial 2 breakfast and brunch bolus insulin. ** represents statistical significance between trial 3 breakfast and brunch bolus insulin. *** represents statistical significance between trial 4 breakfast and brunch bolus insulin. † represents statistical significance between trial 1 brunch bolus insulin and trial 4 brunch bolus insulin. †† represents statistical significance between trial 1 lunch bolus insulin and trial 4 lunch bolus insulin. Data have been treated via repeated measures ANOVA. All statistical significance was accepted at $p<0.05$.

Basal Insulin	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*
Tresiba (U)	25.8 ± 15.4	26.0 ± 15.2	26.0 ± 15.2	26.0 ± 15.2
Breakfast				
Macronutrient	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*
Energy (Kcals)	379.2 ± 158.3	374.9 ± 161.8	374.9 ± 161.8	374.9 ± 161.8
Carbohydrate (g)	51.0 ± 19.2	51.0 ± 19.2	51.0 ± 19.2	51.0 ± 19.2
Protein (g)	14.0 ± 7.5	13.7 ± 7.9	13.7 ± 7.9	13.7 ± 7.9
Fat (g)	11.5 ± 6.1	11.2 ± 6.5	11.2 ± 6.5	11.2 ± 6.5
Bolus Insulin (U)	4.4 ± 1.0	5.4 ± 1.7*	4.4 ± 1.2**	4.4 ± 1.5***
Brunch				
Macronutrient	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*
Energy (Kcals)	281.4 ± 112.7	259.6 ± 153.3	281.4 ± 122.7	281.4 ± 122.7
Carbohydrate (g)	31.5 ± 4.5	28.7 ± 11.4	31.5 ± 4.5	31.5 ± 4.5
Protein (g)	10.1 ± 10.4	9.2 ± 10.9	10.1 ± 10.4	10.1 ± 10.4
Fat (g)	12.4 ± 8.7	11.5 ± 9.5	12.4 ± 8.7	12.4 ± 8.7
Bolus Insulin (U)	3.4 ± 1.2†	2.5 ± 1.5*	2.2 ± 1.1**	2.2 ± 1.2† ***
Lunch				
Macronutrient	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*
Energy (Kcals)	421.2 ± 114.7	431.0 ± 115.3	431.0 ± 115.3	431.0 ± 115.3
Carbohydrate (g)	61.9 ± 21.7	62.0 ± 21.8	62.0 ± 21.8	62.0 ± 21.8
Protein (g)	25.2 ± 10.4	24.9 ± 10.9	24.9 ± 10.9	24.9 ± 10.9
Fat (g)	6.4 ± 3.0	7.6 ± 3.9	7.6 ± 3.9	7.6 ± 3.9
Bolus Insulin (U)	4.5 ± 2.2††	3.8 ± 1.5	2.7 ± 2.1	3.1 ± 2.3††

Mealtime macronutrient energy percentages over the four trials are shown below in Figure 11. Macronutrient energy percentages between trials for breakfast, brunch, and lunch over the four trials were similar as shown below in Figure 13.

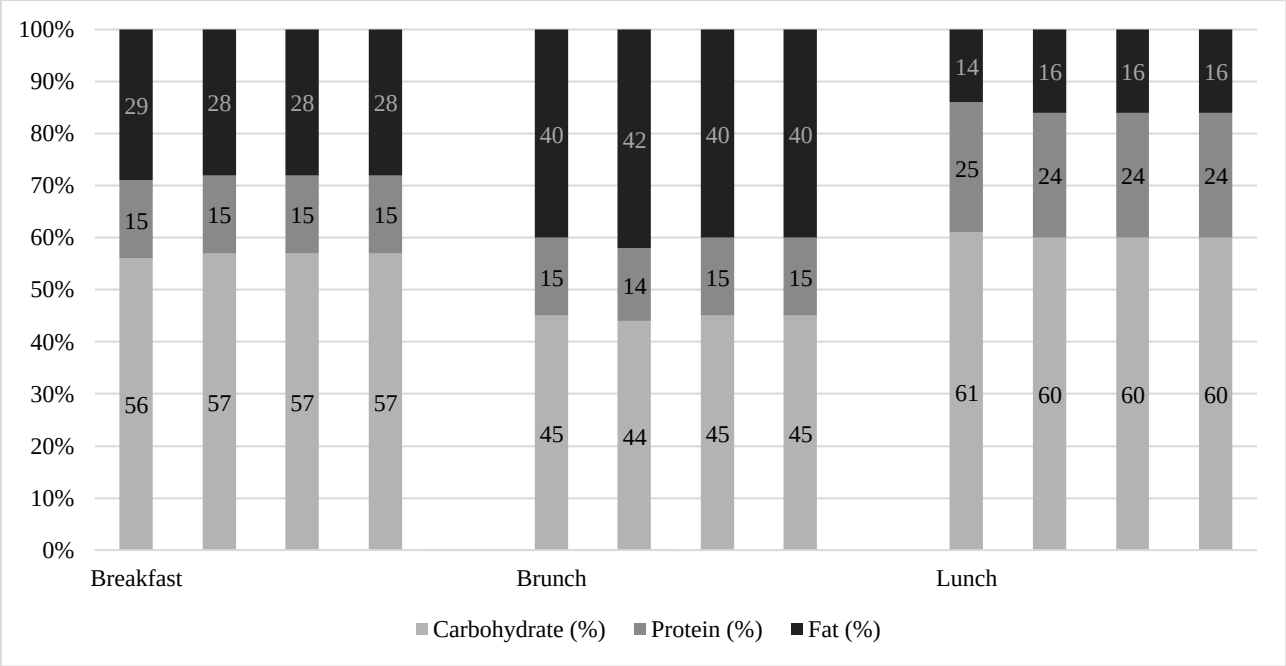


Figure 13. Macronutrient energy intake percent for breakfast, brunch, and lunch over four trials. Reported as a percentage of total energy intake.

