Investigating heart rate variability as a novel non-invasive measure of blood glucose level in type 1 and type 2 diabetes.

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by Luke Jarman[1]

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Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, Faculty of Science, University of Technology Sydney.

Principle supervisor: Associate Professor Sara Lal[1, 2]

Co-supervisors: Associate Professor Roderick Clifton-Bligh[3]

Dr Najah Nassif[2]

Professor Ann Simpson[2]

1. Neuroscience Research Unit, School of Life Sciences, University of Technology Sydney.

2. School of Life Sciences and Centre for Health Technologies, University of Technology Sydney.

3. Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards.

Declaration

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Luke Jarman, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy (Science), in the School of Life Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Signature: Production Note:
Signature removed prior to publication.

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List of Abbreviations

BGL = Blood glucose level

BMI = Body mass index

BP = Blood pressure

bpm = Beats per minute

CGM = Continuous glucose monitoring

ECG = Electrocardiogram

HLA = Human leukocyte antigen.

HF = High frequency

HFnu = Normalised high frequency

HREC = Human Research Ethics Committee

HRV = Heart rate variability

Hz = Hertz

kg/m² = Kilogram per square metre

kJ = Kilojoule

LAQ = Lifestyle Appraisal Questionnaire

LF = Low frequency

LF:HF = Low to high frequency ratio

LFnu = Normalised low frequency

min = Minutes

mmHg = Milligrams of mercury

mmol/L = Millimoles per litre

ms = Milliseconds

ms² = Milliseconds squared

mV = Millivolt

p = P-value

PSD = Power spectral density

QRS = QRS complex (electrocardiogram waveform)

r = Correlation coefficient

RMSSD = Root mean square of successive RR interval differences

RNSH = Royal North Shore Hospital

ROS = Reactive oxygen species.

RR = R-R interval

s = Second

s² = Second squared

SDNN = Standard deviation of NN intervals.

SMBG = Self-monitoring of blood glucose

T1D = Type 1 diabetes

T2D = Type 2 diabetes

UTS = University of Technology Sydney

VLF = Very low frequency

Abstract

Diabetes mellitus is costly to both individuals and health systems and affects almost 500 million adults worldwide. The scientific literature strongly advocates that maintaining blood glucose level (BGL) within the optimal range as the best means of reducing diabetes complications and improving quality of life and health outcomes for those living with diabetes. Optimal management and monitoring of BGL may be significantly improved by combining data on an individual's heart rate variability (HRV) – a non-invasive autonomic marker – with data from currently-available invasive glucose monitoring systems. As part of this PhD candidature and thesis, groups of people with type 1 diabetes, type 2 diabetes, or without any chronic illness, were recruited to investigate correlations between HRV measures and BGL. Correlation analysis demonstrated that multiple different HRV measures were significantly and inversely correlated with BGL measured in a fasting and postprandial state. Multiple linear regression analyses determined that HRV measures account for 27-55% of the total variation in BGL measured in different metabolic states. However, further research is needed. Continuous glucose monitoring (CGM) remains the gold-standard for measurement of BGL in diabetes, however HRV represents a promising area which can add to detection of diabetes and glycaemic events in tandem with CGM.

Chapter 1. Introduction

With the advent of the 21st century, new challenges have emerged for medical science. Driven by global shifts in culture and influence, as well as technological advancements related to food, travel, and entertainment, the lifestyles of modern humans have strayed from those of our Palaeolithic ancestors. Eaten and Konner (1985) were among the first to suggest that though the habits and diets of humans have evolved dramatically over time, the genetic traits passed down with each generation have not experienced the same pressures of natural selection as our Palaeolithic predecessors.[1] As such, the genome of modern humans is more suited to the hunter-gatherer lifestyles that were adapted over 12,000 years ago,[2] resulting in a 'gene-culture' misalignment in the 21st century. In light of this, there is growing recognition in the scientific community that the discordance between modern and Palaeolithic lifestyles is associated with the current rise in 'diseases of civilisation', though the extent to which this is true is debated within the literature.[3, 4] Regardless, diseases attributed to modern ways of life are emerging at alarming rates and represent a significant burden to society, and this must be addressed.

1.1 Diabetes Mellitus: A Disease of Civilisation

There is a growing awareness of the rise of non-communicable diseases related to modern civilisation. In the 1970s, epidemiologists observed an increase in the prevalence of obesity, cardiovascular diseases, and type 2 diabetes (T2D) among indigenous populations that had renounced their traditional lifestyles in favour of 'Westernised' lifestyles,[5, 6] and recent research conducted by Balick and colleagues (2019) supports these findings.[7] This causal relationship was reinforced by O'dea (1984) who demonstrated that Australian Aborigines with T2D who temporarily reverted to their highly-active hunter-gatherer lifestyle and low energy density diet showed significant improvements in blood pressure, glucose tolerance, and lipid profiles.[8] This has also been ratified by more recent research conducted by Frassetto and colleagues (2009).[9] Despite this evidence, adherence to traditional hunter-gatherer diets, which are protective factors against diseases of civilisation, remains in decline.[10] The literature is generally critical of the habits and diets characteristic of Western civilisation, defined by a high-fat, high-sugar diet and a largely sedentary lifestyle lacking in meaningful levels of physical activity.[10, 11] Though some authors contend that trends in physical inactivity have changed little over the last 30-40 years,[12] there is a consensus that the widely popular Western diet is an underlying cause of many emerging diseases of civilisation.[13, 14] Because of this spread, these diseases of civilization are not endemic to Western nations, and many have reached epidemic proportions worldwide.[15, 16]

In the past 20 years, non-communicable diseases have overtaken infectious and parasitic diseases as the leading causes of mortality worldwide (Figure 1.1).[17] Although advancements in medical science have generally improved life expectancy and mortality rates worldwide,[18, 19] the burden of disease remains high.[20] This may also be attributed to the fact that humans are living longer on average, and age is a risk factor for many lifestyle-related diseases.[21] Due to commercial pressures to consume products high in fat and sugar, as well as social pressures to engage in cigarette and alcohol consumption, non-communicable diseases have replaced infectious diseases as the main causes of mortality worldwide, and will present a major challenge to medical science for the foreseeable future.[22] Without adoption of better lifestyles and behaviours, which would require significant cultural changes, it is projected that the burden of lifestyle diseases will continue to grow. T2D is but one of many conditions associated with civilisation. However, it is the fourth biggest cause of lifestyle-related mortality worldwide,[22] and is considered one of the main causes of premature illness and mortality.[23, 24] The objective of this initial chapter is to establish why current management of diabetes mellitus – both type 1 diabetes (T1D) and T2D – is inadequate given its scope, and how the research conducted as part of this PhD candidature may improve it.

Figure 1.1 Total number of global deaths from leading causes in 2000 and 2019.

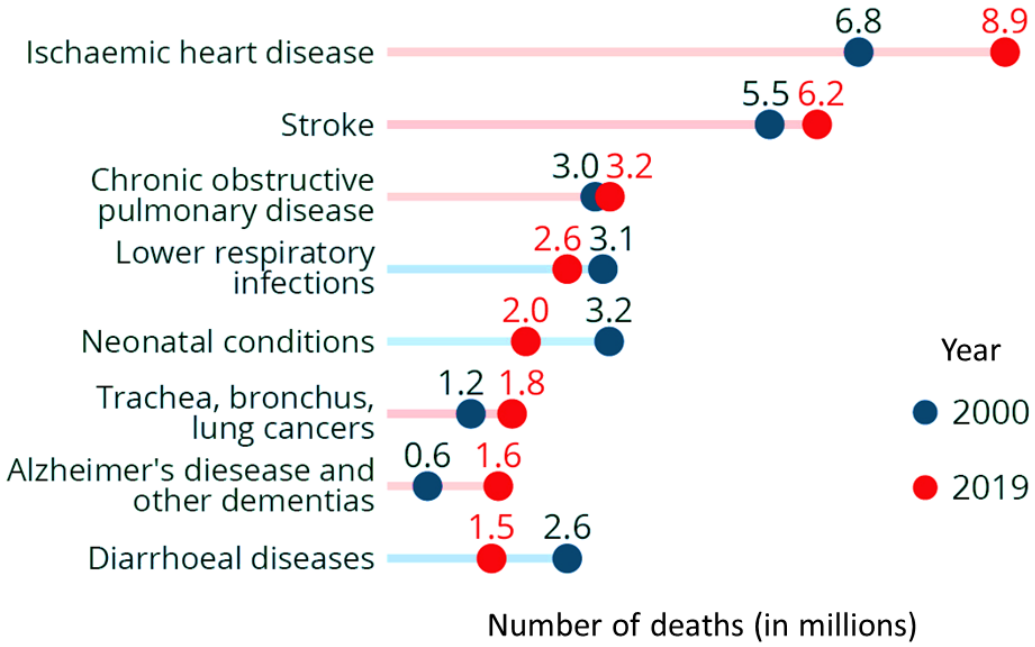

Figure 1.1 illustrates the change in the number of deaths from the leading causes of death in the year 2000 (blue) compared to 2019 (red). Communicable diseases are indicated by the blue lines, and include lower respiratory infections, neonatal conditions, and diarrheal diseases. All others shown are non-communicable diseases and are indicated by pink lines. Non-communicable diseases caused higher mortality rates in 2019 compared to 2000, and communicable diseases caused lower rates, indicating a shift in the causes of mortality in recent decades. Adapted from World Health Organization (2020).[17]

1.2 Epidemiology

Diabetes mellitus – referred to hereon as diabetes – is a group of metabolic disorders characterised by chronic hyperglycaemia, or chronically-elevated levels of blood glucose.[25] Though diabetes has been introduced in this thesis as a Disease of Civilization, this is only true for T2D, which constitutes the vast majority of diabetes cases. Other types, such as T1D which will be distinguished in a later section, are not typically considered 'lifestyle' disorders. However, all types of diabetes share a common end point of dysglycaemia, or abnormal blood glucose, and this is the focus of the thesis. For this reason, the term 'diabetes' is used in this thesis in the context of dysglycaemia and refers to both T1D and T2D. Ranges for blood glucose level (BGL) indicative of diabetes are provided in Table 1.1. In the past, discrepancies in the classification of diabetes between the American Diabetes Association (ADA) and the World Health Organization (WHO) – two leading global authorities on diabetes – caused inconsistencies in epidemiological data.[26] Estimates of some types of diabetes were affected more severely as they were sensitive to the identifying criteria.[27] Now, WHO and ADA use consistent criteria for diagnosing diabetes, providing a gold-standard approach for identification.[28, 29] These criteria are the most widely-accepted ranges for optimal and suboptimal BGL, also referred to as plasma glucose concentration.

Table 1.1 Ranges of blood glucose levels that are considered 'optimal, 'impaired', 'pre-diabetes' or 'diabetes', as determined by fasting blood glucose and 2-hour glucose tolerance test. Fasting is defined as no caloric intake for at least 8 hours. Individuals need to meet criteria for both fasting and 2-hour glucose tolerance ranges for diagnosis. ***** = 2-hour glucose tolerance test is standardised by 75 grams of glucose solution. **mmol/L** = Millimoles per litre. Adapted from the American Diabetes Association (2016) and the World Health Organization (2016).[28, 29]

Diagnosis	Fasting blood glucose (mmol/L)	2-hour glucose tolerance test (mmol/L) *
Optimal	4.0 – 5.5	≤ 7.7
Impaired fasting glucose	6.1 – 6.9	≤ 7.7
Pre-diabetes	5.6 – 6.9	7.8 – 11.1
Diabetes	≥ 7.0	≥ 11.1

Epidemiological evidence suggests that fasting BGL has been on the rise since 1980 (n=2,700,000), increasing by 0.07 millimoles per litre (mmol/L) in men and 0.09 mmol/L in women per decade on average.[30] The current burden of this is already high – the global health expenditure for diabetes was estimated to be 850 billion USD in 2017.[31] On an individual level, diabetes is also the cause of significant emotional distress and financial burden,[32] which is due

to a combination of medical costs and reduced capacity for work.[33] Health expenditure for people living with diabetes is higher for individuals with complications, such as retinopathy and kidney failure, though this depends on various factors such as the type of diabetes and it's duration.[34] To better understand the scope of diabetes, the following sections will discuss the specific attributes which need to be addressed, such as the rising prevalence, concerns with classification, short and long term complications, and limitations of current treatment.

1.3 Prevalence

According to a systematic literature review of 221 data sources conducted by the International Diabetes Federation, the percentage of people affected by diabetes is expected to increase from 8.4% in 2017 to 9.9% by 2045.[31] Figure 1.2 shows that total cases have been steadily rising for the past 30 years. Note the large increase in the Eastern Mediterranean region – the origin of the Mediterranean diet, one of the healthiest modern diets[35, 36] In their 2011 paper, Musaiger reasoned that this was because many people living in the Mediterranean region, particularly those in Arab countries, are renouncing their traditional diet in favour of the Western diet.[37] Prevalence may also be rising as a result of increased screening in some regions.

Figure 1.2 Prevalence of diabetes in major regions between 1980 and 2014.

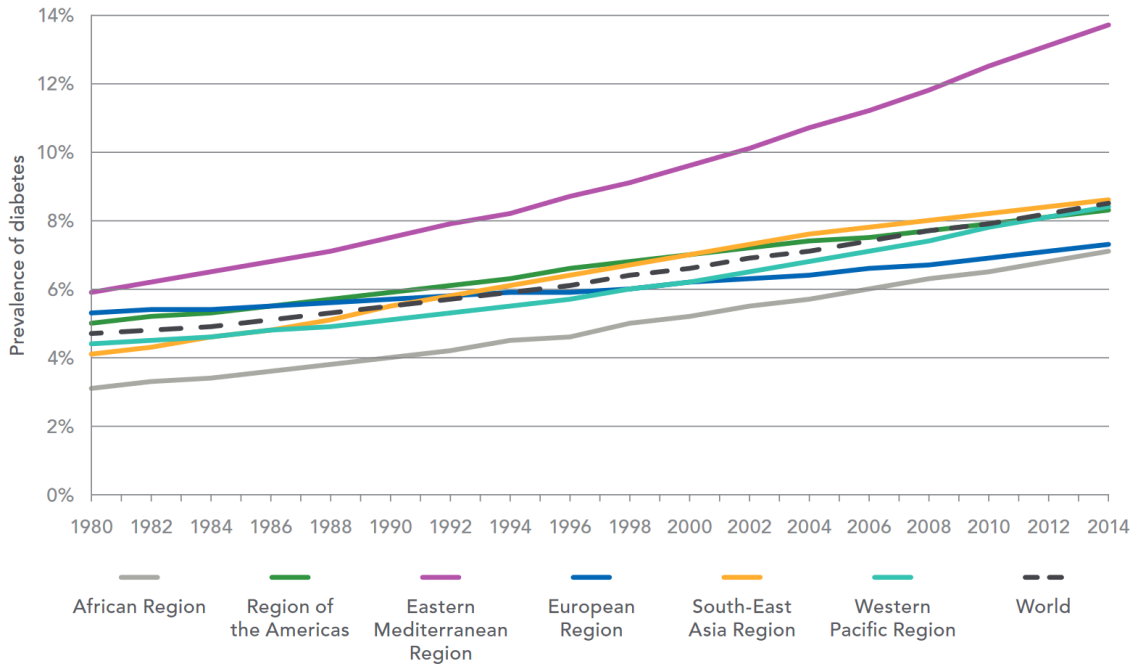

Figure 1.2 portrays the rising prevalence of diabetes across six major regions worldwide, from 1980 to 2014. Diabetes in the South-East Asia Region, of which Australia is part, has been steadily rising over the past 30 years. The Eastern Mediterranean Region is undergoing the highest increase in new cases of diabetes worldwide. The black dotted line indicates average prevalence of diabetes across all regions. Image from World Health Organization (2016).[29]

Data on the prevalence of diabetes in Australia is reported every six months by the National Diabetes Services Scheme, an Australian Government initiative which is administered by Diabetes Australia. As of the 31st of December 2021, there were 1,426,245 people with diabetes registered. Of those, 9% had T1D, 87% had T2D, 3% had gestational diabetes, and less than 1% had some other type of diabetes.[38] Per census data provided by the Australia Bureau of Statistics, the population of Australia at the end of 2021 was 25,766,605.[39] As such, at the end of 2021, 5.54% of all Australians had some type of diabetes. Other studies report lower rates of T1D, such as a recent study in the United States which found that 5.8% of all diabetes cases were T1D and 90.9% were T2D.[40] This discrepancy may be due to many cases of 'insulin-treated T2D' being misclassified as 'T1D' in the Australian Health Survey and National Diabetes Services Scheme. Discrepancies may also arise in cases of 'double diabetes', where people with T1D may also develop metabolic syndrome, which shares clinical features with T2D such as insulin resistance.[41] As such, an individual with T1D may register as both someone with T1D and T2D in the prevalence reports. This raises an important issue with diabetes classification in the literature: many cases of diabetes do not fit perfectly into a single category, and there is some overlap between the different types of diabetes.[42] Future studies should recognise these problems with diabetes classification when approaching study design and when distinguishing sample groups.