3.1.2 Daytime (8h) resting continuous glucose concentration [G_i]

The start, mean, and change from rest (Δ) in [G_i] for the resting 8-hour daytime period for the four trials are shown in Table 6. Mean 8-hour [G_i] were different between the four trials [$F_{(2.821, 835.042)}$=6.806, p=0.000]. Post-hoc analysis using the Bonferroni correction revealed that Trial 4 differed by 0.810 mmol.L^{-1} compared to Trial 1 (p=0.001) and by 0.909 mmol.L^{-1} compared to Trial 3 (p=0.000). The mean glucose variability (mmol.L^{-1}) over 8 hours was different between the four trials [$F_{(2.245, 644.364)}$=19.257, p=0.000]. Post hoc analysis revealed that Trial 1 differed from Trial 3 by 1.566 mmol.L^{-1} (p=0.000), Trial 2 differed from Trial 3 by 0.858 mmol.L^{-1}, p=0.030), Trial 2 and Trial 4 differed by 1.193 mmol.L^{-1}, p=0.007) and Trial 3 and Trial 4 differed by 2.051 mmol.L^{-1}, (p=0.000).

Table 6. Resting 8-hour daytime starting [G_i], mean 8-hour [G_i], and average change in [G_i] for nine participants across four trials. Data are reported as mean ± standard deviation (mmol.L^{-1}). n=9. *represents a significant difference between Trials 1 and 4($p<0.05$). † represents a significant difference between trials 3 and 4 ($p<0.05$). Data have been treated via repeated measures ANOVA.

	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**
Start [G_i] **(mmol.L^{-1})**	8.7 ± 3.0	9.9 ± 3.0	9.7 ± 2.9	8.7 ± 2.6
8-hour mean [G_i]**(mmol.L^{-1})**	9.5 ± 3.6*	9.9 ± 3.6	9.4 ± 3.2†	10.3 ± 3.1*†
Average change in [Gi] **(mmol.L^{-1})**	0.8 ± 3.1	-0.1 ± 3.2	-0.3 ± 3.7	1.6 ± 2.8

3.1.3 Daytime (8h) continuous glucose coefficient of variation

Daytime 8-hour continuous glucose coefficient of variations values for nine participants over four trials along with total mean and standard deviation are shown in Table 7. There was no difference between the 8-hour daytime resting CGM coefficient of variation between the four trial days [$F_{(3, 24)}=0.321$, p=810].

Table 7. Coefficient of variation (%) for 8-hour daytime resting CGM data for nine participants over four trial days, mean and standard deviation. n=9. Data were treated via repeated measures ANOVA.

Participant no.	Trial 1	Trial 2	Trial 3	Trial 4	**Range**
3	26.8	21.0	39.1	24.8	**21.0-39.1**
4	16.1	43.6	39.6	29.1	**16.1-43.6**
6	25.1	28.2	38.2	35.0	**25.1-38.2**
12	49.4	25.4	16.9	29.6	**16.9-49.4**
13	37.1	37.1	23.9	20.5	**20.5-37.1**
15	14.7	26.1	32.2	20.9	**14.7-32.2**
18	21.9	23.5	19.0	21.9	**19.0-23.5**
19	26.3	19.3	18.5	26.3	**18.5-26.3**
21	21.6	40.5	22.8	20.8	**20.8-40.5**
Range	**16.0-49.4**	**19.3-43.6**	**16.9-39.6**	**20.5-35.0**	
Mean	**26.6**	**29.4**	**27.8**	**25.4**	
SD	**10.8**	**8.8**	**9.5**	**5.0**	

3.1.4 Daytime (8-h) glycaemic range

Percent of time in each glycaemic range for the four trials can be shown in Figure 14. There was no difference in glycaemic ranges for Level 2 hyperglycemia [$F_{(3, 24)}$=0.054, p=0.983], Level 1 hyperglycemia [$F_{(3, 24)}$=0.540, p=0.659], time in range [$F_{(3,24)}$ =0.249, p=0.861], Level 1 hypoglycaemia [$F_{(3, 24)}$=1.778, p=0.178].

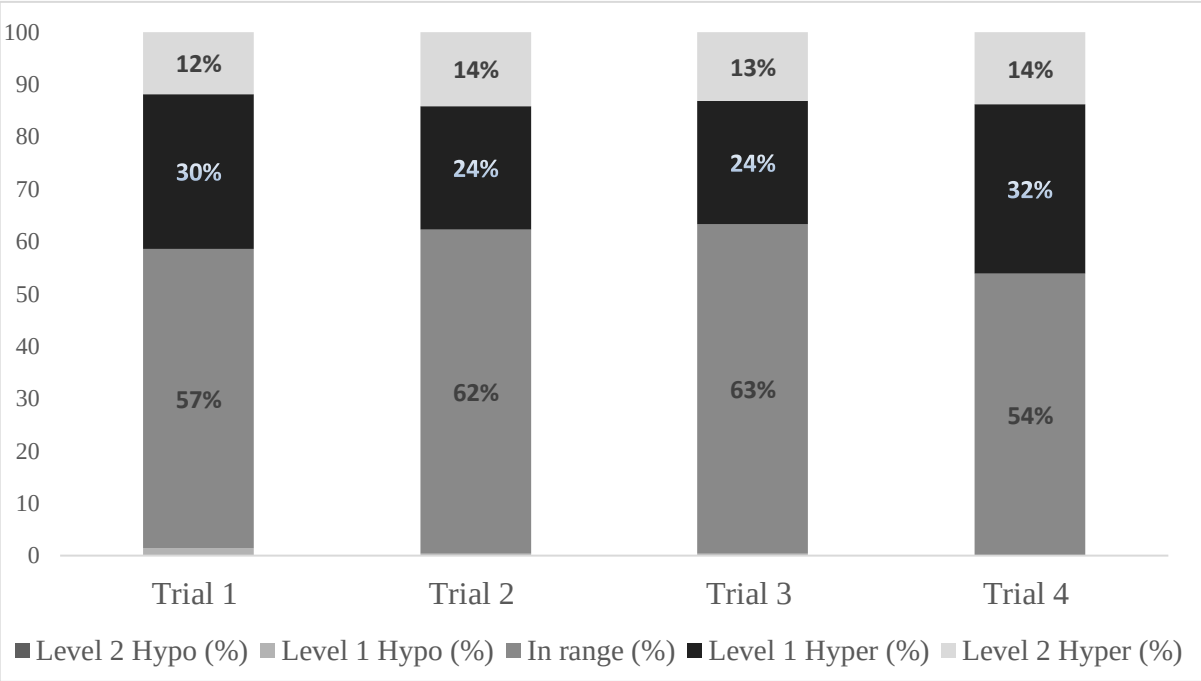


Figure 14. Glycaemic ranges for all nine participants over four trial days. Reported as percent (%) time in glycaemic range.