1.4 Classification

Both ADA and WHO recognise two main types of diabetes which constitute majority of cases: T1D and T2D.[28, 29] Largely, they are caused by defects in multiple genes and, in the case of T2D, also lifestyle risk factors such as obesity.[43] Less common forms of diabetes exist, such as: monogenic forms of diabetes which relate to a defect in a single gene; secondary diabetes which can result from cystic fibrosis or other medical conditions; and latent autoimmune diabetes in adults.[44] There is an emphasis in the literature on separating non-classical forms of diabetes from T1D and T2D, as they have distinguishable clinical manifestations and treatments.[45] Given that T1D and T2D are the most common types of diabetes and collectively account for over 95% of all types of diabetes, these will be the focus of this thesis, and any research conducted as part of the PhD candidature will focus on T1D and T2D. This chapter will also provide evidence as to why T1D and T2D should be studied independently, including the relevance of their distinct aetiology and pathophysiology.[46]

1.4.1 Type 1 Diabetes

T1D and T2D are characterised by a dysfunction of insulin which results in long-term hyperglycaemia. For T1D, this dysfunction is due to a total or near-total deficiency in insulin production.[47] In short, insulin is a hormone which promotes glucose uptake by somatic cells to reduce levels of glucose circulating in the blood. As a result, T1D is associated with chronically-elevated levels of plasma glucose, as the body lacks insulin-related mechanisms to maintain glucose homeostasis.[48] The exact mechanisms of T1D pathophysiology are still widely debated, and this has inhibited the development of effective preventive measures.[49] However, there is a general consensus that insulin depletion is caused by immune-mediated destruction of insulin-producing β-cells in the pancreas.[50, 51] Various genetic, epigenetic, and exogenous risk factors have been implicated in the development of T1D, however causative mechanisms are poorly-understood.[52, 53] This may be because of the sheer number of regions of the human genome which confer susceptibility to the condition, which make it difficult to isolate a single cause, as well as the fact that a human pancreas cannot be studied non-invasively in living subjects.[54]

The human leukocyte antigen (HLA) complex is comprised of 128 genes densely clustered on chromosome-6, and variations in this complex can explain 35-50% of the genetic predisposition to T1D.[55] Familial clustering is prominent in T1D, and the HLA haplotypes which predispose one to T1D are the strongest genetic association to T1D identified to date. However, genetic studies have not been able to identify specific genes of the HLA complex which cause T1D, and there are various non-HLA genes implicated in the pathogenesis of T1D.[56] Increased expression of HLA-1 antigens in the insulin-producing beta cells of the pancreas has been associated with the onset of T1D. It is hypothesised in recent work that HLA-1 antigens increase the action of influent $CD8^+$ T cells specific to defined islet antigens.[57] In this autoimmune model, $CD8^+$ T cells target the beta cells of the pancreas and destroy them, leading to a deficiency in insulin production and thus dysglycaemia.

Studies on hereditary links in T1D, including the inheritance of susceptibility genes, are criticised as being underpowered and thus insufficient to detect complex interactions between genes.[58] Exogenous or environmental agents represent a divided area of study within the literature, as those agents that have been identified so far vary significantly. These include: infection by enteroviruses or retroviruses, pathological gut flora, consumption of certain milk proteins, and exposure to environmental pollutants.[59] A range of genetic and environmental factors are involved in the pathogenesis of T1D, and future research may need to focus on developing current understandings so that more therapeutic options for T1D may be explored.[60]

1.4.2 Type 2 Diabetes

As with T1D, T2D is associated with a dysfunction of insulin that causes chronically high levels of blood glucose. By comparison, T2D is associated with a progressive impairment of insulin secretion and efficacy,[28] which leads to profound, chronic elevations in BGL.[61] Associated with the pathogenesis of T2D is insulin resistance, a term which refers to the way in which long term over-production of insulin, which may be exacerbated by high carbohydrate load of diets, leads to a progressive loss of sensitivity to insulin. The risk factors for developing T2D are more well-known than T1D and, as such, T2D is theoretically easier to prevent compared to T1D.[62] This also facilitates health programs and strategies to target groups at risk of T2D, such as overweight individuals and indigenous populations. Other groups at risk include the elderly, smokers, and heavy drinkers, though generally preventative programs are not targeted at these populations in the context of preventing diabetes.[63] There is also a large genetic component to T2D, and studies in this area need to improve sample sizes, diversify populations, and improve phenotyping and sub-phenotyping.[64]

The traditional risk factors for T2D are well-established, and include increased waist circumference, obesity, smoking, high blood pressure, and physical inactivity.[65, 66] Large-scale clinical trials have shown promising results in reducing the prevalence of T2D in at-risk groups by targeting these specific lifestyle-related risk factors.[67, 68] There are consistent findings that proper management of blood glucose and blood pressure, as well as smoking cessation and weight loss, are important strategies for managing T2D and its complications such as cardiovascular disease.[69, 70] Recent literature also advocates the importance of non-traditional risk factors for cardiovascular disease (Figure 1.3), which are also implicated in T2D. These include insulin resistance, glycaemic variability, thrombogenic factors, and others.[71] In particular, insulin resistance and glycaemic variability are non-traditional risk factors for cardiovascular disease which are also important in the prevention of microvascular and macrovascular complications of diabetes. Future studies may need to consider the complex interactions of these risk factors in the pathogenesis of T2D.

Figure 1.3 Interaction of traditional and non-traditional risk factors in the progression of cardiovascular disease.

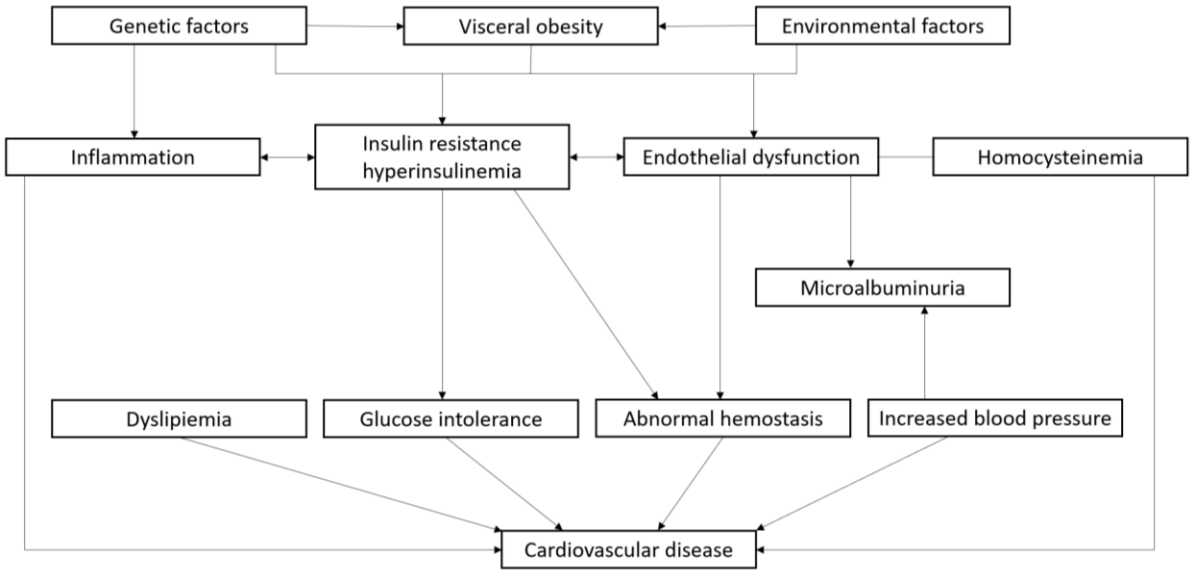

Figure 1.3 shows the complex relationships between the various risk factors present in T2D that are also implicated in cardiovascular disease. Traditional risk factors include: 'increased blood pressure', which has a direct correlation with cardiovascular disease; 'visceral obesity'; and certain 'genetic factors'. The other risk factors featured in this figure are considered non-traditional and represent an emerging awareness of the complex interactions between risk factors and disease onset. Adapted from Martín-Timón et al. (2014).[71]

Evidently, the two main types of diabetes share few similarities in terms of pathophysiology. Due to the spread of poor lifestyles, traditional risk factors for T2D have reached epidemic proportions. Though the mechanisms of its aetiology are debated, T1D is also rising. Reasons for this increase in prevalence are also debated, as correlations between T1D and proposed risk factors such as air pollution and certain infections have been inconclusive,[72, 73] supporting the need for further research. Though T1D constitutes a smaller proportion of all cases of diabetes at only 9%, this statistic has remained static in recent years. This indicates the prevalence of T1D is also increasing in line with the T2D epidemic,[40] which is driven by the rise of traditional risk factors for T2D such as obesity and hypertension.[43] According to the literature, this level of comorbidity and interconnectedness between lifestyle-related diseases and risk factors is difficult to quantify. Additionally, living with diabetes is a risk factor for developing complications of diabetes. More specifically, the severity of complications of diabetes is associated with the amount of time an individual lives with suboptimal levels of BGL.[74] This chapter will now explore the short and long-term complications of diabetes and why prevention of these complications is a core component of reducing the burden of diabetes.

1.5 Complications

Complications of diabetes may arise in the short-term and the long-term, and are the direct consequence of hyperglycaemia.[75] Maintaining optimal BGL reduces the risk for these complications. However, for reasons that will be discussed later in this chapter, many people living with diabetes are unable to optimise their BGL sufficiently. Some reasons include intercurrent illness and infection, or as a consequence of treatment for dysglycaemia. Acute symptoms can arise when BGL rises above the optimal upper limit of 7.7 mmol/L, including acute hyperglycaemia, diabetic ketoacidosis, and the hyperglycaemic hyperosmolar state, and when BGL drops below the lower limit of 4.0 mmol/L, which is known as hypoglycaemia. Roughly one third of all hospitalisations caused by diabetes are due to unmanaged diabetes conditions, representing a significant burden to health care systems.[76]

1.5.1 Short-Term

As one of the defining features of diabetes, persistent hyperglycaemia is the most common symptom experienced by people living with diabetes.[77] Acute symptoms of hyperglycaemia are generally mild, and include feelings of hunger and thirst, as well as frequent urination.[78] The lack of seriousness of these symptoms, combined with the fact that they generally develop slowly over time in T2D, contributes to unawareness of diabetes. In turn, the lack of awareness that these symptoms may be related to diabetes contributes to the large percentage of untreated hyperglycaemia.

1.5.1.1 Hyperglycaemia

In cases of unmanaged or undiagnosed diabetes, the deficiency of insulin activity can result in very high levels of glycaemia, which can cause two serious acute complications: diabetic ketoacidosis, which is particularly prevalent in T1D, and the hyperglycaemic hyperosmolar state, which is the more common presentation for people with T2D. Ketoacidosis is a complication of acute insulin deficiency and is a common acute complication for people living with T1D.[79] It is associated with high levels of circulating ketone bodies (hyperketonaemia) which can cause metabolic acidosis, and people usually experience excessive hunger, thirst, and weight loss.[80] In their systematic review of 19 studies, Marcovecchio (2017) concluded that the prevalence of diabetic ketoacidosis was higher in women, younger people, and in people treated with insulin injections.[81] Most present with a BGL greater than 16.7 mmol/L, and treatment involves insulin therapy to correct the hyperglycaemia, as well as replacing lost fluids.[82] The

literature is critical of additional supplement treatments such as bicarbonate infusion, as they are almost never required. For example, a systematic review of 12 randomized trials conducted by Chua, Schneider, and Bellomo (2011) demonstrated that bicarbonate infusion did not improve treatment outcomes in people with diabetic ketoacidosis.[83] Another acute complication of hyperglycaemia is the hyperglycaemic hyperosmolar state, and treatment involves insulin therapy and correction of fluid deficit.[82] Though ketoacidosis does occur in people with T2D, the more common presentation of acute hyperglycaemia is the hyperglycaemic hyperosmolar state. Diagnosis of this condition is when BGL is greater than 33.3 mmol/L with no metabolic acidosis or ketonemia, and the mortality rate is between 10-20%, or 10 times higher than the rate in people with diabetic ketoacidosis.[84] Stringent monitoring and regulation of BGL is important for prevention.

1.5.1.2 Hypoglycaemia

Just as acute complications can arise from BGL rising above the optimal range, complications can be caused by BGL falling below the optimal lower limit of 4.0 mmol/L. This is more common in cases of diabetes where there is a total dependence on insulin to be supplied exogenously, or externally.[85] Such cases are referred to as 'insulin-dependent diabetes', and do not necessarily refer to T1D, though most cases of T1D are insulin dependent, as well as the more severe cases of T2D. As a result of this trend, severe hypoglycaemia – defined as an event requiring third party assistance – occurs more commonly in T1D compared to T2D. The Global HAT study (n=27,585) reported that people with T1D experience hypoglycaemia at a rate of 73.3 events per year, compared to 19.3 per year for people with T2D. In terms of severe hypoglycaemia, people with T1D experience 4.9 events per year compared to 2.5 for insulin-treated T2D patients.[86]

People with insulin-dependent diabetes are educated on how to measure insulin doses and self-administer them to maintain their BGL in a euglycaemic, or optimal, range. However, due to the multitude of day-to-day factors which influence BGL, including exercise and food intake, this euglycaemic target can be difficult to maintain. It is commonplace for exogenous insulin to reduce BGL below the optimal range, causing hypoglycaemia. This is sometimes referred to as iatrogenic hypoglycaemia because it results from an error in medical treatment. Richard and colleagues (2019) cite three main causes of severe hypoglycaemia in diabetes as missed meals, incorrect use of antidiabetic medication, and mismatch between antidiabetic medication and carbohydrate intake.[87] This is reinforced by Tourkmani and colleagues (2018), who also add physical exercise and alcohol consumption as common causes.[88] Confusion, difficulty

concentrating, and dizziness are known neuroglycopenic symptoms of hypoglycaemia, and occur because glucose is the major source of energy for the brain. Sweating, heart palpitations, and trembling are the autonomic symptoms of hypoglycaemia, and usually occur before the onset of neuroglycopenic symptoms and can act as a 'warning system'.[89] Coma and death result from prolonged severe hypoglycaemia, and loss of consciousness may precede these at undefined levels of BGL.[90] As such, the literature advocates that people living with T1D be aware of the autonomic signs and symptoms of hypoglycaemia and be prepared with a plan of action,[88] for example having a snack containing 15g of carbohydrate and a protein source in between meals to prevent repeated hypoglycaemia.[85] The literature emphasizes the importance of optimizing glycaemia without compromising the safety of people with severe hypoglycaemic events, though this is a difficult balance to maintain.[91]

1.5.1.3 Impaired Awareness of Hypoglycaemia

Impaired awareness of hypoglycaemia, also known as hypoglycaemia unawareness, is a complication of diabetes that results from repeated iatrogenic hypoglycaemia. It involves reduced responsiveness of the autonomic nervous system to low BGL, resulting in a loss of the body's natural alarm system for hypoglycaemia.[92] People with this impaired awareness are six times more likely to experience severe hypoglycaemic events compared to those with optimal awareness.[93] In a study of n=98 young adults with T1D, conducted by Paes and colleagues (2020), hypoglycaemia unawareness was observed in 28% of the subjects.[94] In a cross-sectional study on T2D participants undergoing insulin therapy (n=2,350), Meijel and colleagues (2018) observed impaired awareness of hypoglycaemia in nearly 10% of the sample.[95] Evidently, this unawareness affects a significant proportion of people with diabetes, roughly one in four people with T1D, and roughly one in ten of those with T2D on insulin therapy. This is especially concerning when considering the threat level of hypoglycaemia. Frequent hypoglycaemia leading to hypoglycaemia unawareness is reversible in the short term through stringent avoidance of hypoglycaemia for 2-3 weeks.[92] However, as demonstrated in the HypoCOMPass trial (n=96), awareness of hypoglycaemia was restored after 24-weeks without compromising glycaemic target, and thus autonomic function may take up to 24 weeks or longer to be restored.[96]

One hypothesis is that iatrogenic hypoglycaemia, caused by pharmacotherapy and other factors, is a major cause of hypoglycaemia-associated autonomic failure. According to Cryer (1992), the reduced ability of the autonomic nervous system to activate or respond to critically low BGL reduces the ability of people who rely on autonomic symptoms to self-monitor their own BGL,

leading to recurrent severe hypoglycaemia.[97] Under ideal circumstances, people can recognize the onset of sweating and heart palpitations as early warning indicators of hypoglycaemia. The absence of these indicators creates a cycle where unmanaged hypoglycaemia predisposes one to subsequent instances of hypoglycaemia, further removing defenses against detecting it. This mechanism is still largely misunderstood, and a recent review published by Rickels (2019) determined that there was a lack of consensus on this pathological mechanism.[98] Though autonomic failure is involved in hypoglycaemia unawareness, it is unlikely to be related to cardiac autonomic neuropathy, a long-term complication of diabetes. It has been shown that only subject age and duration of diabetes is associated with hypoglycaemia unawareness, and cardiac autonomic neuropathy is not.[94] Research indicates that people commonly make errors when estimating BGL in very high ranges, such as above 22.0 mmol/L, or in the very low ranges, such as below 3.0 mmol/L, due to the presence of neuroglycopenic symptoms which affect concentration and alertness.[99] As such, self-diagnosis of low BGL can be unreliable, and future research may aim to improve this.

Evidently, the threat of BGL falling below the optimal lower limit is of great concern. A simple solution to this problem is to implement a system of continuous monitoring as part of standard diabetes management. In theory, continuous monitoring is currently the most reliable means of detecting hypoglycaemia early and facilitating self-intervention,[100] but in practice it is expensive and usually reserved for T1D. A more detailed discussion of the strengths and limitations of current glucose monitoring will be provided in a later section. It should be noted at this point that the literature advocates strongly for stringent monitoring of glycaemic events, as it is also associated with improved long-term outcomes,[101] which will be explored in Section 1.5.2.

1.5.2 Long-Term

In the long-term, unmanaged BGL can also lead to life-threatening conditions, as well as increased morbidity and mortality. For hypoglycaemia, the long-term consequences are poorly understood, and this an area of research that is severely lacking. Generally, the main long-term complications of hypoglycaemia are impaired awareness of hypoglycaemia and hypoglycaemia-associated autonomic failure, as well as predisposition to cardiovascular events.[102] Fortunately, almost all episodes of hypoglycaemia occur in people that are taking medication and are thus aware of the risk of iatrogenic hypoglycaemia, so there is increased opportunity to intervene.[87]

As for long-term complications of hyperglycaemia, there is an abundance of research in the literature, and it defines these complications as the result of various factors. Based on estimates provided by Beagley and colleagues (2014), 46% of all people living with diabetes worldwide

are undiagnosed or unaware of their condition, and are thus likely to be untreated cases.[103] The lack of diagnosis is due to progressive increase in BGL over time in people with T2D. As the condition progresses, the individual had an adaptive physiological response to the high levels of glucose which become the new 'normal' for them. As such, symptoms may be absent despite high BGL.[82]. Even in individuals with symptoms, medical intervention may not be a priority for symptoms such as increased thirst and hunger. However, sustained hyperglycaemia causes serious complications in the long term. Among those who are aware of their condition (56% of total diabetes cases worldwide), glycaemic management is better, but not optimal. In an Australian study, roughly half of a large population of diagnosed diabetes cases were unable to maintain their BGL at the optimal target level, such as through medications and lifestyle interventions.[104] The reasons for this will be provided in a later section. At this point, it is only important to note that long-term complications are common in diabetes and the main reason is because of persistent hyperglycaemia. Specifically, when hyperglycaemia remains at chronically high levels for longer than 2 years, causing long-term microvascular and macrovascular damage to the cardiovascular system, kidneys, nerves, and retinas (Figure 1.4). There is abundant evidence from a range of populations which show that duration of diabetes – or the length of time since diagnosis – is strongly correlated with complications of diabetes, and that this correlation with levels of HbA_{1c} is a measure of longer term glycemia.[105-107] The next section will describe these complications and their severity in more detail.