Results show that on average over the four trials, participants spent 13% of the time in level 2 hyperglycemia, 27% in level 1 hyperglycemia, 59% of the time in range, 1% of the time in level 1 hypoglycaemia and 0% in level 2 hypoglycaemia.

3.1.5 Daytime (8h) ECG alterations

Results show that there was no difference in ECG metrics over the four trials during the 8-hour resting period as shown below in Table 8.

Table 8. Comparison of ECG responses during an 8-hour resting period between four trials. n=9. Data are reported as mean ± standard deviation.

Parameter	***Trial 1***	***Trial 2***	***Trial 3***	***Trial 4***	***P value***
HR (bpm)	69 ± 9	71 ± 13	70 ± 10	69 ± 9	0.832
QT (ms)	377.6 ± 23.4	381.4 ± 22.8	378.4 ± 20.6	375.0 ± 27.2	0.387
QTc (ms)	394.4 ± 18.1	397.5 ± 18.5	395.4 ± 16.3	391.5 ± 20.4	0.558
SDNN (ms)	68.5 ± 30.4	73.7 ± 34.2	70.6 ± 31.9	71.3 ± 33.0	0.649
rMSSD (ms)	37.1 ± 18.4	40.2 ± 23.1	39.2 ± 20.0	38.7 ± 21.4	0.725
pNN50 (%)	16.0 ± 14.6	18.7 ± 18.2	17.8 ± 16.2	17.5 ± 16.6	0.747
LF (ms^2)	1661.2 ± 1479.7	1875.9 ± 1636.3	1669.1 ± 1553.6	1874.0 ± 1650.1	0.405
HF (ms^2)	577.8 ± 571.6	686.7 ± 749.9	619.3 ± 685.2	670.0 ± 677.3	0.643
LF/HF Ratio	4.6 ± 4.1	5.6 ± 5.6	4.6 ± 4.3	5.8 ± 5.5	0.435

3.2 Low-intensity exercise test

3.2.1 Pre-exercise insulin administration and nutritional intake

There was no difference in pre-exercise energy (kcal), carbohydrate (g), protein (g), fat (g), and bolus insulin (U) intake between the four trials [$F_{(1, 12)}$=1.00, p=0.337] as shown in Table 9.

Table 9. Pre-exercise meal energy, macronutrient, and basal insulin intake over four trials. Reported as mean ± standard deviation. n=10. Data have been treated via repeated measures ANOVA.

	Trial 1	***Trial 2***	***Trial 3***	***Trial 4***
Energy (kcal)	502.4 ± 59.1	506.6 ± 60.7	506.6 ± 60.7	502.3 ± 63.6
Carbohydrate (g)	80.0 ± 10.1	79.5 ± 11.1	79.5 ± 11.1	78.0 ± 10.1
Protein (g)	18.7 ± 10.6	20.7 ± 14.0	20.7 ± 14.0	20.5 ± 14.1
Fat (g)	10.0 ± 4.6	9.8 ± 4.5	9.8 ± 4.5	10.1 ± 4.2
Bolus Insulin (U)	3.6 ± 2.1	3.6 ± 2.2	3.5 ± 2.7	3.1 ± 2.1

3.2.2 Low-intensity exercise glucose

Mean glucose concentrations for hyperglycemia and euglycemia during low-intensity exercise is shown below in Table 10. Mean glucose concentrations during hyperglycemia and euglycemia were different (p<0.001).

Table 10. Average glucose concentrations for hyperglycemia and euglycemia. Reported as mean ± standard deviation. n=10. * represents the significance between hyperglycaemic and euglycemic conditions. Data have been treated via T-test.

Glycaemic conditions	**Mean [G_i) (mmol.L^{-1})**
Hyperglycemia	13.2 ± 2.3*
Euglycemia	7.8 ± 1.0*

3.2.3 Low-intensity exercise ECG alterations

In individuals with type 1 diabetes during a low-intensity bout of exercise QT (ms), QTc (ms) and HF (unit) were lower during hyperglycaemia compared to euglycemia as shown in Table 11. There was no change in SDNN (ms), rMMSD (ms), pNN50 (%), LF (ms^2), and LF/HF between hyperglycaemic and euglycemic conditions as shown below in Table 11. Changes in ECG responses from the start of exercise to the end of exercise were similar between conditions.

Table 11. Comparison of ECG responses during hyperglycaemia and euglycaemia. Data was reported as normally distributed. Data are reported as mean ± standard deviation. n=10. * represents a significant difference between hyperglycaemic and euglycaemic QT (ms). ** represents a significant difference between hyperglycaemic and euglycaemic QTc (ms). *** represents a significant difference between hyperglycaemic and euglycaemic HF (ms^2). Data were treated via repeated measures T-test.

Parameter	Hyperglycaemic	Euglycaemic	P value
HR (bpm)	84.5 ± 0.8	84.1 ± 0.7	0.451
SDNN (ms)	78.3 ± 3.3	76.0 ± 3.4	0.053
rMSSD (ms)	25.7 ± 1.6	26.4 ± 1.9	0.215
pNN50 (%)	7.6 ± 1.0	7.8 ± 1.2	0.294
QT (ms)	356.3 ± 3.2	363 ± 1.9	**0.01***
QTc (ms)	397.2 ± 2.7	408 ± 1.9	**<0.01****
LF (ms^2)	712.5 ± 74.6	800.9 ± 103.5	0.292
HF (ms^2)	291.2 ± 37.1	370.8 ± 52.8	**<0.01*****
LF/HF Ratio	13.4 ± 4.6	3.4 ± 0.3	0.148

3.3 Nocturnal 6-hour period

3.3.1 Post-exercise and pre-bed insulin administration and nutritional intake

There was no difference in post-exercise meal energy (p=0.333), carbohydrate (p=0.333), protein (p=0.333) or fat (p=0.333) nutritional intake between the four trials as shown in Table 12. There was a difference in bolus insulin (U) administration (p<0.001). There was no difference in pre-bed snack energy (p=0.457), carbohydrate (p=0.333), protein (p=0.278), and fat (p=0.669) nutritional intake between the four trials.

Table 12. Post-exercise meal and pre-bed snack insulin administration and nutritional intake. n=10. Data were treated via repeated measures ANOVA.

Post-exercise meal				
	Trial 1	***Trial 2***	***Trial 3***	***Trial 4***
Energy (kcal)	489.6 ± 50.5	490.1 ± 58.5	490.9 ± 58.5	496.2 ± 58.6
Carbohydrate (g)	80.1 ± 9.5	79.8 ± 10.1	79.8 ± 10.1	79.8 ± 10.1
Protein (g)	17.8 ± 9.1	19.0 ± 11.9	19.0 ± 11.9	19.0 ± 11.9
Fat (g)	9.3 ± 4.7	8.9 ± 4.2	8.9 ± 4.2	9.5 ± 4.4
Bolus Insulin (U)	0.06 ± 0.02	0.03 ± 0.01	0.06 ± 0.01	0.03 ± 0.01
Pre-bed snack				
	Trial 1	***Trial 2***	***Trial 3***	***Trial 4***
Energy (kcal)	278.5 ± 138.0	288.2 ± 121.0	288.2 ± 121.0	278.5 ± 138.0
Carbohydrate (g)	30.5 ± 8.8	32.1 ± 3.9	32.1 ± 3.9	30.5 ± 8.8
Protein (g)	7.5 ± 8.2	8.1 ± 7.9	8.1 ± 7.9	7.5 ± 8.2
Fat (g)	13.4 ± 9.4	13.7 ± 13.7	13.7 ± 13.7	13.4 ± 13.4

3.3.2 Nocturnal 6-hour glucose concentrations

6-hour nocturnal [G_i] and coefficient of variation values over the four trials can be found below in Table 13. There was no difference in 6-hour nocturnal glucose values over the four trials [$F_{(2.031, 402.142)}$=2.048, p=0.126]. There was no difference in the 6-hour nocturnal coefficient of variation between the four trials [$F_{(3, 24)}$=0.612, p=0.614].

Table 13. Nocturnal interstitial glucose concentration [G_i] and coefficient of variation (%) over the four trials. Reported as mean ± standard deviation. n=10. Data were treated via repeated measures ANOVA.

	Trial 1	***Trial 2***	***Trial 3***	***Trial 4***
Mean [G_i] (mmol.L^{-1})	10.2 ± 3.3	10.9 ± 4.2	11.2 ± 4.5	11.3 ± 3.8
CoV (%)	32.6	38.3	40.1	33.4

3.3.3 Nocturnal 6-hour glycaemic ranges

Nocturnal glycaemic range percentages can be found in Figure 15. There was no difference in time in Level 2 hyperglycemia [$F_{(1.692, 15.228)}$=0.338, p=0.684], Level 1 hyperglycemia [$F_{(3, 27)}$=1.136, p=0.352], time in range [$F_{(1.610, 14.491)}$=0.466, p=0.596], Level 1 hypoglycaemia [$F_{(1.182, 10.634)}$=1.071, p=0.338] or Level 2 hypoglycaemia [$F_{(3, 27)}$=1.000, p=0.408].