Figure 1.4 Summary of the short and long-term complications of diabetes and how they relate to diabetes awareness and long-term management.

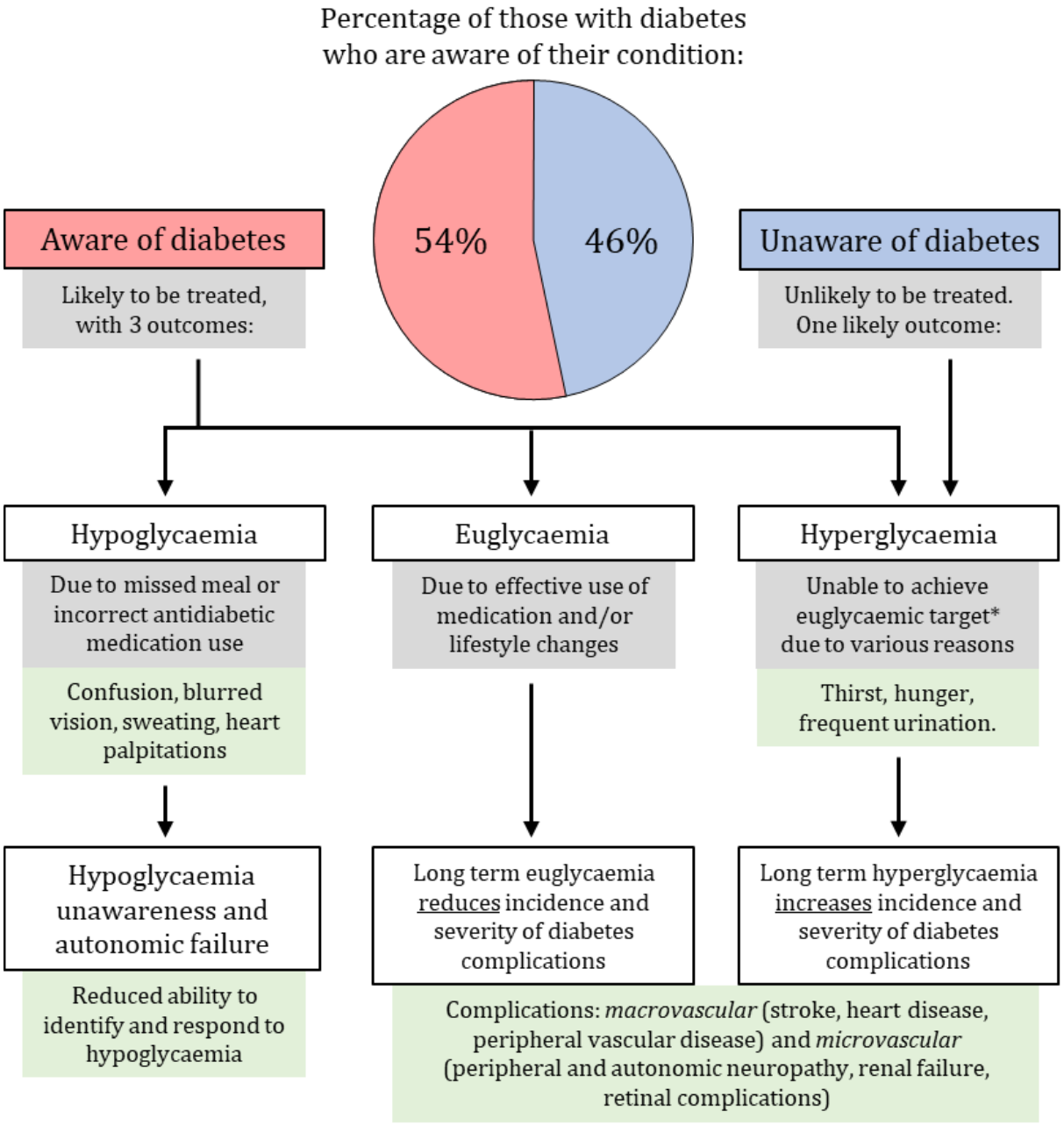

Figure 1.4 presents a flow-chart of how sustained glycaemia relates to prevalence of diabetes complications. An estimated 46% of all cases of diabetes worldwide affect people who are unaware of their condition. In these cases, treatment is unlikely. Without effective intervention, chronic hyperglycaemia, which is inherently part of diabetes, leads to serious, life-threatening diabetes complications. Of those who are aware, the effectiveness of their interventions is correlated with less severe complications. Those who are being treated with antidiabetic medication or glucose-lowering drugs in general should be aware of the risks of hypoglycaemia. *Euglycaemic target refers to HbA_{1c}, which is a biomarker for long-term glycaemia described in a later section. Image adapted from information presented in previous sections.

1.5.2.1 Macrovascular

Macrovascular complications affect arterioles, capillaries, and venules, which play an important role in supplying blood to organs. As such, the main macrovascular complications of diabetes are cardiovascular diseases, and include stroke, peripheral vascular disease, and coronary heart disease.[74] There is some debate over whether microvascular complications precede macrovascular complications in terms of pathogenesis and development, or whether they are distinct entities which progress separately.[108] Al-Wakeel and colleagues (2009) and Krentz and colleagues (2007) concluded that both types of complications develop simultaneously.[109, 110] It is also possible that macrovascular and microvascular complications develop simultaneously and independently, though more research is needed to clarify the interaction between risk-factors of diabetes and it's complications.

1.5.2.2 Microvascular

End-stage renal disease, retinopathy, and neuropathy are the common microvascular complications of diabetes.[111]. An estimated 80% of all end-stage renal disease cases are caused by diabetes or hypertension, and of those related to diabetes, 91% were due to T2D.[74] Figure 1.5 summarises the general process by which hyperglycaemia leads to tissue damage in certain organs. Pettus and colleagues (2019) determined that suboptimal glycaemia in T1D adults (n=31,430) was associated with higher prevalence of acute complications, such as severe hypoglycaemia and diabetic ketoacidosis, as well as chronic complications, such as neuropathy and nephropathy.[112] Brownlee (2005) has suggested that certain cells in the renal nephrons, eyes, and neurons of the autonomic nervous system cannot regulate their internal glucose concentrations efficiently, and thus are susceptible to damage from hyperglycaemia.[113] Consequently, the progression of diabetes is associated with a decline in autonomic modulation of the heart and cardiovascular disease.[114-116] Clinical research has shown that autonomic function is significantly diminished in diabetes compared to people without diabetes.[117] There is widespread acceptance that chronic hyperglycaemia causes irreparable damage to nerves of the autonomic nervous system, and diabetes is the most common cause of neuropathy.[118]

Figure 1.5 Flow chart for the pathogenesis of tissue damage in chronic hyperglycaemia.

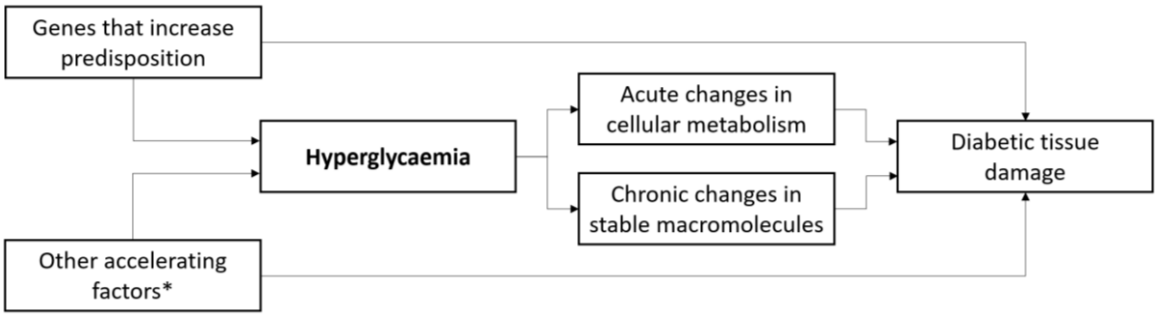

Figure 1.5 outlines the process of hyperglycaemia-induced tissue damage. There is a strong genetic component to diabetes, which contributes to hyperglycaemia as well as cellular damage. Hyperglycaemia is associated with changes in cellular metabolism and structure. *Other accelerating factors include hypertension and hyperlipidaemia, which act independently to exacerbate hyperglycaemia and accelerate tissue damage. Adapted from Brownlee (2005).[113]

1.5.2.3 Diabetic Autonomic Neuropathy

As with most adverse complications of diabetes, diabetic autonomic neuropathy is more common with chronic hyperglycaemia, and there is a strong agreement in the literature that duration of diabetes is correlated with increased risk for diabetic autonomic neuropathy.[107, 119] This reinforces the current understanding that untreated hyperglycaemia accelerates tissue damage in diabetes. Degeneration of the autonomic nervous system is caused by the accumulation of oxidising agents which compromise vascular supply to the nerves.[120] Consequently, diabetic autonomic neuropathy is associated with the development of diabetic foot disease, ulcers, and vascular disease,[121, 122] and longitudinal research published by Morbach and colleagues (2012) reports that the prognosis of subjects with foot ulcers is especially poor.[123] In their literature review, Vinik, Erbas, and Casellini consider diabetic autonomic neuropathy to be one of the least understood complications of diabetes.[115] One of the problems that has been identified in the treatment of diabetic autonomic neuropathy is the lack of simple methods for evaluating autonomic function.[124] Based on the MONA LISA Hypothesis (Most Obesities k/Nown Are Low In Sympathetic Activity), the measurement of sympathetic activity – a component of the autonomic nervous system that can be partially measured by low frequency heart rate variability – may be useful in monitoring the progression of complications.[125] Lagani and colleagues (2018) advocate for the development of prognostic tools, such as models which include clinical data on age, sex, smoking habits, blood pressure and HbA_{1c}, as these are strongly correlated with diabetes complications.[126] Hyperglycaemia is a leading cause of diabetic autonomic neuropathy, and people with diabetes who are affected by diabetic autonomic neuropathy have increased mortality.[127, 128]

1.5.3 Neurophysiology of Blood Glucose Level and Autonomic Activity

The autonomic nervous system modulates glucose metabolism and BGL through its two counter regulatory branches.[129] The sympathetic nervous system acts to increase BGL through direct innervation of the liver and skeletal muscles to increase glucose production, as well as through activating the adrenal medulla and pancreas to produce epinephrine and glucagon, respectively.[129] During exercise or exposure to cold, the sympathetic nervous system may also be involved in increasing glucose uptake by skeletal muscles, despite unchanged BGL.[130] This is likely related to survival mechanisms, rather than conventional glucose homeostasis. The autonomic nervous system can also directly innervate the liver in response to pancreatic glucagon or insulin release, and the effects of direct sympathetic neural innervation of the liver are stronger compared to parasympathetic innervation.[131] The parasympathetic nervous system effectively decreases BGL by increasing insulin release from the pancreas, as well as by directly innervating the liver.[132] Evidently, autonomic innervation of endocrine organs is an important component of glucose regulation. Activation of the vagus nerve, the main parasympathetic nerve, leads to increased gastric acid secretion to facilitate digestion, and also leads to decreased heart rate, a staple of parasympathetic control.[129] Insulin and glucagon, as well as epinephrine and norepinephrine, cause neural activation of the liver. Epinephrine is released by the adrenal medulla and the sympathetic nerve terminals, and rapidly increases BGL by promoting liver glucose production and inhibiting insulin-mediated glucose uptake in skeletal muscles. By comparison, norepinephrine contributes little to liver glucose production, but is involved in glucose uptake and use in skeletal muscles independently of insulin.[132]

Over the past 20 years, there has been a shift in the fundamental ways in which diabetes affects quality of life. Mortality rates for people with diabetes are declining, and there have been decreases in the incidence of classic complications such as cardiovascular diseases and lower limb amputation.[133] Current understandings of the underlying mechanisms of diabetes complications are insufficient, and future research should consider how this affects the development of better therapeutic options and treatment outcomes.[134] Based on the information provided in this section, improving access to technologies that can evaluate autonomic function in everyday clinical practice may be invaluable in the early detection, and thus early intervention, of diabetic tissue damage, as the peripheral nervous system is an early target of hyperglycaemic damage.[135] The role of the autonomic nervous system in glucose regulation is an important component of this PhD candidature, and will be discussed in a later section. Now that the nature and severity of diabetes complications have been established, it is clear these represent a significant burden to both individuals and national health systems.

1.6 Importance of Glycaemic Management

There is a strong consensus in the literature that stringent management of BGL, which involves maintaining BGL within the target glycaemic range, significantly reduces the risk and severity of diabetes complications.[136-138] This has been reinforced by the ADVANCE trial[139] (n=11,140) and the follow-up study of the Veterans Affairs Diabetes Trial[140] (n=1,791), both of which demonstrated a reduction in microvascular and macrovascular complications of diabetes in groups randomly assigned to intensive glucose management compared to less intensive. Large-scale longitudinal studies have reinforced this understanding as well. In the United Kingdom Prospective Diabetes Study (n= 4,209), T2D subjects undergoing intensive insulin therapy to manage their BGL over 10 years showed a 24% reduction in microvascular disease, a 15% reduction in myocardial infarction, and a 13% reduction in all-cause mortality compared to the group treated with a simple dietary restriction.[141] The results from the Diabetes Control and Complications Trial and its long-term observational follow-up, the Epidemiology of Diabetes Interventions and Complications study (n=1,441), also demonstrated that stringent glycaemic management, facilitated by intensive insulin therapy and vigilant glucose monitoring, substantially reduce the long-term complications of diabetes in people living with T1D.[142]

1.6.1 Glycaemic Variability

There is growing recognition that glycaemic variability, attributed to large, acute fluctuations in BGL, such as those associated with glucose spikes after a meal, is also relevant in the pathogenesis of autonomic complications. Glycaemic variability indicates the presence of glycaemic excursions,[143] and BGL falling outside the optimal limits leads to complications in the short and long term, as discussed. As a therapeutic target, it is under-recognized, and recent literature reviews advocate for the importance of targeting glycaemic variability as a means of reducing the risk of complications of diabetes.[144] In the Verona Diabetes Study, higher variability in long term fasting BGL was shown to be associated with high total mortality, as well as higher mortality from cardiovascular disease and cancer.[145] Greater glycaemic variability is associated with lower autonomic function in T2D, and stringent glucose monitoring and intervention is crucial in reducing glycaemic variaiblity.[146] Nusca and colleagues (2018) contend that the two major aspects of glycaemic variability are as follows: the magnitude of glucose fluctuations, including the height from the nadir to the peak; and the length of time between glucose fluctuations. Both are important in glycaemic variability, which is associated with coronary heart disease and other vascular complications.[147] Damage from glycaemic variability occurs at a cellular level, and there are several pathways in which this is theorized to occur.

1.6.2 Oxidative Stress and Hyperglycaemia

A popular long-held theory is that complications of diabetes, particularly neuropathy, are the result of an increase in oxidative stress.[148] To elaborate, there are four key processes which link hyperglycaemia and neuropathy in diabetes, and they all involve excess production of reactive oxygen species (ROS) by the electron transport chain in the mitochondria. The electron transport chain is a major source of ROS, including superoxide, hydroxyl radical, and hydrogen peroxide, and these arise from oxidative phosphorylation, glucose autoxidation, and multiple enzymatic processes, which are all heightened in hyperglycaemia.[149] This is because high BGL leads to glucose molecules accumulating in the cells of the muscles, adipose, and pancreas, where these cellular processes which produce ROS are increased. ROS are likely helpful in cell signaling, acting as second messengers in intracellular signaling pathways. However, in excess, these cause damage to the membranes, protein structure, lipids, and DNA of cells, and thus contribute to oxidative stress.[149] As such, chronic hyperglycaemia leads to degeneration of autonomic nerves by way of affecting the biochemistry of free radicals.

Oxidative stress causes harm to many types of cells, however, there are certain types of cells which show signs of damage sooner than others in response to chronic hyperglycaemic conditions. Clinical studies have shown that parasympathetic neuropathy can have a relatively early onset in T2D.[150] It has been proposed that increased oxidative stress, caused by hyperglycaemia, affects the parasympathetic nerves first because their myelinated preganglionic fibres are longer and thicker compared to sympathetic nerves.[151] This is related to the work of Ewing and Clarke (1985) who proposed a battery of five non-invasive reflex tests for early detection of autonomic neuropathy in diabetes. Since parasympathetic neuropathy occurs early in T2D, Ewing and Clarke (1985) proposed the 'Ewing battery' which includes the Valsalva manoeuvre, lying to standing heart rate response, deep breathing heart rate response, postural blood pressure change, and sustained handgrip test.[152] Worsening of autonomic function can be detected using the Ewing battery, a gold-standard in clinical practice, and early detection is important in diabetes as autonomic neuropathy is linked to myocardial ischemia, coronary artery disease, and stroke.[153]

Autonomic neuropathy in diabetes also presents low-grade chronic inflammation. Cytokines such as interleukin-1, 6, and 8, as well as C-reactive protein, TNF-α, and monocyte chemoattractant protein-1, contribute to inflammatory reactions.[154] These are produced by activated immune cells and resident macrophages and adipocytes, and play a key role in inflammatory signalling. Activation of receptor-mediated inflammatory signalling leads to increased oxidative stress, which as discussed contributes to degeneration of autonomic nerves, particular those of the parasympathetic nervous system.[155] Several therapeutic drugs have

demonstrated reductions in microvascular complications in T2D by reducing inflammation. For example, melatonin reduces elevated levels of proinflammatory cytokines and increases blood circulation to the nerves.[156] Targeting inflammation signalling in chronic hyperglycaemia has important clinical implications for reducing the risk and severity of autonomic neuropathy. Given the background of diabetes complications and the cellular processes involved, it is clear that stringent glycaemic management is a priority in reducing the current cost of diabetes on individuals and society. Now that it is clear why glycaemic management is important, the next sections will explain how this is possible and discuss current limitations.

1.6.3 Treatment

Adopting the best course of treatment of diabetes is complicated by a range of factors. Primarily, therapies will differ based on the type of diabetes, as pathological differences between T1D and T2D lead to profound differences in treatment.[46] The severity of the diabetes as well as other comorbidities are also key factors to consider. Although therapeutic strategies differ significantly, T1D and T2D are both characterised by high levels of blood glucose that cannot be controlled by the body's own insulin. Left untreated, chronic hyperglycaemia leads to debilitating, life-threatening complications in the long-term. As such, it is possible to generalize the main objective of diabetes treatment as such: maintain target glycaemia by use of antidiabetic mediation in combination with lifestyle modification.[157] The extent of diet and exercise changes, as well as the nature of the pharmacotherapy, can differ significantly among people with diabetes.

Most cells in the human body require insulin signalling to take in glucose from the blood. In conditions defined by insulin dysfunction, such as diabetes, the lack of signalling causes glucose to reach high levels in the blood.[158] Therefore, pharmaceutical insulin therapy is a common method for supplying exogenous insulin to people living with diabetes who cannot use or produce their own. This usually applies to those living with T1D, as insulin production is limited, but it may also apply to end-stage T2D where insulin resistance has progressively worsened, and pancreatic insulin supply may also be limited. According to Riddle and colleagues in their 2021 consensus report, pharmaceutical therapies targeting glycaemic management in diabetes have improved greatly in recent years.[159] This section aims to describe the role of medications in dealing with dysglycaemia (Figure 1.6), as well as explain why insulin therapy is important in diabetes management.

Figure 1.6 Anatomical structures involved in glucose, insulin, and glucagon regulation.

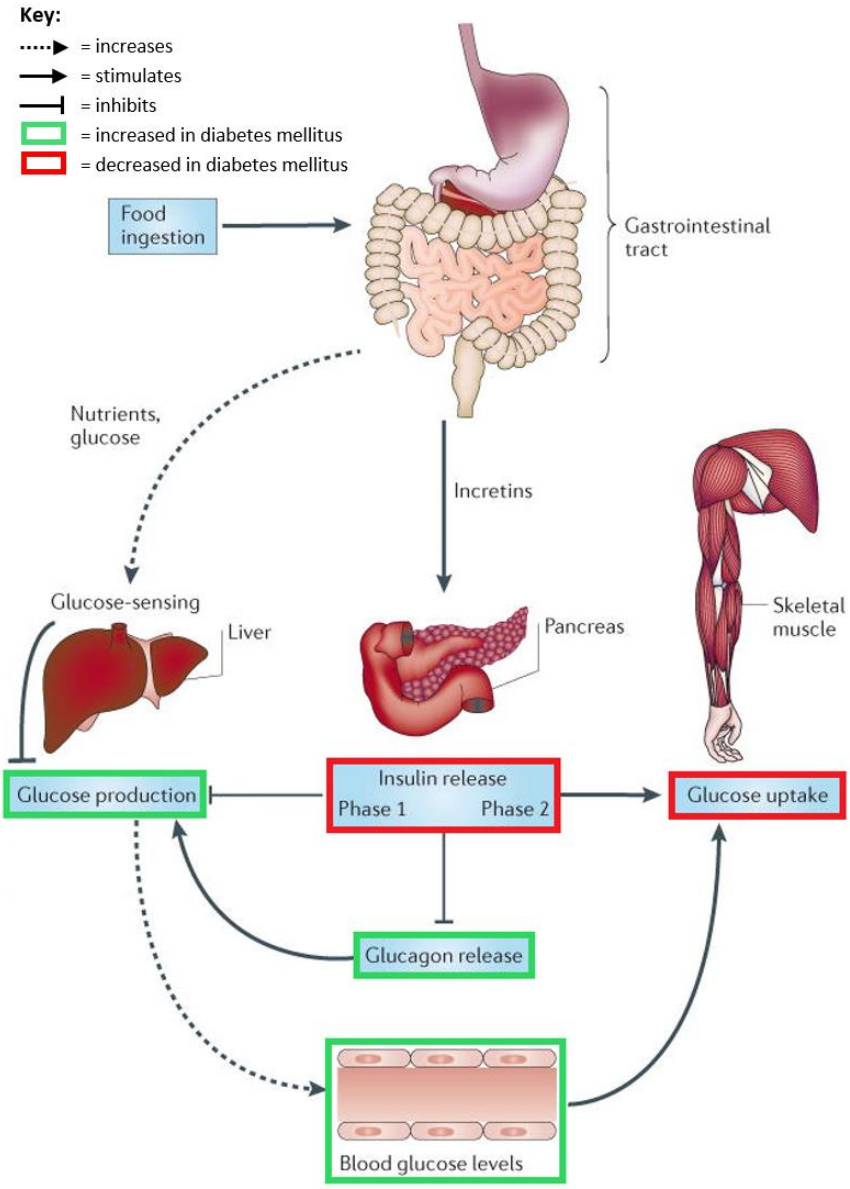

Figure 1.6 illustrates the production, regulation, and role of insulin in maintaining glucose homeostasis. Following the absorption of glucose-containing nutrients in the gastrointestinal tract, glucose sensors in the liver detect an increase in blood glucose concentration. In a person without diabetes, this is followed by the release of insulin from the pancreas to promote glucose uptake by somatic cells, such as those in skeletal muscle, and thus reduce blood glucose to an optimal level. In diabetes, reduced insulin activity causes glucose levels to rise, without a mechanism for maintaining homeostasis. Adapted from Grayson, Seeley & Sandoval (2013).[48]

1.6.3.1 Type 1 Diabetes Treatment

As T1D results in a total or near-total deficit of insulin, the condition is managed with insulin therapy paired with frequent monitoring of BGL to remain vigilant of hypoglycaemia.[160] Insulin therapy can be achieved with 3-4 injections of basal (slow-acting) and prandial (fast-acting) insulin per day, or continuous insulin infusion.[161] Automated insulin delivery, combined with currently available CGM, significantly improves the length of time people with T1D can have target glycaemia. With T1D, complete remission is possible through pancreas transplant, though novel research is also exploring the potential for gene therapy to restore insulin production in T1D and result in remission.[162] Regardless of the method, the main objective of T1D treatment is clear: maintain BGL in the optimal range for as long as possible and reduce glycaemic variability. Among common diabetes treatment strategies, more stringent methods are correlated with improved glycaemic management; however, based on a meta-analysis of 37 articles, there is little data on whether engagement with treatment improves quality of life in diabetes.[163] Future research may need to consider how current treatment methods do not necessarily lead to better quality of life.

In T1D, the effects of a pancreas transplant on long-term complications is an area of research that is lacking, though available literature suggests that a transplant is effective in restoring euglycaemia in T1D and improving quality of life.[164] However, the availability of donors and the lifelong immunosuppression therapy are major factors which limit the viability of transplant surgery for most people living with T1D.[165] Surgery in general is invasive and is generally not applicable in most cases. There is growing interest in islet cell transplant and stem cell generation of islet cells prior to gene therapy, though currently this is not used in clinical settings. Transplanting islet cells has shown improvements in insulin independence up to five years following the transplant, with minimal complications.[166] 20-year follow-up data is available for a cohort of n=29 females and n=20 males.[167] At the end of the follow-up, from the year 2000 to 2020, 86% of the subjects had no albuminuria, 12% had microalbuminuria, and 2% had macroalbuminuria. Two subjects (4.08%) died during the follow-up period. Since pancreas transplantation has been in clinical use for longer, there is 30-year follow-up data for subjects. In a cohort of n=2,796 subjects with T1D, simultaneous pancreas-kidney transplant improved survival and resulted in an almost twofold lower 10-year mortality rate.[168] Data from the International Pancreas Transplant Registry of n=18,159 pancreas transplants demonstrated that simultaneous pancreas-kidney transplanting is superior to pancreas transplants alone in terms of survival. At 5-year, 10-year, and 20-year follow-up, survival rate was 80%, 68%, and 45%, respectively, for simultaneous pancreas-kidney transplants versus 59%, 39% and 12%, respectively, for pancreas transplants alone.[169]

Transplants restore euglycemia in people with T1D, and in turn these reduce the progression of diabetes complications, such as retinopathy. However, large epidemiological data has shown that early metabolic control is critical in the overall development of complications of diabetes, despite the short-term benefits provided by intensive glycaemic control. This is hypothesised to be due to 'metabolic memory', a phenomenon where cellular pathways, such as excessive cellular ROS at the level of glycated-mitochondrial proteins, maintain stress signalling and contribute to long-term diabetes complications.[170] Metabolic memory is one issue which has been used to justify the importance of early intervention and prevention of diabetes, as early control is critical in preventing the progression of complications.

1.6.3.2 Type 2 Diabetes Treatment

Conversely, management of T2D typically involves improving physical activity and diet in combination with pharmacotherapy.[67] Lifestyle changes include: daily physical activity;[171] dietary changes and restrictions,[172] such as minimising processed foods and high energy density foods;[173] and reducing alcohol consumption and smoking.[174] The objective of these lifestyle interventions is to improve glycaemic management in the short term and restore insulin sensitivity in the long term, which in theory can result in remission from T2D.[175] Such treatment is considerably less invasive and more affordable compared to surgery, though it is important to note that sustained remission of T2D is very rare, even with intensive lifestyle and pharmaceutical intervention,[176] though it is more common with surgery.[177] Remission is described as achieving target glycaemia at least three months after ceasing antidiabetic medication, as the person's natural homeostatic processes have been restored and optimal glycaemia can be achieved without pharmacotherapy.[159]

Lifestyle interventions are often difficult to maintain, and weight that is initially lost can be regained over time as people with T2D age. As such, pharmacotherapy is considered a first-line therapy, and some drugs are even used in the prevention of T2D. A common therapeutic agent in the treatment of prediabetes and T2D is metformin, an oral glucose-lowering drug which helps to draw glucose out of the bloods by enhancing insulin action in the liver and skeletal muscles.[178] Metformin use is associated with reduced oxidative stress in T2D subjects compared with subjects treated with lifestyle interventions[179] or with sulfonylureas.[180] Albumin in the blood is an important antioxidant, and it has been proposed that an added benefit of metformin use is the protection it provides to serum albumin. By this process, people with T2D treated with metformin show reduced oxidative stress and glycation.[180] Metformin use also provides sustained cardiovascular benefits, which are unlikely related to its glucose-lowering effects

since this sustained benefit has not been observed with sulfonylureas or insulin therapy.[181] Clinical research has suggested that metformin may improve sympathovagal balance in T2D.[182]

For most cases of T2D, maintaining euglycaemia – or optimal levels of BGL – involves medication and lifestyle interventions. However, there is data which supports more non-conventional options. According to a meta-analysis of 10 studies (n=17,532) conducted by Billeter and colleagues (2018), metabolic surgery such as bariatric surgery prevents microvascular complications to a better extent in T2D than treatment by medication, which includes insulin therapy and glucose-lowering medications.[183] Chang and colleagues (2014) confirm in their meta-analysis and review (n=161,756) that bariatric surgery works to slow or even reverse the progression of T2D by restricting hunger, and thus also caloric intake.[184] However, as with any surgery the invasive nature of bariatric surgery provides a risk for serious complications, limiting the application of this treatment.

Insulin therapy is required when non-insulin therapy, such as metformin or other glucose-lowering drugs, is unable to maintain glucose homeostasis and euglycaemia.[185] In T2D, multiple drugs may be used to achieve euglycemia, and insulin can be added on to multiple oral therapies, and in T1D treatment is typically just insulin therapy. In a person without diabetes, homeostasis is the process by which BGL is maintained within a narrow range (Figure 1.7), between 4.0 and 7.7 mmol/L.[186] As with other homeostatic systems, such as core body temperature, BGL must be maintained within its optimal range to ensure an individual's health.[187] Intensive insulin therapy reduces the risk of complications in the long-term, but researchers warn that the risks of severe hypoglycaemia in the short-term may outweigh this.[188] In insulin-dependent diabetes, it is important to consider diet and medication guidelines set by the physician to reduce the incidence of hypoglycaemia, as glucose monitoring systems cannot always be relied on for the detection of hypoglycaemia. The reasons for this unreliability will be explored in depth in a later section which discusses the applications of glucose monitoring.

Figure 1.7 Hormonal regulation of blood glucose level in people without diabetes.

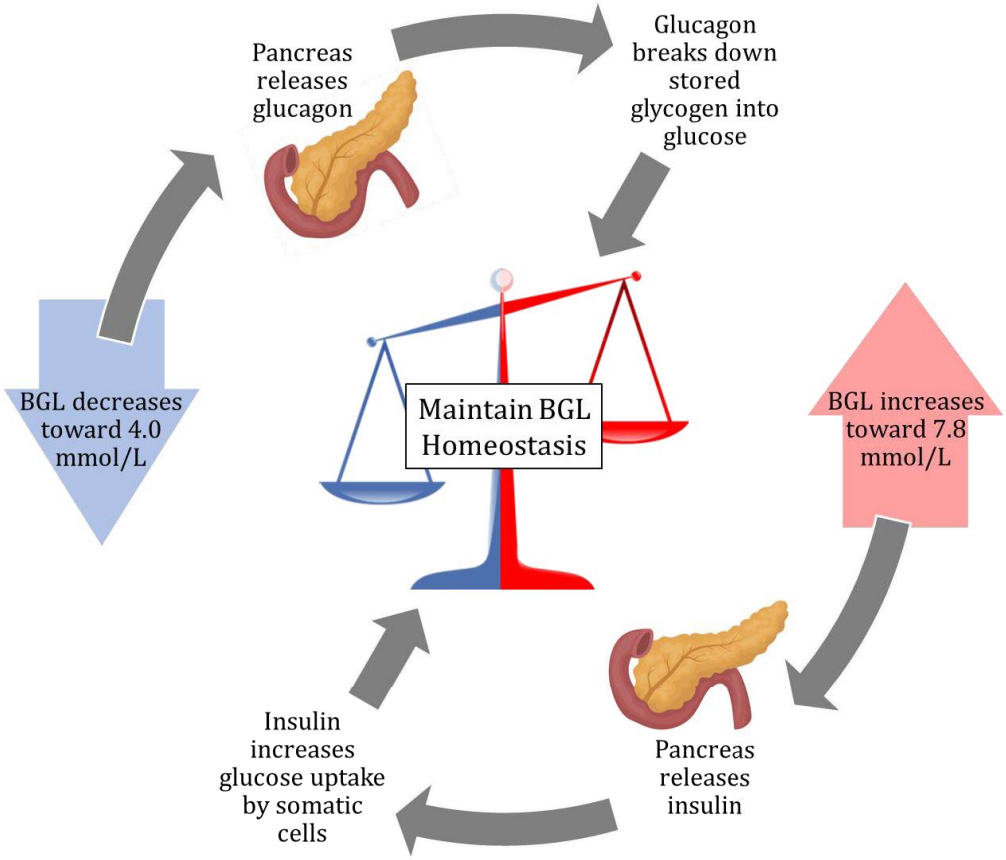

Figure 1.7 portrays the role of glucagon and insulin in maintaining glucose levels within a optimal range. In a fasting state, where nutritional sources of glucose are restricted for at least eight hours, homeostatic processes seek to maintain an individual's BGL above 4.0 mmol/L through the breakdown of stored glycogen in the muscles and liver into glucose. BGL is maintained below 7.8 mmol/L during any metabolic state by releasing insulin, which increases the uptake of glucose by somatic cells. **BGL** = Blood glucose level. **mmol/L** = Millimoles per litre. Adapted from Roder and colleagues (2016).[189]

To summarise, a dysfunction in the role of insulin reduces the ability of people with diabetes to optimise their BGL when it rises above 7.7 mmol/L. Treatment of diabetes revolves heavily around an individual's metabolic state, such as whether they are fasting, eating, exercising, or at rest, to name a few. Some examples of this have been discussed already, such as the need to take antidiabetic medications before consuming a meal to reduce the inevitable postprandial glucose spike. This requirement also aims to reinforce a routine with those taking antidiabetic medication, as research from Vervloet and colleagues (2013) demonstrated that disruptions to a regular routine can be detrimental to overall treatment efficacy.[190] As diabetes management is heavily focused on meeting glycaemic targets and reducing glycaemic variability, metabolic state is an important factor when deciding when to take medications and when to self-measure BGL. To elaborate, measuring BGL in the morning before breakfast is an example of 'fasting

BGL', as the absence of food or medications in the last eight hours results in a glucose baseline.[189] The importance of fasting and postprandial BGL are highlighted in the literature as they are key indicators of health. For example, fasting BGL is useful for diagnosing diabetes and monitoring its progression, and postprandial BGL is predictive of cardiovascular complications in diabetes.[191-193] The ability to measure BGL in different metabolic states may to relevant to the development of future measures of BGL, and thus is relevant to this thesis. This will also be relevant to the methodology chapters, where it will be discussed how BGL can be measured in fasting and postprandial states.

1.6.4 Homeostasis

Consider that with any physiological system, there is significant minute-to-minute variation due to ongoing homeostatic processes. In the case of glucose homeostasis, BGL fluctuates in response to metabolic demands, such as the change between exercising and being at rest, as well as in response to the digestion of energy sources after consuming a meal.[194] These are just two of the main pressures which cause BGL to fluctuate across the day. In the absence of these – a state which is referred to as 'fasting' – BGL remains very stable in a person without diabetes: between 4.0 and 5.5 mmol/L.[189] Following the consumption of a meal, an individual would expect their BGL to fluctuate between 5.6 and 7.7 mmol/L. Therefore, individuals who present with a fasting BGL greater than 5.5 mmol/L or a postprandial BGL greater than 7.7 mmol/L are classified as having impaired glycaemia and may have diabetes or pre-diabetes (refer to Table 1.1). Clinicians may also order a 'random' blood glucose assessment, where BGL is assessed at an undefined point where a person is not necessarily fasting nor postprandial. The objective of this is the determine whether a person's BGL is above 11.1 mmol/L, because BGL above this threshold at any point is indicative of diabetes and warrants further investigation with a glucose tolerance test. Current literature indicates that random BGL is difficult to interpret, as it may be affected by confounders such as recent food intake and medications,[194] and so for the purposes of this thesis the focus will be on BGL assessed in fasting or postprandial states.