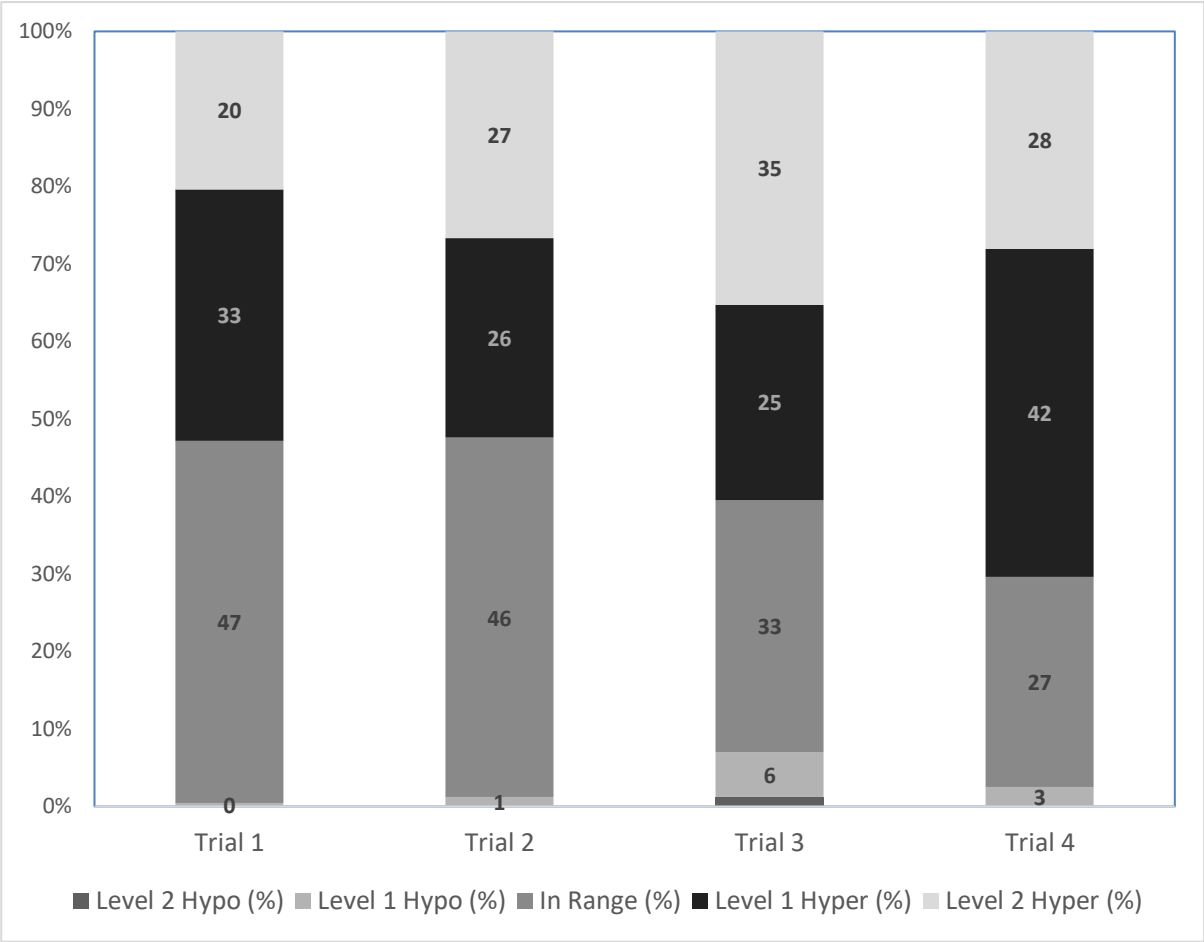


Figure 15. Nocturnal glycaemic range percentages over four trials. Data reported as the time taken in each glycaemic range as a percentage of total time.

3.3.4 Rate of nocturnal continuous glucose drops

Rate of nocturnal continuous glucose drops (FAST v SLOW) start and end of drop glucose concentrations and rate of change in glucose concentrations are shown in Table 14. The average duration of glucose drop was 40±17 minutes which was time matched between FAST and SLOW conditions. There was no difference between starting glucose concentrations between the two conditions ($p=0.11$) nor ending glucose concentrations between conditions ($p=0.36$). FAST rates of change in glucose concentration were different from the SLOW rates of change ($p<0.05$).

Table 14. Continuous glucose values for the start and end of the drop, mean glucose concentration, and the rate of change in glucose concentration for the FAST compared to the SLOW rate of glucose change conditions. Reported as mean ± standard deviation. n=10. * represents the significance between the FAST and SLOW conditions ($p<0.05$). Data were treated via repeated measures ANOVA.

Rate of glucose drop condition	**Start of drop glucose conc. ($mmol.L^{-1}$)**	**End of drop glucose conc. ($mmol.L^{-1}$)**	**Mean glucose conc. ($mmol.L^{-1}$)**	**Rate of change in glucose conc. ($mmol.L^{-1}.min$)**
FAST	13.7 ± 3.6	11.9 ± 3.6	12.8 ± 3.9	0.045 ± 0.020*
SLOW	12.0 ± 2.8	11.4 ± 2.7	11.7 ± 2.9	0.012 ± 0.014*

In the 'FAST' condition 7/10 participants started and ended with glucose values in hyperglycaemic zones, with 1/10 starting in hyperglycemia and ending in euglycemia, and 2/10 euglycemic-euglycemic. In the 'SLOW' condition 7/10 participants started and ended in hyperglycemia and 3/10 started in hyperglycemia and ended in euglycemia.

3.3.5 ECG alterations during the nocturnal period

Results revealed that ECG alterations during the 6-hour nocturnal period included heart rate (bpm), RMSSD (ms), and pNN50 (%) were different between the FAST and SLOW conditions as shown below in Table 15. ECG responses at the start and end of the drops were similar between 'FAST' and 'SLOW' conditions.

Table 15. ECG alterations between FAST and SLOW drops in interstitial glucose during a 6-hour nocturnal period. Reported as median ± IQR. n=10. Data were normally distributed. * represents the significance in heart rate (bpm) between FAST and SLOW conditions. ** represents the significance in RMSSD (ms) between FAST and SLOW conditions. *** represents the significance in pNN50 (%) between FAST and SLOW conditions. Data were treated via repeated measures T-tests.

Parameter	**FAST Median (IQR)**	**SLOW Median (IQR)**	**P value**
Heart Rate (bpm)	61 ± 17	62 ± 14	**<0.01***
QTc (ms)	408.0 ± 13.0	406.0 ± 10.0	0.660
SDNN (ms)	72.3 ± 47.6	69.9 ± 46.7	0.113
RMSSD (ms)	46.0 ± 41.1	54.3 ± 39.2	**<0.01****
pNN50 (%)	18.9 ± 43.2	27.5 ± 41.5	**<0.01*****
LF/HF Ratio (ms^2)	2.4 ± 3.7	2.2 ± 3.6	0.499

4. General Discussion

4.1 Summary of aims and findings

The title of this secondary analysis thesis is to further the understanding of interstitial glucose on ECG metrics in people with type 1 diabetes. The study aimed to identify ECG changes at rest, during a low-intensity exercise test, and during the nocturnal period in individuals with T1DM. Detecting early ECG changes within individuals with T1DM is useful to help manage the onset of CAN, which is a common complication and the greatest mortality of T1DM and is generally considered to have irreversible effects (Anaruma et al, 2016). ECG and heart rate variability metrics used in this study are clinically well-established methods of measuring the onset of CAN in individuals with T1DM (Serhiyenko & Serhiyenko, 2018; Vinik et al, 2013).

4.1.1 Hypothesis

The null hypothesis states there is no clear relationship between interstitial glucose concentration or rate of change of interstitial glucose concentrations in resting or dynamic scenarios on ECG metrics in people with type 1 diabetes. This study rejects the null hypothesis as the results reveal that interstitial glucose concentrations can impact ECG metrics.

4.1.2 Daytime 8-hour resting period

The 8-hour resting data aimed to explore the reproducibility of CGM and ECG alterations in individuals with type 1 diabetes. Findings revealed that although there was a small difference in 8-hour mean interstitial glucose between trials 1 and 4 and trials 3 and 4 there were no ECG alterations.

4.1.3 Low-intensity exercise test

Participant continuous glucose data were explored during the low-intensity exercise test and participants with both a euglycemic and hyperglycaemic zone test over the four trials were included in this section. The aim of the low-intensity exercise test was to explore the effect of parasympathetic withdrawal on ECG metrics in individuals with type 1 diabetes under euglycaemic and hyperglycaemic conditions. The mean interstitial glucose between the euglycaemic and hyperglycaemic conditions was different and resulted in ECG alterations of reduced QT (ms), QTc (ms), and HF (ms2) during hyperglycaemia compared to euglycaemia. It is well-established that hyperglycaemia can result in reduced QT (ms) and QTc (ms) at rest however it has not been explored during parasympathetic withdrawal making this a novel finding.