1.6.5 Metabolic State

Though BGL is most often assessed in a fasting state in a clinical setting, there is a strong consensus in the literature that postprandial BGL is a better predictor of diabetes complications. Cavalot and colleagues (2006) contend that postprandial BGL, but not fasting BGL, predicts cardiovascular events in T2D, with stronger prediction in women compared to men.[195] Some

research suggests that fasting BGL is independently associated with cardiovascular disease risk, however this only holds true for groups in the 'high-optimal' range of BGL, between 5.3 and 5.6 mmol/L.[193] A more recent study conducted by Jingjing and colleagues (2017) found that postprandial BGL performed better in screening for coronary heart disease compared to fasting BGL and HbA_{1c}.[192] Clinical research has also shown that specifically targeting and lowering postprandial BGL in T2D, compared to fasting BGL, leads to better glycaemic management overall and better HbA_{1c}.[191] Clearly, postprandial BGL offers key insights into diabetes prognosis, and a convenient method of measuring postprandial BGL may provide meaningful information to people currently living with diabetes. To reiterate, the specifics of how fasting and postprandial BGL are measured will be described in the methodology chapters. As previous sections have explored in detail how diabetes can be treated by current standards, the next section will explore how diabetes – and blood glucose – can be monitored, as this is critical to making clinical decisions and improving treatment efficacy.

1.7 Invasive Glucose Monitoring: The Current Standard

Stringent monitoring of blood glucose is strongly advocated for people living with diabetes for several reasons. The first reason has been discussed throughout previous sections: stringent BGL monitoring is a requirement for the continued health and wellbeing of certain people living with diabetes (Section 1.5). This is most relevant for those with T1D or end-stage T2D on insulin therapy, as the reliance on external insulin is associated with increased risk for severe hypoglycaemic events. In these groups of people, glycaemic excursions occur often in the high range of BGL (hyperglycaemia) and in the low range (hypoglycaemia), and current guidelines recommend BGL be assessed 4-10 times per day.[196] Vigilant monitoring of BGL is therefore critical for detecting these glycaemic excursions early and intervening.[197] The literature advocates strongly that glucose monitoring be a priority even for those with early T2D and other non-severe forms of diabetes.[198] It is important that individuals take responsibility for monitoring their glycaemia, as diabetes is a chronic condition that affects everyone differently. Current literature advocates for frequent monitoring of blood glucose and proactive intervention to achieve target glycaemia and improve quality of life in both the short-term and long-term.[136-138]

Technologies used to monitor glucose levels can be categorised by the frequency in which they assess glucose levels. Intermittent methods are characterised by their ability to only provide 'snapshot' data, such as a blood glucose assessment which directly measures BGL at a specific point in time, and continuous methods offer constant monitoring of BGL, which may be useful

for vigilant monitoring of potential hypoglycaemia.[199] Both technologies fill different roles and have their own uses in T1D and T2D, and each have different advantages and disadvantages. As a rule, there is a trade-off between accuracy and frequency when it comes to current methods of monitoring of BGL. For example, technologies that are more accurate are impractical to use frequently,[200] and technologies that measure glucose levels frequently, such as continuous systems, are less accurate, as they measure interstitial glucose instead of blood glucose.[201] However, continuous monitoring systems have the advantage of being more useful for detecting hypoglycaemic events, as they may occur unexpectantly and require constant monitoring.

Intermittent measures of BGL are praised in the literature for their accuracy but criticised for their invasive nature.[202] They are defined as 'intermittent' because they provide only a snapshot measure of BGL and cannot provide information on BGL over long periods of time. Though it is possible to take many intermittent measurements in quick succession, allowing for changes in BGL data to be tracked similarly to continuous measures, this is impractical due to the invasive nature of these measures. This will become clearer in the following sections, as the nature and role of these intermittent technologies is discussed. There are a range of devices capable of measuring or estimating BGL. The following sections will focus on those which are most used, and which have the most significant clinical significance at present.

1.7.1 Blood Glucose Assessment

A core feature of many chronic conditions that do not require constant hospitalisation or care is that there is a heavy reliance on the individual to self-monitor their condition. In the case of T1D and T2D, this involves self-administering prescribed medication, adopting lifestyle recommendations set by their primary care physician, and self-monitoring of blood glucose (SMBG). However, the ability of a person to monitor their own BGL is based on the strengths and limitations of the blood glucose monitoring tool, as well as how well they understand the tool itself. Therefore, tools that are more convenient or easier to can be more effective compared with tools which require more education for effective use.

According to WHO guidelines, SMBG is synonymous with the blood glucose assessment – the most commonly-used intermittent measure of BGL.[203] SMBG describes the routine of performing blood glucose assessments at specific intervals in one's own home, such as before or after meals, and using the information to adjust their medication or lifestyle in conjunction with advice from their primary care physician. The blood glucose assessment requires a glucose meter, or glucometer, to assess a small sample of blood typically drawn from the finger. As such, it is often referred to as the 'fingerspot' check. Capillary BGL measured at the fingertip

correlates strongly with BGL measured in the arteries.[199] BGL is recorded by the glucometer in millimoles per litre, and the optimal ranges are dependent on the metabolic state of the individual when they perform the blood glucose assessment. For example, fasting BGL ranges between 4.0 and 5.5 mmol/L in people without diabetes, and is above 7.0 mmol/L in people with diabetes before taking medication.[47]

In n=30 non-insulin-treated T2D subjects, SMBG led to significant improvements in HbA_{1c} compared to the control group, and was an effective tool for determining antidiabetic drug dosage.[204] Importantly, the authors of this study confirmed that detailed training was not required for subjects with suboptimal glycaemic to interpret the results of SMBG and adjust their drug treatment. Per the 2011 Consensus Report from the Coalition for Clinical Research – SMBG Scientific Board, SMBG is most effective when subjects can use it as a tool to guide their own treatment, and both patients and health care providers should be educated on how to interpret and respond to information provided from SMBG.[205] Amongst the board members, there was also a consensus that the non-insulin-treated T2D trials support the efficacy of SMBG, and that further studies are required to assess the cost-benefits of SMBG across different end points, for example not limited to HbA_{1c}.

1.7.1.1 Strengths

The blood glucose assessment may be performed in a clinical setting by physicians as part of a routine check-up, similar to blood pressure monitoring, though it is more commonly used in SMBG as the primary home-monitoring tool.[202] Studies from Halldorsdottir and colleagues (2013) and Tack and colleagues (2012) indicate that glucometers are performing accurately in the context of diabetes management,[206, 207] and there are a wide range of affordable and cost-effective glucometers convenient for at-home SMBG.[208] Though there are minor variations in SMBG approaches in the literature, SMBG almost always involves a glucometer and blood glucose assessment, and there are no significant clinical differences between the different SMBG approaches in T2D (n=453) according to a randomised trial conducted by Farmer and colleagues (2007). [209]

For glucose monitoring, the complexity of the diabetes is related to how often BGL needs to be monitored. For example, in cases of insulin-dependent diabetes, which includes most cases of T1D as well as cases of end-stage T2D, the individual's health and wellbeing depends on their ability to supply themselves with insulin in the form of medication.[47] Dieting and exercise are insufficient as the primary means of lowering BGL to optimal levels. Allemann and colleagues (2009) advocate for stringent BGL monitoring in insulin-dependent diabetes, including blood

glucose assessments before and after meals, as well as continuous monitoring of BGL.[210] These tools empower insulin-dependent individuals with the means to self-monitor spikes in BGL after meals, as well as respond to hypoglycaemic events instigated by their medication to prevent hospitalisation. Nagelkerk, Reick, and Meengs (2006) claim that empowering people living with diabetes with the knowledge of specific diet strategies and plan of care are important for improving SMBG and overall glycaemia.[211] This may be related to an individual's tendency to "take what I think works for me", as it has been observed that a person's understanding of their diabetes treatment, including their knowledge of the benefits and risks, is positively correlated with how closely they follow that treatment.[212] Improving the accessibility and ease of use of current SMBG technologies may therefore also improve people's knowledge of their condition, including diabetes progression, glycaemic management, and treatment effectiveness.

1.7.1.2 Limitations

Though the blood glucose assessment is an accurate estimate of BGL, glucose concentration is only recorded at the one specific point in time.[28, 29] As a consequence, this recording can be significantly affected by recent food ingestion or recent exercise, as well as acute illness.[213] It is important to control for these confounding variables when assessing BGL using the blood glucose assessment, because the glucometer will provide only a single measurement, and it is impractical to perform additional assessments in a short period of time.[214] A common method of controlling for these confounders is for subjects to refrain from any food or drink, except water, in the eight hours leading up to their appointment. This allows for the blood glucose assessment to record a fasting BGL. Clinical research has shown that SMBG is an effective tool on its own and can improve glycaemia in diabetes without intensive drug treatment.[215] Specifically, SMBG leads to better glycaemic management in the short term, but over time that management shows diminishing returns. To elaborate, a systematic review and meta-analysis found significant reductions in long-term glycaemia in people with T2D after 12 weeks and after 24 weeks of SMBG, but changes in long-term glycaemia were non-significant after 52 weeks.[216]

There is considerable variability in the guidelines of different international diabetes organizations regarding optimal SMBG and what it involves.[217] The lack of consistent guidelines may lead to confusion about the most optimal frequency and timing of SMBG. Additionally, some studies indicate that the cost-benefit analysis of SMBG is unfavourable. The results from a parallel group randomised controlled trial (n=453) suggest that SMBG after 12 months leads to increased healthcare costs and is unlikely to be cost-effective when used routinely.[218] This is ratified by a systematic review of the literature by Clar and colleagues (2010) which found that

SMBG is not cost-effective and only offers limited improvements in glycaemic influence in non-insulin dependent diabetes, including T2D treated with oral antidiabetic medication or diet alone.[219] However, a meta-analysis of seven randomised controlled trials found a significant decrease in overall glycaemia in response to SMBG, providing large-scale results which suggest that people who utilise SMBG have better glycaemia compared to those who do not.[220]

Clearly, there is value to SMBG. It is suggested that SMBG may lead to more cost-effective glycaemic management when it is cheaper, more convenient, and when it is supplemented with healthcare education.[219] Cost effectiveness is generally evaluated in the context of an individual's current glycemia. SMBG is important for those with highly-fluctuating glycaemia, such as people with T2D who take oral medications which cause hypoglycaemia, but has limited benefit when glycaemia does not fluctuate significantly or where HbA1c is <7.5%.[221] Where insulin treatment is involved, and an individual is more reliant on exogenous insulin, SMBG is cost effective and essential to managing the dosing of insulin.

1.7.2 Continuous Glucose Monitoring

Continuous glucose monitoring (CGM) describes any technology capable of assessing BGL in a constant manner without pause. This allows for changes in BGL to be tracked in real-time, as data is continuously transmitted from a sensor, which is directly inserted into the tissue of interest, to a receiver which can display data as a glucose level.[199] The major advantage of CGM is that life-threatening increases or decreases in BGL can be detected as long as the device is being worn, which can be all hours of the day if necessary. CGM systems with better alarm features, such as the Dexcom G6, are associated with superior avoidance and intervention of glycaemic events.[222] As discussed, CGM is strongly recommended in insulin-dependent diabetes, as the reliance on externally-delivered insulin increases the individual's risk of hypoglycaemia.[28] CGM systems measure glucose levels in real-time and can identify severely low blood glucose, which can occur unexpectantly or even when asleep, which is known as nocturnal hypoglycaemia.[223]

CGM systems – devices which incorporate CGM technology – allow people to monitor their BGL vigilantly for glycaemic events and intervene in a timely manner. The use of a CGM system is associated with more optimal glycaemia.[224] Most commercially-available CGM systems function by measuring interstitial glucose concentrations (Figure 1.8), which is the concentration of glucose surrounding cells.[225] In a steady state, levels of glucose in the interstitial space are strongly correlated with capillary and arterial glucose levels, however they lag behind arterial concentrations only when BGL is changing rapidly, such as after the consumption of a meal.[226]

Unlike the blood glucose assessment, which directly measures glucose concentrations from a fingerspot blood sample which is strongly correlated with arterial BGL, a CGM sensor relies on minimally-invasive means to estimate arterial BGL. Based on data from Medtronic MiniMed CGM system, one of the most common systems in use, interstitial glucose concentration estimates can lag behind BGLs by 4-10 minutes.[225]

Figure 1.8 Measurement of interstitial glucose concentration using a continuous glucose monitoring system.

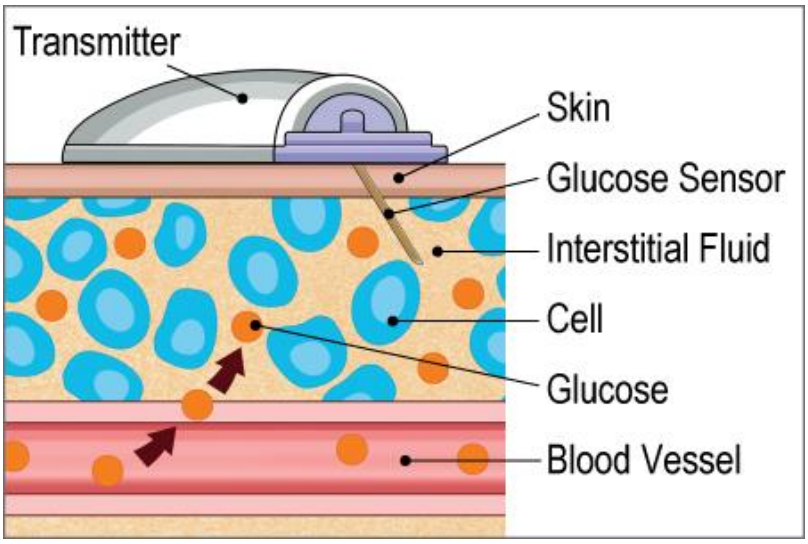

Figure 1.8 highlights the components involved in the continuous monitoring of interstitial glucose concentration, a close approximation of blood glucose concentration. The glucose sensor is inserted beneath the skin where it can contact the interstitial fluid. It then relays glucose data back to the continuous glucose monitoring system, which can process the data and report values digitally through an interface. Adapted from Medtronic MiniMed, Inc (2017).[227]

1.7.2.1 Strengths

A major strength of CGM is that is has been consistently shown to increase glycaemic management and quality of life in T1D[228] and T2D[229] with better outcomes seen in those who use CGM for at least 6 days per week.[230, 231] This is not specific to insulin-dependent diabetes, as researchers also advocate the use of CGM in early T2D groups that have not progressed to insulin therapy.[232] At present, blood glucose is monitored in T2D on a more intermittent basis because the threat posed by hypoglycaemia is much lower[233] and lifestyle-interventions take longer to become effective in achieving target levels of glycaemia.[67] Some justify the use of CGM in T2D because it is beneficial in reducing glycaemic variaiblity.[198] Studies have consistently shown that glycaemic variability is associated with dangerous microvascular complications, and this variability occurs in early T2D as well as end-stage.[234] Glucose variability it also associated with nocturnal hypoglycaemia episodes.[235] CGM can detect large variations in blood glucose and

empower individuals with the information to intervene.[236] This is not possible by measuring HbA_{1c} levels, as this only provides meaningful information about long-term trends in BGL, and the retrospective analysis does not allow for immediate intervention. Though convenient in some aspects, many SMBG tools used in T2D lack the ability to track daily glucose fluctuations, which may be more relevant in ideal management of diabetes.[237]

Many CGM systems are programmed to alert the wearer with an alarm when they detect glycaemic excursions. This allows for the wearer to intervene in a timely manner, and is especially useful in people with hypoglycaemia unawareness.[238] Few alternatives to CGM exist for insulin-dependent diabetes, as hypoglycaemia onset can occur quickly, and can cause confusion, drowsiness, and can lead to coma.[239] In T1D, CGM is often paired with SMBG, such as using blood glucose assessments to calibrate their CGM systems and to provide more accurate reference values when BGL is fluctuating rapidly after a meal. Some modern CGM systems in use, such as the Dexcom G5 and G6, are approved for non-adjunctive use, meaning they do not require calibration with routine blood glucose assessments.[240, 241] This removes one of the core problems with CGM and increases its viability for use in both T1D and T2D.

To summarise, a review of recent studies has shown that there has been steady improvement in the reliability of CGM systems, in that devices require less calibration and more accurately detect glycaemic excursions.[242] Moreover, many recent studies are attempting to address specific concerns with CGM. For example, the PRECISE Study[243] and the PRECISE II[244] trial demonstrated the use of a CGM system that is intended to be used with sensors that last for 90 days, to reduce the discomfort and pain associated with weekly sensor replacements. To summarise, CGM is ideal in both T1D and T2D, in people with and without insulin-dependence. This is because hypoglycaemia, which is more prevalent in insulin dependent diabetes, is not the only clinically significant complication that can be reduced with CGM. Glycaemic variability, caused by sudden spikes in BGL, can also be reduced with CGM, and this is responsible for significant risk of complications in all diabetes cases.[144] Clearly, the ability to constantly track BGL changes in real-time is a major strength.[238] However, for reasons that will be presented in the following section, modern CGM systems face many problems that make it difficult for people with diabetes and their primary care physicians to justify their use.

1.7.2.2 Limitations

CGM systems provide valuable data – irreplaceable in many circumstances – which forms the basis of many clinical decisions for people living with diabetes. However, concerns about the accuracy and reliability of CGM systems have been prominent since their introduction.[245]

Generally, performance of CGM systems is poor in the very low BGL range,[246, 247] raising concerns that hypoglycaemia may not be detected in time or at all in some cases. Two widely-used CGM systems show low accuracy in the hypoglycaemic range.[248] For these reasons, people with diabetes often cannot rely on readings from CGM systems alone when making treatment decisions, such as insulin dosage and timing, and may need to rely on glucometers (SMBG) because of their higher accuracy, even at very low ranges of glycaemia.[245, 249] Though some specific CGM systems do not require calibration with a blood glucose assessment, many people with T1D continue to utilise SMBG, or the intermittent blood glucose assessment method, routinely because of its superior accuracy.[238] For example, it can be used if a person is experiencing autonomic symptoms of early hypoglycaemia, such as sweating and heart palpitations, in circumstances where their CGM system has not yet detected the glycaemic excursion due to lag-time.[136] The concept of this 'lag-time' is illustrated in Figure 1.9. Severe hypoglycaemia represents a significant threat to those with insulin-dependent diabetes, and timely intervention of these glycaemic events is key to improving quality of life and preventing subsequent hypoglycaemic events.

Though there have been major improvements in the reliability and accuracy of CGM systems in recent years,[250, 251] the core problems of affordability and convenience have yet to be solved by any current CGM system. The issue of affordability is prevalent across most commercial CGM systems. CGM systems have a high upfront cost and maintenance costs, and the question of cost-effectiveness is difficult to answer when the benefit of using a CGM system is potentially lifesaving, depending on the severity of diabetes. Generally, though, the literature is in consensus that CGM is cost effective relative to SMBG and other health interventions.[252] Mean total lifetime costs of running a Dexcom G6, for example, are an estimated 18% higher compared to SMBG, and the mean quality-adjusted life years are 16% higher compared to SMBG, so there is an approximately equal value to the higher cost of using a CGM system.[253] This cost analysis is largely dependent on the severity of the insulin-dependence, and differs greatly between individuals. The results of the DIAMOND randomized trial show that CGM is cost-effective below a certain threshold, depending on the price an individual is willing or able to pay for their healthcare, with increased glycaemic management and reduced hypoglycaemic events.[254] This is ratified by other studies which have concluded that CGM systems are cost effective in people living with T1D,[255] and also people with T2D who do not use insulin,[256] but people must be able to pay the initial out-of-pocket expenses to achieve the better quality of life associated with long-term CGM use. However, many individuals, particularly those in developing nations, may simply be unable to afford the high initial cost of the CGM system, as

well as the maintenance costs such as replacing sensors, even if it does provide superior quality of life.

Figure 1.9 Line graph comparing the glucose levels recorded by a continuous glucose monitoring system, a glucometer, and a laboratory glucose analyzer.

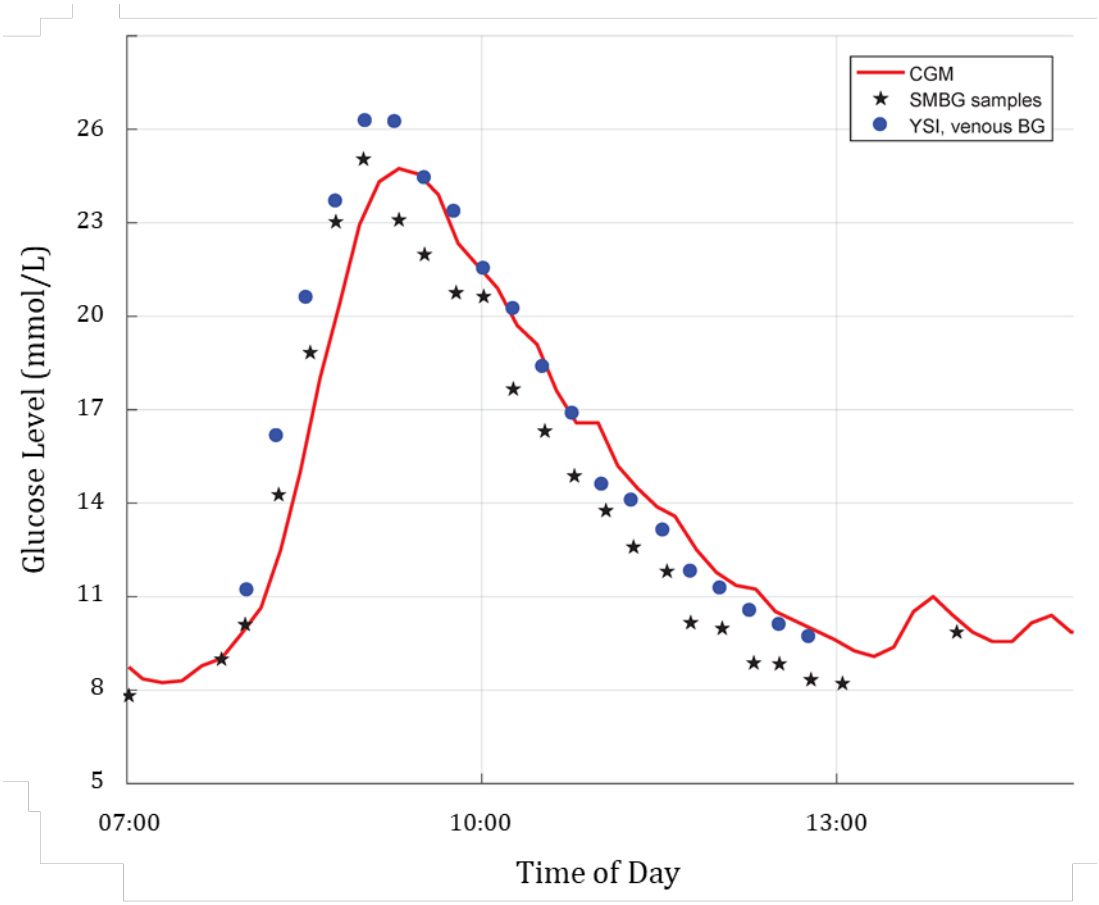

Figure 1.9 shows the readings observed by three different glucose monitoring technologies. The red line indicates data recorded by a CGM system, the black stars are data points measured from multiple blood glucose assessments (SMBG) performed in quick succession, and the blue circles represent data from a YSI 2300 START Plus laboratory glucose analyzer. The CGM system used in this example does not necessarily represent the accuracy of all CGM systems – it is simply an example showcasing a common problem with many devices. As seen, the readings from the CGM system (red line) lag behind the other two measures when glucose levels are changing rapidly, such as between 08:00 and 09:00am when the subject consumed a meal. The red line also lags behind the other two measures around the 12:00pm mark, when glucose levels drop into the low range. Adapted from Schrangl and colleagues (2018).[257]

1.7.3 Additional Measures

At present, there are a range of measures of blood glucose that are used in many different clinical settings. Two additional measures of glycaemia that have yet to be discussed in this section are the HbA_{1c} assessment and the glucose tolerance test. These do not fit neatly under

the definitions of 'intermittent' or 'continuous' as they are assessed in laboratory settings and provide unique measures of glycaemia. Compared to CGM and SMBG, these additional measures are less relevant to this thesis as they cannot provide information that can be used in real-time to make clinical decisions – they are outside the scope of this PhD candidature. However, they still provide meaningful information and are used routinely in diabetes diagnosis and monitoring. As such, they will be discussed only briefly to provide additional context for glucose monitoring.

1.7.3.1 Glucose Tolerance Test

The glucose tolerance test is the gold-standard for diagnosing diabetes, prediabetes, and gestational diabetes, as well as and impaired glucose tolerance.[258] It involves a fasting BGL assessment, followed by oral ingestion of a standardized glucose load (75 gram glucose solution diluted in water).[259] Blood glucose is assessed at standard times at 0, 1 hour, and 2 hours post-consumption. The results of the 2-hour oral glucose tolerance test may indicate normal, impaired, or diabetes levels of blood glucose.[259] Additional measurements may be taken during the two hour period, for example BGL measured at the 30-minute and 60-minute mark can predict risk of diabetes and all-cause mortality.[260] Some authors contend that BGL assessed at the 60-minute mark is a stronger predictor of lifetime risk of diabetes and diabetes complications compared to the 2-hour mark.[261] The glucose tolerance test is a strong clinical marker of diabetes and the gold-standard for diagnosis.

1.7.3.2 HbA_{1c} Assessment

One of the key outcomes used to assess diabetes progression is HbA_{1c}, or the percentage saturation of haemoglobin by glucose molecules carried in the blood.[262] This is a biomarker found in the blood which represents glycated haemoglobin levels and is routinely assessed in a laboratory setting from a person's blood sample.[263] HbA_{1c} is often viewed as the long-term average of an individual's BGL over the past 120 days, which is the lifespan of the average red blood cell.[264] As such, it can provide longitudinal averages of BGL, and this can be used to determine the effectiveness of the person's treatment and lifestyle interventions in maintaining the glycaemic target. HbA_{1c} levels are strongly correlated with fasting BGL,[265] and they are often interpreted to represent 'mean blood glucose', however this is a distinguishable metric which can be measured using a glucose monitoring system. An analysis of 387 participants across three randomized trials demonstrated that HbA_{1c} may sometimes underestimate or overestimate mean glucose,[266] and therefore they are distinguishable metrics with specific

clinical uses. Many authors agree that an assessment of HbA_{1c} is sufficient as an estimate of long-term glycaemia, as supported by the results of the Diabetes Control and Complications Trial,[267] and this assessment is usually conducted every six months for people with T2D. However, an open cohort study conducted by Ohde and colleagues (2018) concluded that HbA_{1c} monitoring is sufficient once every 12 months for people with stable glycaemia, and this should become the clinical standard to reduce over-testing.[268] Though there are minor discrepancies here in the literature, there is a clear consensus that long-term measures of BGL are useful in tailoring specific treatments to individuals to achieve glycaemic targets, supporting their use in clinical settings.

The HbA_{1c} assessment is a useful diagnostic tool for diabetes,[269] and is also the gold standard for monitoring long-term glycaemic management in diabetes.[264] The available evidence for the prevention of diabetes complications is to maintain HbA1c below 7%, and there is significant literature to support this, such as the Diabetes Control and Complications Trial in T1D[270] and the United Kingdom Prospective Diabetes Study for T2D.[271] However, some authors contend that HbA_{1c} levels are insufficient in predicting the risk for diabetes complications. Frequent glycaemic excursions, which occur mostly around meals and when antidiabetic medication is taken incorrectly, also contribute to glycaemic variability, but do not necessarily contribute to HbA_{1c}.[232] As discussed, glycaemic variability is associated with the progression of complications such as retinopathy, and this is speculated to be related to hyperglycaemia-induced oxidative stress.[272] Though this physiological model is theoretical, new evidence suggests that reducing glycaemic variability may aid with maintaining target HbA_{1c} in reducing risk of complications.[144] To add to this, neither the HbA_{1c} assessment nor the blood glucose assessment are able to accurately measure short-term fluctuations in BGL.[273] This is important because significant, acute variations in BGL contribute to glycaemic variability, and are associated with diabetes complications.[274, 275] The literature advocates for the prevention of glycaemic spikes that arise commonly around mealtimes, as a means of reducing glycaemic variability.[144] Systems which track BGL continuously and allow real-time analysis of that data may therefore have another advantage over the static, snapshot readings provided by intermittent measures of BGL.[276] It is important to consider, however, that the relationship between glycaemic variability and diabetes complications is new and has only a few studies to support it. Further research is needed in this area.

The glycaemic target for diabetes, which has been referenced throughout this chapter, is generally defined by $HbA_{1c} < 7.0\%$.[262] For some people with diabetes, such as those also living with known cardiovascular disease, this target may be stricter, such as < 6.5%.[277] Prevention of long-term complications of diabetes is an important goal for individuals and their physicians to

work toward, and can be achieved through tracking of HbA_{1c} with routine assessments.[278] T2D is a progressive condition, and complications are often viewed as an inevitability as opposed to a risk. Treatment with oral medication that lowers BGL is a frontline treatment, and consequently most patients with T2D will eventually require treatment with multiple drugs, known as poly-pharmacy, and also in combination with insulin.[279] The literature stresses the importance of meeting HbA_{1c} targets at all stages of diabetes, though this becomes more complicated as diabetes progresses, due to the increased likelihood of comorbidities and concomitant medications with age and diabetes progression.[280] The results from the GUIDANCE study (n=7,597) indicate there are many barriers to effectively meeting HbA_{1c} targets.[281] As with similar studies, only approximately half of their total sample were able to meet the optimal glycaemic target of HbA_{1c} < 7.0%. There were findings in this study that the authors claimed were counterintuitive, for example, subjects using diabetes medication and antihypertensives were less likely to meet targets for optimal glycaemia and blood pressure, respectively, suggesting that treatments are being allocated correctly, but on their own are not effective in terms of ensuring clinical endpoints. The authors Stone and colleagues (2013) also considered it surprising that the presence of one or more macrovascular complications was a predictor for optimal glycaemia, and suggested that this was because these people were more likely to have frequent appointments with their health care providers and more intensive care compared to subjects without any macrovascular complications.

In terms of early detection and screening for diabetes, HbA_{1c} is also important. A reading of HbA_{1c} that is greater than or equal to 6.5% is indicative of diabetes. These percentages roughly equate with long-term averages in BGL and can be converted to millimoles per litre. For example, an HbA_{1c} value of 6.5% equates to an average of 7.7 mmol/L BGL over the past 120 days.[282] An individual with < 7.0% HbA_{1c} may be described as an unmanaged diabetes case, but they will not be considered in remission from diabetes unless they are able to maintain this glycaemic threshold in the absence of medication.[263] As discussed, sustained remission from diabetes is rare without surgery, but possible. A HbA_{1c} level above 7.5% indicates a person's diabetes is unmanaged, as they are at risk of long-term complications, and a person with HbA_{1c} between 7.0% and 7.5% is not necessarily unmanaged, but their glycaemic is not optimal and they should aim to reduce their glycaemia to the target level of 7.0%.[283] However, a large proportion of people living with diabetes do not consistently reach the target of HbA_{1c} < 7.0%.[175] Many factors contribute to this, however the literature has identified that a key factor in the inability of many people living with diabetes to maintain target glycaemia is that current glucose monitoring tools are limited. The inability of people with diabetes to meet glycaemic targets is not entirely caused by limitations of glucose monitoring tools – lifestyle interventions

are often difficult to meet due to the social and commercial pressures of the Western lifestyle, as discussed in the first section of this chapter, and treatments should also be improved. However, this thesis focuses only on glucose monitoring tools. The following sections will provide further evidence for why current standards in glucose monitoring are insufficient given the scope of diabetes.

1.7.4 Achieving Glycaemic Targets

A core concept of this chapter is that glucose monitoring, when applied properly, leads to significant improvements in glycaemic management and allows people to achieve target glycaemia for longer periods. This ultimately leads to a better quality of life as well as a reduced risk for acute and chronic diabetes complications. As diabetes is a rising global health issue and a leading cause of premature death and illness, extra significance is awarded to technologies which aim to reduce the burden of diabetes, including those which aim to improve glucose management.[23] However, despite the overwhelming consensus that stringent glycaemic management is the most ideal course of diabetes management, many people living with diabetes are unable to achieve consistent glycaemic management.[284] This is determined by the ability of a person to maintain their HbA_{1c}, or long-term blood glucose, below 7% (Section 1.7.3.2). A large-scale study concluded that almost 50% of the Australian diabetes population were unable to maintain their HbA_{1c} levels at the recommended level of <7.0%.[104] Other studies suggest this is a global trend, and confirm that about half of all people living with diabetes are unable to reach this glycaemic target.[284] Furthermore, this trend is more apparent in groups that are insulin dependent. Between 2016 and 2018 in the United States, only 21% of people with T1D receiving specialized care achieved the glycaemic target of HbA_{1c} < 7.0%.[285] According to Rickels (2020), advancements in glucose monitoring and insulin delivering technology in recent years have not led to corresponding improvements in glycaemic management for insulin-dependent adults or youths with diabetes.[98] Grenier and colleagues (2016) agree that there is an important opportunity to improve on the current standards in diabetes care and treatment outcomes.[286]

To reiterate, the main concern is that 7% HbA_{1c} represents a major clinical threshold in diabetes, and that the inability to maintain HbA_{1c} below this threshold is associated with increased risk for diabetes complications. The DIALECT-1 study found that 64% of a T2D group (n=450) were unable to achieve target glycaemia, and the authors attributed this to increased resistance to insulin and antidiabetic medication, which occur over time as diabetes progresses.[175] Despite this, it has been discussed that the inability to achieve target glycaemia is not necessarily an inevitability of diabetes progression, but is something that can be amended

by improving medication and glucose monitoring.[287] There is growing recognition that the major problems of CGM systems and SMBG are key drivers of not meeting optimal glycaemic targets.[288]

1.7.5 Barriers

Barriers to diabetes management generally refer to factors which reduce the effectiveness of management strategies and overall treatment effectiveness. However, of particular importance to the literature are barriers specific to SMBG. Since so much of diabetes management relies on day-to-day self-medication and self-monitoring performed by the individual, effective SMBG is of critical importance to proper diabetes management.[289, 290] However, several systematic reviews have recognised that there are many barriers to SMBG which reduce its effectiveness, and research in this area is severely lacking given the scope of diabetes.[291-293] The review conducted by Saha (2019) labelled current evidence of barriers to SMBG as inadequate and weak.[292] Even among primary care physicians, attitudes toward the adequacy of SMBG lack a consensus.[294] However, there is an association between education and efficacy in regards to SMBG. The use of smartphone technology, integrated with healthcare systems, was also shown to be associated with better use of SMBG and increased effectiveness of diabetes management.[292] This is promising because it indicates that convenience is important to people who rely on themselves to monitor their own chronic condition, such as SMBG in diabetes. Improving convenience of SMBG technologies may also improve use, and thus improve treatment outcomes.[295] This is reinforced by Ng and colleagues (2020) who list inconvenience as a major barrier to SMBG. Other barriers listed in this 2020 paper include fear of pain and injection, fear of side effects of medication, and concern that resorting to insulin indicated the individual had reached end stage diabetes.[291] Though SMBG by blood glucose assessment is generally cheaper than CGM, many people with T1D or T2D still report high cost as a barrier to SMBG.[296] Consequently, SMBG is low in people with diabetes, and it is even lower in groups with less severe symptoms, such as those who are at low risk of hypoglycaemia or acute hyperglycaemic.[297]

Another barrier to maintaining target glycaemia is the fact that many people with diabetes are undiagnosed or otherwise unaware of their condition. Approximately 50% of all people with diabetes are unaware of their condition, and though rates of undiagnosed diabetes are lower in developed nations (25-33%),[298] they are estimated to be as high as 75% in some developing nations.[103] As discussed, this is likely related to the rising prevalence of diabetes worldwide and the fact that symptoms of hyperglycaemia can be quite mild and have a slow onset.[82] The

probability that people who are unaware of their diabetes are actively managing their condition are near zero. As such, the prevalence of undiagnosed diabetes and current limitations of SMBG and CGM are key factors that are implicated in the inability of half of all people living with diabetes to achieve target glycaemia. The development of a new glucose monitoring technology which addresses the concerns facing current SMBG and CGM could lead to significant improvements in glycaemic management in those with diabetes. If implemented as a routine health assessment conducted by general practitioners and other primary care physicians, this technology could also reduce the prevalence of diabetes unawareness.