4.1.4 Nocturnal 6-hour period

The nocturnal subsection of this study aimed to identify ECG alterations during "FAST" and "SLOW" rates of change in interstitial glucose concentrations in individuals with type 1 diabetes. Findings showed that during a "FAST" drop compared to a "SLOW" drop-in interstitial glucose heart rate (bpm), rMSSD (ms), and pNN50 (%) were significantly reduced.

This suggests that rate of change in interstitial glucose could reduce heart rate variability although additional research is required. Research by Serhiyenko & Serhiyenko (2018) states that reduced heart rate variability is a clinical indication for the early onset of CAN in individuals with T1DM and therefore is an important finding.

4.2 Screening protocols

Individuals with a clinically abnormal 12-lead resting ECG conducted during the screening visit, as stated in the methods section, were excluded from participating in this study. Therefore, findings reveal that even individuals with clinically normal 12-lead resting ECG, free from disease, still had ECG alterations during glycaemic stages as stated above. This reaffirms the importance of clinically detecting these early ECG changes as having type 1 diabetes alone could increase the risk of developing CAN and other cardiovascular complications.

4.3 Hyperglycaemia

Guidance on glycaemic control targets for adults with type 1 diabetes recommends >70% of the time per day in the euglycemic range (3.9-13.9 mmol.L^{-1}), <4% of the time in level 1 hypoglycaemia (3.0-3.8 mmol.L-1), <1% of the time in level 2 hypoglycaemia (<3.0 mmol.L-1), <25% in level 1 hyperglycemia (10.1-13.9 mmol.L-1) and <5% in level 2 hypoglycaemia (>13.9 mmol.L-1) (Battelino et al, 2019). During the 8-hour daytime period on average participants in all trials did not meet the recommended time in range and spent too much time in level 2 hyperglycemia with on average participants during trials 1 and 4 also spending above the recommended guidance in level 1 hyperglycemia. Participants for all four trials during the nocturnal period also spend significantly above the recommended duration in hyperglycaemic zones with only 35% of the 6-hour nocturnal period spent in euglycaemia, 31.5% spent in level 1 hyperglycaemia and 27.5% spent in level 2 hyperglycaemia. This is important to note as a factor in the results of this study and also as spending over the recommended duration in hyperglycemia can result in developing diabetic complications previously discussed such as cardiovascular disease, neuropathy, retinopathy, and nephropathy.

During the resting period, trial 4 mean glucose concentration was higher than trial 1 and trial 3 resulting in trial 4 mean glucose concentration of the 8 hours to sit within a level 1 hyperglycaemic range. Bolus insulin administration exploration during this period revealed that trial 4 brunch bolus insulin was slightly higher alongside a similar feeding and therefore could explain why trial 4 average glucose concentration was higher during the resting period.

4.4 Glucose variability and rate of change

Findings show that during the nocturnal period rate of change in glucose reduced ECG parameters including heart rate (bpm), rMSSD (ms), and pNN50 (%). These findings are in agreement with research from Moser et al (2020) who also found that the rate of change in glucose variability resulted in ECG alterations which have been linked to the onset of cardiac complications such as CAN. These findings highlight the potential effect GV has in explaining sudden unexpected cardiac events in healthy individuals with type 1 diabetes for example 'dead in bed' syndrome and require further research. Effects of GV on potential cardiac complications include absolute insulin deficiency, erratic absorption of exogenous insulin, incomplete suppression of hepatic glucose production, and altered counterregulatory (Suh & Kim, 2015). Future research should further explore the relationship between GV, ECG alterations, and these physiological effects in individuals with type 1 diabetes.

4.5 ECG parameters

HR

Heart rate was unchanged between the same participants under four very similar trial visits even though during trial 4 the average interstitial glucose was higher and in a level 1 hyperglycaemic range at rest. During the low-intensity exercise test and therefore parasympathetic withdrawal heart rate was also unchanged between hyperglycaemic and euglycaemic conditions. This is in disagreement with research that has shown that hyperglycemia can cause an increase in HR by activating the sympathetic influence of the ANS

and releasing hormones such as adrenaline and noradrenaline which activate beta-adrenergic receptors in the heart and have a direct influence on increasing HR (Haas & McDonnell, 2018).

However, heart rate (bpm) was statistically significantly reduced during a 'FAST' compared to a 'SLOW' rate of drop in interstitial glucose during a 6-hour nocturnal period. Therefore, suggesting that the rate of change and more specifically FAST compared to SLOW drops in interstitial glucose can result in a reduced heart rate (bpm). It is important to note that both FAST and SLOW conditions started and ended in hyperglycaemic ranges. A research study found that the rate of rise in glucose altered heart rate at rest (Moser et al, 2020). It was concluded that this could be an increased stress response to a faster change in glucose, therefore causing a rise in heart rate (Moser et al, 2020). Interestingly, our findings reveal that a faster drop in glucose resulted in a reduction in heart rate at night. These changes may be due to the effect circadian rhythm has on cardiac metabolism. Cardiac metabolism is influenced by circadian rhythm by levels of metabolites, metabolic flux, and response to nutrients due to feeding, fasting, waking, and sleep (Zhang & Jain, 2021). Cardiac metabolism could explain some differences between day and night response to interstitial glucose, however, this complex area needs additional extensive research. It is also important to note that participants staying overnight in a laboratory environment may disrupt their normal circadian rhythm.

QT

QT interval (ms) was unchanged between four similar trials during an 8-hour resting period. During parasympathetic withdrawal (low-intensity exercise test), the QT interval (ms) was reduced during hyperglycaemia compared to euglycaemia.

It is relatively well established that hyperglycaemia can change QT interval in individuals with type 1 diabetes, however, it is unknown how hyperglycaemia can affect QT interval during parasympathetic withdrawal. Our finding of a reduction in QT interval during hyperglycaemia compared to euglycemia is therefore a novel observation. Hyperglycaemia could affect cardiac metabolism due to an increased prevalence of glucose to produce ATP however further research is required in this area to conclude the reasoning for this change (Bing, 1965).