Engaging with recommended treatment and lifestyle changes is an important consideration for any chronic condition, but it is especially relevant in diabetes. This is because lifestyle and diet interventions can be difficult to maintain over long periods of time,[299] and medications such as glucose-lowering drugs can lose their efficacy over time as diabetes progresses.[300] This can lead to increases in medication dosage or addition of other medications, such as insulin, to influence BGL. Consequently, even those who do engage strongly with their treatment plan may not be able to prevent their diabetes from progressing or worsening.[301] The feeling of frustration associated with this is one of the most commonly reported barriers to stringent diabetes management – another is frustration from lack of glycaemic management.[211] The effect of these barriers may be alleviated by improving SMBG techniques, such as improving their ease of use, as SMBG in general is associated with better glycaemic management.[210] The results of a large international study (n=5,104) indicated that use of SMBG was 44% in T1D adults and 24% in T2D adults.[302] Other studies have reported similar trends in T1D[303] and in T2D.[304] The need to overcome barriers to diabetes management is a core component of this PhD candidature, as it can improve treatment outcomes and lead to a significantly reduced burden from diabetes. To summarise the previous sections: complications of diabetes are highly prevalent and represent a significant burden to those living with diabetes, and SMBG is an important tool for both T1D and T2D to improve glycaemia and reduce these complications.

1.7.5.1 Invasive Technologies

A common element to all the measures of glycaemia discussed so far is that they are all invasive. This refers to their nature as tools which can only function by breaking the skin and accessing the tissues underneath. This is an unfortunate component of current diabetes management and glucose monitoring. The most accurate measures of BGL currently available require direct access to the blood, which is invasive and painful, and tools that estimate BGL by accessing alternative tissues, such as skin, mucous, and saliva, are less accurate.[200] These will be discussed

further in the following sections. CGM represents a strong middle-ground, where a sensor requires subcutaneous access for continuous monitoring, which is only minimally invasive, and the trade off in accuracy is acceptable.[201] However, even with minimally invasive technologies, there is still a requirement that the skin layer is penetrated. The pain associated with these procedures represents a psychological barrier to diligent BGL monitoring,[302] and is a common reason for suboptimal CGM.[305] This problem is not exclusive to CGM however, as all commercially-successful and clinically-relevant glucose monitoring tools are invasive, including the blood glucose assessment used in SMBG.[306] Research by Tanaka and colleagues (2018) demonstrates that people who experience pain from SMBG place less value on SMBG, and pain is associated with lower health-related quality of life, higher mental distress, and higher HbA_{1c} regardless of their number of daily blood glucose assessments.[307] According to one study, people who experience fewer barriers to SMBG, such as pain, inconvenience, and cost, are more likely to use SMBG (n=933).[308] The literature agrees that the invasive nature of all commercially-available glucose monitors is cause for inefficiency in diabetes management.[288] A non-invasive alternative to current SMBG standards can improve routine glucose monitoring as it removes a key barrier, which is pain and fear of invasive procedures.[288] However, such a technology would need to match or improve on the accuracy and reliability of current glucose monitors, whilst meeting certain other criteria of ideal monitoring.

1.7.6 Ideal Monitoring

Firstly, glucose monitoring should ideally be continuous in nature. As discussed, a clear strength of CGM systems is they can track glucose levels in real-time and provide meaningful information to people with diabetes and their physicians for optimising treatment. This is true for both T1D and T2D. CGM provides better glycaemic management than non-continuous measures[309, 310] – it is simply a matter of weighing the costs against the benefits, as current CGM systems are expensive. The ability to monitor BGL constantly cannot be understated in its importance. It allows for individuals to intervene when their glucose levels elevate or drop outside the target range. This can occur frequently and unexpectantly in response to meals, exercise, and medication, and can occur even when a person is asleep. It is not sufficient to simply maintain BGL at an optimal range for long periods of time – these spikes in BGL, even if they are very short in duration, should also be avoided to reduce the risk of diabetes complications. Combined with the fact that hypoglycaemia is prevalent in insulin-dependent diabetes, and may present at unpredictable times, it should be clear that glucose monitoring is more effective when it is continuous in nature.[311]

Secondly, glucose monitoring should provide data in real-time. According to a meta-analysis, CGM is more effective and leads to better glycaemic management compared to SMBG when it provides glucose data in real-time, but not when it is retrospective.[312] This means that a continuous measure of blood glucose should ideally provide information immediately to the individual, and many studies have indicated that integrating real-time CGM with smartphone applications may significantly improve convenience and thus efficacy of stringent glucose monitoring.[295, 313] Systems which alert people with diabetes in real-time to the onset of hypoglycaemia provide a significant decrease in the rate of severe hypoglycaemia, compared to systems with no alert.[314] Additionally, a major concern with current glucose monitoring tools is that they are all invasive. An ideal measure of BGL would combine elements of SMBG and CGM and be non-invasive. Therefore, an ideal glucose monitoring system should be continuous, non-invasive, accurate, cost-effective, and provide data in real-time with little to no lag time.[315] The ability to predict glucose levels in the near future may also be beneficial. Of significance to the present research is a technology that is both continuous and non-invasive, as no commercially-available technology has managed to achieve this to date, and this would represent a significant improvement for those currently utilising SMBG, as well as some who utilise CGM.[311]

1.8 Non-Invasive Glucose Monitoring: The Future Standard

Emerging technologies seek to develop an ideal method for monitoring BGL, which would involve a non-invasive, accurate measure of BGL that provides information continuously and in real-time. SMBG and CGM are the most used methods of glucose monitoring in T1D and T2D and are both invasive. Non-invasive technologies are those which analyse physiological variables, including glucose-containing fluids such as saliva and urine, without drawing fluid out of the skin.[316]. They aim to avoid the pain and irritation associated with invasive and minimally invasive devices, though it is important to note that some of these fluid-based technologies, such as those which rely on urine samples, can only provide intermittent measures of glycaemia due to limited supply.[317] Figure 1.10 summarises the invasive technologies discussed so far (left side) as well as the emerging non-invasive technologies (right side), some of which will be discussed in the next section. At present, none of these are sufficiently accurate and at best only estimate BGL.[318]

Figure 1.10 Current methods for invasive and non-invasive measurements of blood glucose.

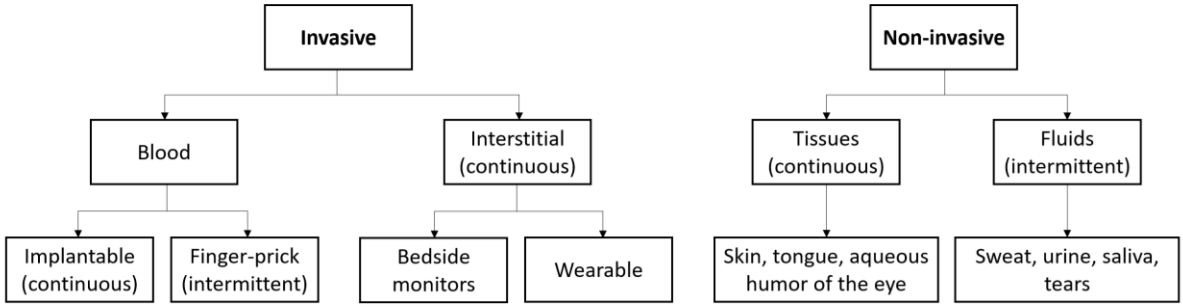

Figure 1.10 broadly outlines the different types of invasive and non-invasive technologies used to measure blood glucose. Invasive systems involve inserting a sensor through the skin to achieve direct access to the blood or interstitial fluid. Non-invasive systems use sensors that do not require the skin to be broken to access the target tissues or fluids. The HbA_{1c} assessment (not shown here) is also an invasive technology as it requires a blood sample. Adapted from do Amaral & Wolf (2008).[319]

1.8.1 Absorption Spectroscopy

Many efforts to develop a non-invasive measure of BGL employ some form of absorption spectroscopy, including near-infrared or mid-infrared.[320] Changes in the absorption of light at different wavelengths reflect characteristics of glucose molecules in the blood.[321] However, the optimal range of the electromagnetic spectrum to use in these investigations remains a controversial topic, and there is no standardised method. The use of wavelengths in the mid-infrared region, mostly between 8382 and 9708 nm, produces more distinct glucose peaks, compared to spectra produced at lower wavelengths, but there is limited light penetration.[319] Studies conducted in the therapeutic range between 600 and 2500 nm, such as the visible and near-infrared spectrum, are favourable because they allow for deep tissue analysis.[319] However, molecules other than glucose absorb in the near-infrared range as well, such as water and fat. In general, the precision of this technology is too poor for clinical use,[226] and requires significant work and modification to be viable.

1.8.2 Fluorescence

The use of fluorescent measurements in the development of a non-invasive measure of BGL is another emerging technology. It relies on the principle that glucose levels in tears reflect similar concentrations to those in blood.[319] Some of the major benefits of fluorescence include its extreme sensitivity to glucose and its resistance to fluctuations in ambient light intensity.[322] Additionally, this technology is promising due to its convenience, as the sensor can be

incorporated into clear contact lenses that can be worn by the individual.[323] However, since glucose levels are tracked with a 30-minute lag time, this may be a concern as current CGM systems have much shorter lag times.[319] Due to the poor accuracy of these emerging technologies, Tronstad and colleagues (2018) suggested a multi-sensor approach may work better, whereby information from multiple non-invasive devices, such as fluorescence sensors, bioimpedance, and skin temperature measurements, is combined to improve accuracy in detecting glycaemic events, making them fit for clinical use.[324] Though interest in this area is still gathering, more larger-scale studies are required to address some of these concerns.

1.8.3 Reverse Iontophoresis

Another technology that was developed in the pursuit of a non-invasive measure of BGL is reverse iontophoresis. This relies on a transdermal method, whereby small amounts of interstitial fluid are extracted through the skin with a device.[325] Devices can produce glucose estimations at 10 minute intervals, and this is comparable to current CGM systems. Traditionally, iontophoresis is used to electrically-charge drug molecules to transport them across the skin and into the blood, and thus this technology works in reverse by drawing fluid out of the initial space onto the surface of the skin, where it can be analysed.[326] The Food and Drug Administration approved a wrist-watch device capable estimating BGL by reverse iontophoresis, though the use of this technology in this example is technically minimally invasive, as fluid is being drawn through the skin.[327] Some of the problems with reverse iontophoresis include long calibration periods, skin irritation, and poor accuracy resulting from movement, exercising, and sweating.[319]

1.8.4 Summary

In conclusion, though much interest has been gathering in these areas, the development of a non-invasive, continuous marker of BGL has, to date, been unsuccessful. A recent systematic review and meta-analysis conducted in 2021 concluded that there were no minimally or non-invasive glucose monitoring systems fit for routine monitoring of hypoglycaemia.[288] In this paper, the authors Lindner, Kuwabara, and Holt state that current systems are not sufficiently accurate for detecting hypoglycaemia in routine use.[288] Lin and colleagues (2017) have also highlighted the lack of a commercially successful non-invasive glucose monitor, despite new technologies emerging in the past 10 years.[318] As such, there is an important opportunity to develop a novel non-invasive measure of BGL. If sufficiently accurate, this technology could

significantly decrease medical costs and deaths related to diabetes, as the convenience of non-invasive monitoring may improve glycaemic management and therefore glycaemic control.[328]

1.9 Glucose Prediction: Novel Applications

With the advent of machine learning, artificial neural networks, and deep learning algorithms, new computer-based approaches to glucose monitoring are emerging. Models are trained on large amounts of data, and when used in a clinical setting these models can provide an estimate of an outcome variable (such as BGL) based on several predictor variables, which could be related to medical history, demographics, or other physiological variables. There is a consensus that some of the problems with current CGM systems, including lag time and low accuracy in the hypoglycaemic range, can be improved by integrating machine learning algorithms and other predictive models into CGM systems.[329, 330] These models aim to improve the accuracy and reliability of current glucose monitors, such as CGM systems, to deliver more personalised care to individuals with diabetes[331] and allow for more timely detection and intervention of hypoglycaemia and hyperglycaemia (the outcome variables).[332] However, a core problem of these models is that they rely on data recorded by glucose monitors that are already in use, including CGM systems. Although machine learning models have led to more accurate prediction of dysglycaemia in combination with glucose monitors, glucose monitors are still invasive in nature and are not used routinely outside of T1D. Most people living with diabetes do not use CGM systems and will not benefit from algorithms which increase their accuracy. Therefore, this thesis is interested in predictive models that use information from non-invasive tools, as it is the invasive nature of current glucose monitoring systems that is cause for concern.

1.9.1 Based on Alternative Measures

Alternative means of predicting BGL – such as those which do not involve a CGM system or SMBG – represents a novel but growing area of interest in the literature. One example is the use of diabetes alert dogs, which can be trained to detect the onset of hypoglycaemia by smell.[333] However, Los and colleagues (2016) demonstrated that service dogs tend to raise an alert with a high false-positive rate, and by comparison a CGM system detects hypoglycaemia significantly earlier than a trained dog, with a median difference of 22 minutes.[334] As such, there is a need for a non-invasive marker of diabetes, with good potential for screening diabetes risk as well as the ability to estimate or predict BGL.

Based on work from Jung (2016), machine learning may be able to predict a person's risk of hypoglycaemia using information that can be entered into a smartphone, such as surveys, interviews, and diaries.[335] In a hospital setting, the risk of hypoglycaemia may be predicted using electronic medical records alone. A machine learning model developed by Ruan and colleagues (2020) was trained on a data set from 17,658 subjects with diabetes using their oxygen saturation, medications, type of diabetes, and type of procedures the person was undergoing.[336] Though the work of Jung (2016) and Ruan and colleagues (2020) may have limited applications, they highlight the importance of demographic data – such as age, height, and weight – and medical history in strengthening the value of predictive models. Recent work from Lama and colleagues (2021) indicates that there is still current interest in the capabilities of machine learning to predict glycaemia. Their algorithm was able to predict the risk of developing T2D in people without diagnosed diabetes based on their demographic and physiological data. They concluded that the strongest predictors of diabetes risk were body mass index, waist-hip ratio, age, systolic and diastolic blood pressure, and family history of T2D.[337] Foss-Freitas and colleagues (2019) contend that heart rate may be an important predictor of hypoglycaemic events, as their algorithm learned from data from a continuous heart rate monitor and provided useful predictions.[338] This is significant because heart rate monitors are non-invasive, relying on access to the surface of the skin only. The relevance of cardiac rhythms in predicting BGL is growing in recognition in the literature and is based on the principle that autonomic control of the heart reflects autonomic control of BGL. In theory, autonomic activity may predict BGL.

1.9.1.1 Neural Regulation of Blood Glucose

Both the central and peripheral nervous system are involved in glucose homeostasis. However, neural regulation of the pancreas, liver, and gastrointestinal tract, which are the primary organs involved in actioning glucose homeostasis, is the domain of the autonomic nervous system.[339] The autonomic nervous system is the branch of the peripheral nervous system which regulates the metabolic, visceral, and vascular systems of the body. As such, neurodegeneration of autonomic nerves, as seen in diabetic autonomic neuropathy, affects many parts of the body.[115] Combined, the autonomic nervous system and the somatic motor system constitute the entire neural output of the peripheral nervous system.[340] The somatic motor system controls skeletal muscles, and the autonomic nervous system controls every other tissue and organ in the body that is innervated, including secretory glands, the heart, blood vessels, organs of the digestive and excretory systems, and many others.[340] There are two counter regulatory branches which comprise the autonomic nervous system (Figure 1.11). Activation of the sympathetic nervous

system is associated with increased heart rate, vascular constriction, and dilation of the pupils, and is often characterised as the 'fight or flight' response.[341] Activation of the parasympathetic nervous system has an opposite effect, lowering heart rate, increasing digestion by dilating blood vessels in the gut, and constricting the pupils. It is thus characterised by the 'rest and digest' state.[342, 343] At rest, restorative and vegetative functions aim to conserve energy by way of parasympathetic innervation of the heart and other organs.[344]

As the autonomic nervous system is responsible for restoring glucose homeostasis through innervation of the pancreas, liver, and gut, the ability to measure sympathetic or parasympathetic innervation of these organs may theoretically provide meaningful predictions of glycaemia, or indicate if current BGL is high or low. However, autonomic control of these organs cannot be directly measured by non-invasive means.[345] This is due to the complex neural processes involved and the lack of a dedicated technology that can non-invasively measure autonomic input to the organs of interest.[346] However, autonomic activity in general is strongly correlated with autonomic control of the heart, which can be non-invasively measured.[347] Though there are various means of assessing autonomic function in a clinical setting, such as the Valsalva manoeuvre, there is a clear consensus in the literature that heart rate variability (HRV) is the best non-invasive measure of autonomic activity.[348-351]

Figure 1.11 Sympathetic and parasympathetic innervation of the human body.