Research has shown that there is an increased prevalence of sudden nocturnal death in individuals with type 1 diabetes which has been referred to as ‘dead in bed’ syndrome. This makes investigating ECG alterations under various changes in interstitial glucose during the nocturnal period in people with type 1 diabetes important (Gill et al, 2008). Our results found the rate of drop (FAST V SLOW) of interstitial glucose does not change the QT interval in individuals with type 1 diabetes. Investigation of interstitial glucose during in nocturnal period in our study reveals that both the start and end of drop glucose concentrations were still in hyperglycaemic ranges which could explain why the QT interval was not changed, however further research is required.

QTc

QTc (ms) was unchanged during four similar trial visits during an 8-hour rest and the rate of change of glucose during the 6-hour nocturnal period also did not change QTc (ms). However, QTc (ms) was reduced during hyperglycaemia compared to euglycaemia during parasympathetic withdrawal shown in the low-intensity exercise test.

Our results at rest contrast some research studies as it is generally established that hyperglycaemia altered QTc at rest (Christensen et al, 2010; Mezquita-Raya et al, 2018 & Nguyen & Nguyen et al, 2012). During the 8-hour resting period, even though trial 4 interstitial glucose was higher than the other trial visits and sitting within the hyperglycaemic range (>10mmol.L^{-1}) QTc did not change. However, exploring average mean glucose values over the 8-hour resting period over the four trials average glycemia differed by <1mmol.L^{-1} between the lowest and highest mean interstitial value, with trials 1, 2, and 3 sitting at the higher end of the euglycaemic range, which could explain why QTc was not statistically altered between the four trials during the resting period. The study design used in this thesis also differed from other research studies. This study was able to compare the same participants over 4 separate 8-hour periods, whilst other studies used different design methods such as matched pairs. Even though they controlled for factors such as age, gender, and fitness level this design is not as strong as analysing the same individual under near identical conditions in a controlled environment.

Research has shown that nocturnal hypoglycaemia causes prolonged QTc intervals (Gill et al, 2008; Robinson et al, 2004 & Murphy et al, 2004). However, analysis of nocturnal glycemia

within this thesis shows that even though we were exploring the rate of drop in glucose, for both 'FAST' and 'SLOW' conditions the start and end of drop glucose concentration were within hyperglycaemic ranges which could explain why QTc interval was not altered. Therefore, future research could further the understanding of the effect of the rate of drop on ECG metrics by looking at drops from within different glycaemic ranges including hypoglycaemia.

SDNN

SDNN (ms) was unchanged during the four similar resting trials, glycaemia had no effect on SDNN (ms) during the low-intensity exercise test and was also unchanged during the different rates of glycaemic change during the night-time period. Research has suggested that SDNN is more accurate during longer 24-hour ECG recordings, and therefore RMSSD and pNN50% may be considered more reliable metrics over shorter recordings and could explain why SDNN was not significantly changed in this study (Shaffer & Ginsberg, 2017).

RMSSD

RMSSD (ms) was unchanged at rest during four similar trial visits and there was no difference in RMSSD (ms) during hyperglycaemia compared to euglycaemia during parasympathetic withdrawal. However, RMSSD (ms) was reduced during the FAST drop compared to the SLOW drop in interstitial glucose during the 6-hour nocturnal period, suggesting that the rate of change at night does affect this heart rate variability metric.

Research by Moser et al (2020) explored the rate of glucose change on HRV metrics at rest and found that rapid increases in glucose resulted in a decrease in RMSSD. However, in contrast

to our findings, results showed rapid drops in blood glucose led to greater HRV. A strength of this research compared to Moser et al (2020) is that we used a repeated measure design using the same participants on different trial visits however Moser et al (2020) comparisons were between different participants. Rapid changes in glucose have been suggested to cause a greater stress response which could be resulting in altered HRV in individuals with type 1 diabetes (Moser et al, 2020). During an increased short-term stress response many physiological systems are affected for example musculoskeletal, neuroendocrine, and importantly the cardiovascular system (Dhabhar, 2018). Therefore, it is of interest to further investigate the effect of these short-term changes on long-term cardiovascular health in individuals with type 1 diabetes.

pNN50

PNN50 (%) was unchanged at rest, during parasympathetic withdrawal however was reduced during the FAST rate of change in interstitial glucose compared to a slower rate of change during the nocturnal period.

However, a study by Moser et al (2020) found that PNN50 (%) was reduced during a rapid rise in glucose at rest but found no difference at different glycaemic ranges. Therefore, further suggesting that in our study it could be the change in glucose that is altering HRV metrics due to a potential increased stress response of the body during increased glucose disturbances as described above in the RMSSD section (Moser et al, 2020).

LF

A study that investigated the effect of a bout of aerobic exercise on heart rate variability in individuals with type 1 diabetes also found no change in LF (ms) (Anaruma et al, 2015). However, therefore were differences in the methodology used in this study as it was 30 minutes of 40- 60% intensity aerobic exercise, which is different from the 3-minute low-intensity exercise test used in this research study, as the sympathetic nervous system will be dominant during aerobic exercise compared to parasympathetic being dominant during the exercise bout in this thesis making it difficult to compare results (Anaruma et al, 2015 & Fornasiero et al, 2018).

HF

HF (ms) was unchanged at rest, however, was reduced in hyperglycaemia compared to euglycaemia during the low-intensity exercise test. Interestingly, clinical monitoring has revealed that a decrease in HF suggests and decrease in parasympathetic dysfunction, which in this study was more apparent during hyperglycaemia compared to euglycaemia (Serhiyenko & Serhiyenko, 2018).

LF/HF ratio

LF/HF ratio is a frequency domain heart rate variability metric that represents the ratio of low-frequency to high-frequency power. The LF/HF ratio was unchanged during rest, during parasympathetic withdrawal, and also by the rate of change of interstitial glucose during the nocturnal period.

4.6 Heart rate variability

In conclusion to the metric analysis above, key findings in this study were hyperglycaemia during parasympathetic withdrawal rate of change in interstitial glucose during the nocturnal period, resulting in a reduction in heart rate variability in individuals with type 1 diabetes. Heart rate variability is the physiological ability to respond to external stressors and therefore a higher heart rate variability is generally desirable (Shaffer & Ginsberg, 2017). Research has found reduced heart rate variability to be associated with the onset of CAN and although we cannot conclude this from this study, these findings are important observations that require further investigation (Serhiyenko & Serhiyenko, 2018).

4.7 Thesis strengths

A strength of this secondary analysis research participants attended the laboratory for the 8-hour daytime period, low-intensity exercise, and during a 6-hour overnight period on four separate occasions. This study design enables comparisons between the same individuals under extremely similar and well-controlled conditions. Due to participants being in controlled laboratory conditions over a 23-hour period basal and bolus insulin administration and nutritional intake for breakfast, brunch, lunch, pre-exercise meal, post-exercise meal, and pre-bedtime snack were able to be controlled and analysed which is essential when investigating and understanding the effect of interstitial glucose in individuals with type 1 diabetes. Due to the nature of the laboratory environment, physical activity outside of the exercise test was also controlled which is highly important as explained in the ‘glucose disturbances with exercise in

T1DM' section within this thesis, exercise, and physical activity can alter interstitial glucose responses and also ECG responses which are the two primary dependent variables within this study (Houlder & Yardley, 2018, Riddell & Perkins, 2009 & McCarthy et al, 2019).

This study consists of three interrelated sub-sections: rest, low-intensity exercise, and nocturnal analysis. This is a strength of the study as it allows for the exploration of each section with the acknowledgment of the other sections. For example, exploring the reproducibility of CGM and ECG alterations during rest acts as a degree of control before investigating nocturnal changes within interstitial glucose in the same participants with type 1 diabetes.

The design of this study includes a novel low-intensity exercise test at 20 watts. The aim of this test was to isolate parasympathetic withdrawal before the onset of sympathetic activity becomes the dominant branch during higher-intensity exercise. Research has shown that the energy demands during a 3-minute 20-watt exercise test are low and therefore the sympathetic 'fight or flight' response, increased cardiovascular output, or utilization of other resources would not be required (McCorry, 2007). A study exploring autonomic control using HRV spectral analysis during exercise found that as soon as exercise started parasympathetic influence decreased however the sympathetic influence didn't start increasing until exercise intensity exceeded the predetermined ventilatory threshold (Yamamoto, Hughson & Peterson, 1991). Therefore, this novel exercise test could be used in future research to expand the understanding of parasympathetic withdrawal.

4.8 Thesis limitations

Faulkner et al (2019) found differences in heart rate variability in non-hispanic black versus non-Hispanic white adolescents with type 1 diabetes, therefore suggesting results may not be reliable for non-white individuals limiting the transferability of research. However, research has suggested that just a reduction in HRV is associated with the onset and CAN and no specific standard values have been established for the diagnosis of CAN (Mogensen et al, 2012). For completeness, future research should aim to include participants from a range of ethnic backgrounds to ensure the reliability of results within the general population.

Due to the nature of secondary data, the analysis could be improved during the nocturnal period by including additional metrics to coincide with resting and low-intensity exercise test metrics to allow for further comparison.

4.9 Suggestions for future research

To ensure complete standardisation between trials, bolus, and mealtime macronutrient intake could be controlled further. However, due to the nature of diabetes, this is extremely hard to completely control as small alterations and corrections in bolus insulin and CHO are sometimes essential for ethical reasoning and the well-being of participants.

Future research during nocturnal ECG monitoring could also add the factor of analysing the effect of sleep stage within the sleep cycle into the design to investigate whether the stage of sleep for example light sleep, deep sleep, or REMs during changes in interstitial glucose has

an effect on ECG alterations in individuals with T1DM. This is of interest as research has found ECG alterations during different stages of sleep but has not been researched under different glycaemic levels in individuals and could help to develop an understanding of ‘dead in bed’ syndrome in individuals with type 1 diabetes (Sun et al, 2020).

Future research could also further investigate novel aspects of this thesis including using a repeated measure method where the same participants were investigated under different trial visits, the low-intensity exercise test, and the rate of change of interstitial glucose on ECG metrics at a wider range of glycaemic ranges including hypoglycaemia. As the hyperglycaemia section of this discussion states, a large proportion of time within the study was spent in hyperglycaemia and therefore ECG alterations during lower glycaemic ranges and hypoglycaemia were not fully explored.

4.10 Conclusions

4.10.1 General conclusion

In conclusion, interstitial glucose resulted in some ECG under different glycaemic and time ranges in a healthy cohort of individuals with type 1 diabetes. At rest, a small difference in 8-hour mean interstitial glucose resulted in no ECG alterations over four trial visits in the same individuals with type 1 diabetes. However, during a 20-watt low-intensity exercise test hyperglycaemic conditions resulted in ECG alterations of reduced QT (ms), QTc (ms), and HF (ms^2) compared to the same individuals performing the same exercise test in euglycaemia. During nocturnal periods the rate of change in interstitial glucose, a 'FAST' rate of reduction significantly reduced heart rate (bpm), rMSSD (ms) and pNN50(%) compared to a 'SLOW' rate of reduction.

4.10.2 Key impacts of findings

The participants involved in this study were deemed healthy and had well-controlled diabetes which previously may be thought unlikely to have long-term complications such as cardiovascular autonomic neuropathy. However, this study reveals that even in a healthy population, type 1 diabetes alone serves as a risk factor for ECG alterations under certain glycaemic conditions. Detecting these early changes in a clinical setting could be vital in preventing the onset of irreversible complications of the disease such as cardiac neuropathy. Future research should outline clinical protocols to help detect these early signs of future cardiac complications that can be used in routine assessments for individuals with type 1 diabetes.

Findings revealed that ECG metrics particularly HRV metrics were reduced during parasympathetic withdrawal when glucose levels were raised compared to euglycemic. Research has shown the relationship between reduced HRV and the onset of cardiac implications. Therefore, this raises the question as to the potentially damaging impact of exercising in hyperglycemia on cardiovascular health in individuals with type 1 diabetes (Serhiyenko & Serhiyenko, 2018).

Findings reveal the relevance of GV on nocturnal ECG alterations and future research should further explore the relationship between GV, ECG alterations, and sudden nocturnal cardiac events such as ‘dead in bed’ syndrome in healthy individuals with type 1 diabetes. Future research should aim to quantify target nocturnal GV values for clinicians to advise during routine diabetes care.

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