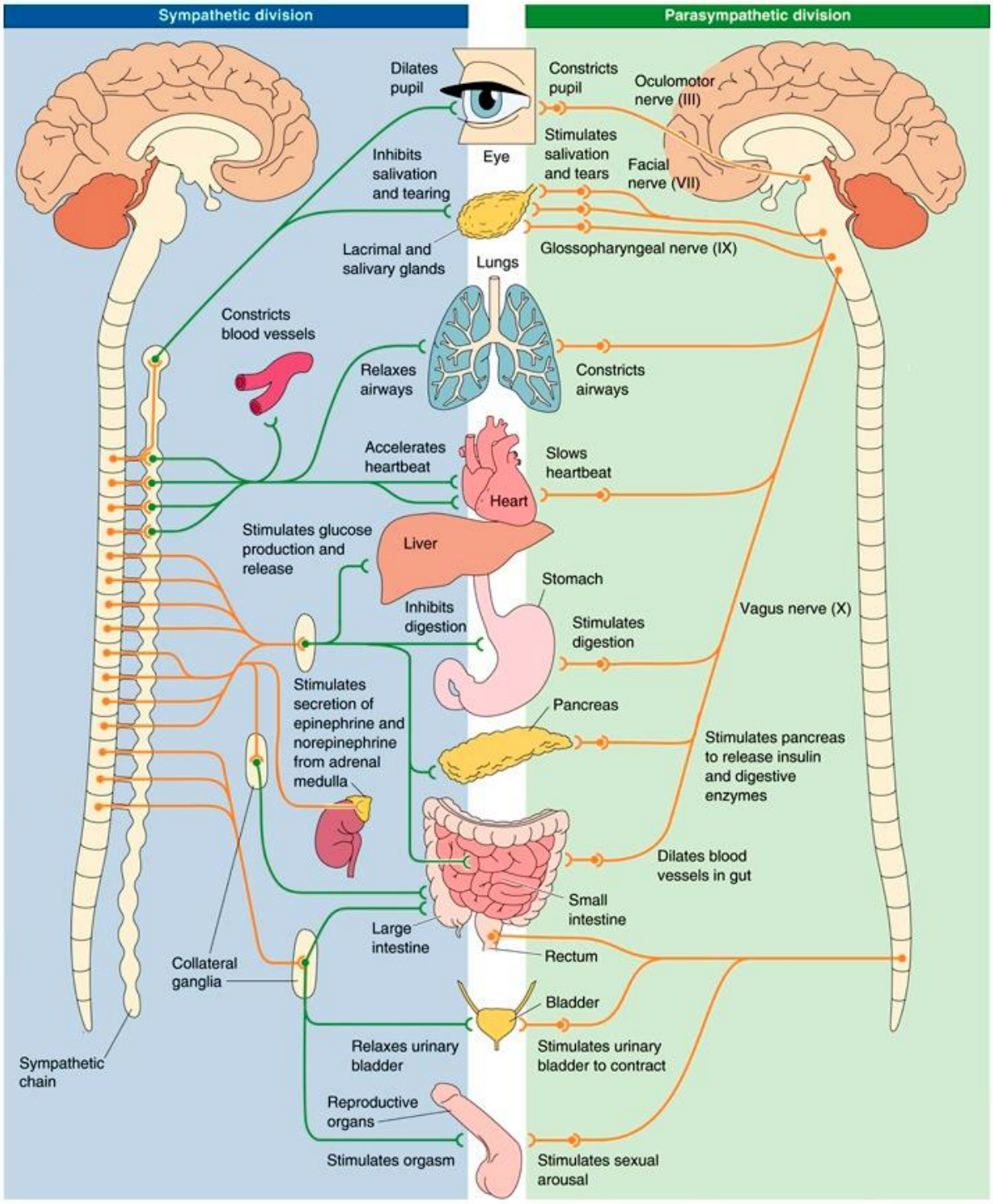

Figure 1.11 illustrates the two branches of the autonomic nervous system: the sympathetic and parasympathetic divisions. These branches maintain homeostasis of internal systems through opposite, counter regulatory actions. For example, sympathetic innervation of the stomach inhibits digestion, whilst parasympathetic innervation stimulates digestion. Adapted from Bear et al. (2016).[340]

1.10 Heart Rate Variability: An Autonomic Marker

Understanding how HRV is distinguishable from heart rate is relevant to this thesis. An individual's heart rate is a measure of how many times their heart contracts or beats per minute. Acute increases in heart rate can be related to exercise, as there is increased metabolic demand, or even stressful periods, as the nervous system prepares for a 'fight-or-flight' scenario in response to a perceived threat.[352] Heart rate also fluctuates naturally in a resting state, synchronised with the respiratory cycle. When breathing in, heart rate increases momentarily to increase the efficiency of the exchange of oxygen and carbon dioxide between the fresh air and the alveoli in the lungs.[353] Heart rate increases when inhaling, as the return of blood to the heart decreases due to increase in thoracic pressure on blood vessels. This leads to the corresponding increase in heart rate, as per Frank Starling Law. When exhaling, pressure in thoracic cavity decreases, which increases blood flow to the heart, and therefore a decrease in heart rate to maintain blood pressure.[354] When exhaling, heart rate decreases momentarily to allow for a rest period. This is known as respiratory sinus arrhythmia, a reflection of vagal or parasympathetic control of the heart.[355] The effects of metabolic state, respiration, and the autonomic nervous system on the cardiac cycle are well-known topics in the literature, with new understandings emerging in novel areas.[356] In the 1990s, it was determined that the optimal rhythms of a heart are not like a metronome; rather, they are complex and non-linear.[357] This variability in heart rate, or HRV, describes the natural tendency of the cardiac cycle to vary between subsequent cycles, a process which reflects the body's ability to adapt to environmental and psychological challenges. Optimal HRV is associated with good health and cardiac adaptability and resilience.[358] Blood pressure, including systolic and diastolic indices, is another example of a physiological variable which fluctuates on a minute-to-minute basis in response to changing demands.[359, 360]

Of significance to this thesis is the notion that HRV is assessed through non-invasive means, usually an electrocardiogram (ECG) or heart rate recording, and that HRV is the best marker of autonomic activity. This is due to a combination of various factors, such as accuracy and reliability, the ability to assess it non-invasively, and the ability to measure specific components of autonomic tone. The latter point is possible because different HRV measures reflect different autonomic influences over cardiac activity, including sympathetic and parasympathetic influences.[361] These unique HRV measures are not measured in beats per minute. Rather, they are calculated by applying complex mathematical equations (see Section 2.1.5.1 and 2.1.5.2 in Methods for more details) to an ECG waveform or heart rate recording.[362] Figure 1.12 illustrates the main features of a typical ECG waveform, from which computer programs can extrapolate HRV measures. Specifically, the calculation of HRV measures requires a recording of many R-R

intervals, which can also be achieved with a simple heart rate monitor. A heart rate monitor measures the pulse wave, and from this the pulse rate is determined. An R-wave on an ECG waveform denotes the electrical activity of the heart during its main ventricular contraction, or heartbeat, and an R-R interval denotes the time in milliseconds between two successive heartbeats. Although the main rhythm of the heart is determined by the sinus node – the natural pacemaker of the heart – it is the autonomic nervous system which determines the final heart rhythms through the innervation of sympathetic and parasympathetic nerve fibres.[363] Parasympathetic innervation of the heart is achieved via the vagus nerve, though this nerve shows dysfunction in the early stages of diabetic autonomic neuropathy.[364] It has been proposed that the vagus nerve is an early target of neuropathy because it is the longest nerve of the autonomic nervous system.[365] The vagus nerve is also continuously active as it is an integral autonomic nerve, and as such is more susceptible to free radical damage, which is increased with hyperglycaemia.

Figure 1.12 Electrocardiogram waveform with QRS complex and R-R intervals.

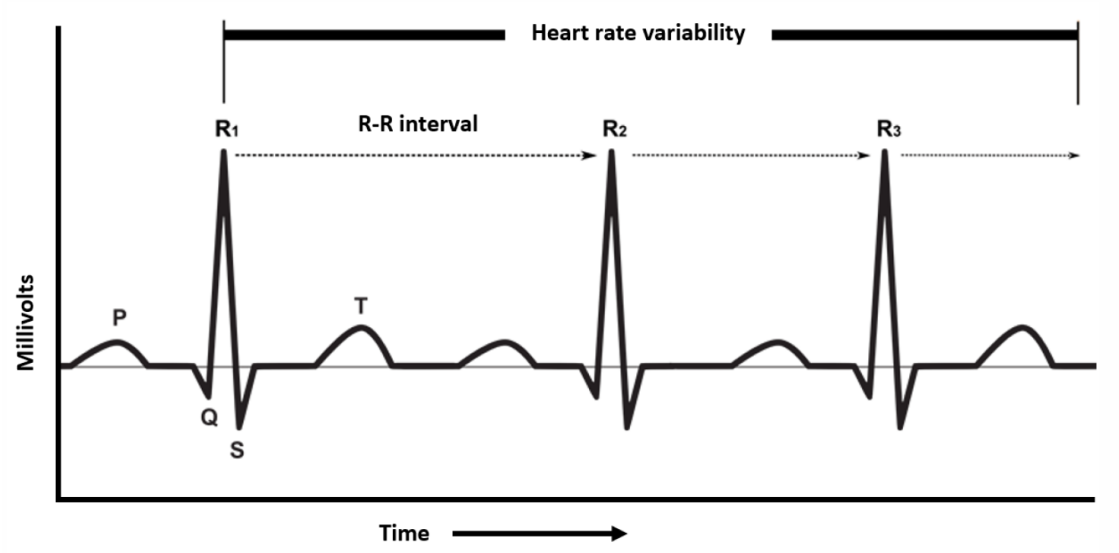

Figure 1.12 illustrates how heart rate variability can be determined from an ECG waveform. A typical waveform provides five 'landmarks' of cardiac activity, including the 'P-wave', 'T-wave', and the 'QRS complex'. **P** = atrial depolarisation. **Q** = depolarisation of interventricular septum. $\mathbf{R_{1,2,3}}$ = depolarisation of the main ventricular mass, which is the 'heartbeat'. **S** = ventricular depolarisation. **T** = ventricular repolarisation. The R-wave is the most important component of an ECG waveform in determining heart rate variability. The QRS interval precedes ventricular contraction and measures the electrical activity of the ventricle. Adapted from Ortiz Guzmán et al. (2012).[366]

1.10.1 Applications in Diabetes

Autonomic neuropathy is a major complication of diabetes, driven by unmanaged hyperglycaemia and duration of diabetes,[350] as long-term hyperglycaemia causes neurodegeneration in the peripheral nervous system.[367] Neuropathy of the autonomic nerves is also driven by hypoglycaemia, and reduced autonomic function then predisposes individuals to further hypoglycaemic events.[368] This can lead to a cycle of impaired awareness of hypoglycaemia, as described in Section 1.5.1.3. Neuropathy is an independent risk factor for myocardial infarction and other major cardiovascular events, and early detection of neuropathy is essential in reducing the risk of morbidity and mortality in people living with diabetes.[369] The ADA advocates the importance of monitoring autonomic function in diabetes due to the severe nature and prevalence of neuropathy.[370] Though some clinicians prefer a symptomatic approach to monitor the onset or progression of neuropathy, HRV can reliably detect autonomic decline before it becomes symptomatic, providing the opportunity for early intervention and prevention of further autonomic decline.[371] Recent interest in the applications of computational methods in medical science has indicated that models derived from machine learning may be embedded with clinical information systems to predict a person's risk of developing complications.[372] This may assist with specific treatment and improving treatment outcomes.

There is a consensus that lower HRV correlates with impaired autonomic activity, and all measures of HRV are reduced in diabetes.[373-376] This represents a large area of research. Significant decreases in HRV related to diabetes occur within the first 5-10 years of diabetes onset.[350] HRV is an excellent measure of vagal tone, which represents the function of the primary parasympathetic nerve: the vagus nerve.[344] Cardiac vagal tone is a predictor of parasympathetic tone in diabetes, and since it can be measured non-invasively with an ECG or heart rate monitor, it represents a convenient method of monitoring autonomic decline in diabetes.[377] Other authors concur that cardiac vagal tone is sensitive to autonomic neuropathy in diabetes, and thus HRV provides an affordable, accessible, and easily applicable screening method for autonomic dysfunction.[378] There is significant literature to justify the use of HRV in the prognosis of autonomic neuropathy in diabetes.[348, 352, 379] Though HRV may be a useful tool for primary care physicians to monitor the progression of a major long-term complication of diabetes, this thesis is only interested in the novel application of HRV measures in monitoring glycaemia.

1.10.2 Applications in Glucose Monitoring

In cross-sectional studies, HRV measures are inversely associated with BGL. In general, people with diabetes have suboptimal glycaemia, and this is associated with increased hypoglycaemia and hyperglycaemia. Glycaemic excursions contribute to autonomic neuropathy, and as demonstrated by Deshmukh and colleagues (2021), this is not reversible with euglycaemia restored by islet cell transplantation in T1D.[380] People who live with persistent hyperglycaemia and reoccurring hypoglycaemia, have lower HRV measures, which indicates poorer autonomic function.[381, 382] This relationship is strongest in cases of unmanaged glycaemia, as well as long-term cases of diabetes. This correlation may be due to a range of independent risk factors such as obesity, hypertension, and increasing age which all contribute to both lower HRV and higher BGL.[313, 371] Measures of HRV and BGL are also general indicators of health, which may explain why many different cross-sectional studies agree that there is an association between the two variables. As such, it has been proposed that HRV, determined by an ECG or heart rate monitor, may be a useful non-invasive measure of general glycaemic health. Machine learning may be useful in strengthening the ability of HRV measures to predict diabetes or high BGL in an individual. The use of HRV measures as predictors of glycaemia has been proposed previously in the Neuroscience Research Unit at the University of Technology Sydney (UTS) by Rothberg and colleagues (2016).[383] This PhD candidature is a continuation of this research, and aims to further investigate the viability of non-invasive HRV parameters as a measure of BGL in people with or without diabetes.

1.11 Basis for Research

This chapter has so far discussed the global nature of diabetes as well as the specific threats posed by diabetes and their significance. To generalize: diabetes causes significant burden to affected individuals and to world governments, both developed and developing, and due to a combination of various cultural and commercial pressures, the prevalence of diabetes is projected to continue increasing.[384, 385] There is a question of whether the global response to diabetes is adequate, given the large scope of diabetes, especially T2D.[384, 386] In this thesis, however, the focus is on whether current standards in diabetes management are adequate. Given the information provided in this chapter, current management strategies are inadequate for those living with diabetes. Current technologies need to be improved, or a new technology needs to be introduced, to address this inadequacy. The aim of this thesis is to address some of the main concerns facing current standards in glucose monitoring by proposing an alternative marker of diabetes and BGL which may have clinical utility. A non-invasive measure of BGL,

relying on predictive models trained on alternative physiological data points, such as cardiac rhythms, is clearly an area of interest in the literature. A novel non-invasive technology may be implemented in clinical settings alongside the glucose tolerance test, blood glucose assessment, or even used in combination with CGM to improve accuracy and sensitivity of these existing tools for managing dysglycaemia.[387] Though there are many non-invasive measures of BGL under development, such as those which record glucose levels in the saliva and sweat, none are commercially successful or clinically relevant. In this thesis, a novel predictor of BGL is proposed, and the aim is to justify further research into HRV measures as novel markers of BGL.

The long-term effects of suboptimal BGL on autonomic function are well-established within the literature, [352, 367] and HRV is widely considered to be the best marker of autonomic activity.[348-351] Also, HRV is more sensitive to glycaemic fluctuations compared to conventional assessments of autonomic function, including the orthostatic test, the Valsalva manoeuvre, and the controlled and deep breathing test.[388] The current literature on associations between HRV and BGL is limited,[350] and is summarised in Table 1.2. Though limited, current literature has established that measures of HRV are diminished in T1D and T2D, and that HRV is inversely associated with BGL. A recent systematic review and meta-analysis of 25 studies (n=2,932) conducted by Benichou and colleagues (2018) reinforced this consensus. The main link that requires further investigation is whether HRV measures can predict short-term fluctuations in glucose levels, including hypoglycaemia and hyperglycaemia, as well as non-pathological fluctuations in BGL such as those seen after a meal. During certain glycaemic excursions, such as hypoglycaemia, there is a distinct autonomic response defined by autonomic symptoms, such as heart palpitations and sweating. HRV, measured non-invasively, may be able to identify distinct patterns in autonomic activity preceding hypoglycaemia or responding to declining BGL. In general, the ability for HRV measures to predict glycaemia is a novel area that requires further research. Specifically, it is only well-known that HRV is related to BGL when assessed cross-sectionally, and not whether short-term fluctuations in BGL may also correspond with changes in HRV measures. This may lead to the development of an regression model capable of estimating the direction and magnitude of BGL based on non-invasive means, as suggested by previous work.[389]

On a minute-to-minute basis, this relationship between HRV measures and BGL is complex. Acute changes in autonomic activity precede certain glycaemic events, such as the increase in BGL after a meal (postprandial BGL). The work of D'alessio and colleagues (2001) in rhesus macaques demonstrated parasympathetic innervation of the pancreas increases the secretion of insulin, which lowers BGL in anticipation of a postprandial glucose spike.[390] This suggests an association between autonomic activity whilst eating and postprandial BGL. However, no study

to date has demonstrated an association between postprandial BGL and HRV measures recorded in a prandial state (whilst eating). Such research could justify the development as HRV measures as predictors of postprandial BGL. Furthermore, in response to hypoglycaemia, the autonomic nervous system increases sympathetic innervation of the pancreas to secrete glucagon and restore euglycaemia.[391] Therefore, HRV measures observed during specific time periods or metabolic states may provide meaningful information for estimating current or future BGL. Currently, there is a lack of consensus regarding the specific changes in autonomic activity, reflected by HRV measures, during hypoglycaemia and hyperglycaemia. The aims and hypotheses in this thesis are based on these gaps in the literature.

Table 1.2 Summary of key findings from studies assessing frequency-domain heart rate variability parameters and blood glucose levels in groups with diabetes and groups without diabetes. *All correlations are significant. **BGL** = Blood glucose level, **CGM** = Continuous glucose monitoring, **ECG** = Electrocardiogram, **HF** = High frequency, **LF** = Low frequency, **LF:HF** = low to high frequency ratio, **T1D** = Type 1 diabetes, **T2D** = Type 2 diabetes.

Authors and year	Sample group	Sample size (n)	Mean age (years)	HRV method	BGL method	Was BGL inversely related to LF and HF?	Limitations
Singh et al. (2000)[392]	Adults without diabetes	1779	47.4	2-hour ambulatory ECG	Fasting BGL	Yes. Also, BGL inversely correlated with LF:HF.	Data was pooled for all 3 groups. HRV vs. BGL was not analysed in each group separately.
	Impaired fasting glucose	56	54.9				
	Diabetes	84	55.4				
Weissman et al. (2006)[393]	Pregnant adults without diabetes	15	Not shown.	ECG	Oral glucose tolerance test	Mostly yes. Increase in BGL led to decrease in HF and increase in LF:HF. LF was not correlated with BGL.	Autonomic processes are complicated by pregnancy. Small sample size.
Stein et al. (2007)[394]	Adults without diabetes	1089	72	24-hour Holter ECG	Fasting BGL	Yes	Sample was mostly elderly (mean age 72).
	Diabetes	178					
Jarczok et al. (2013)[395]	Adults without diabetes	2441	41.9	24-hour ambulatory heart rate recording	Fasting BGL	Yes	Did not control for medications or illness. Predominately male (75%).
Tarvainen et al. (2014)[350]	Adults without diabetes	189	64	20-min ECG	Fasting BGL	Only for LF power. HF power not significantly correlated.	Older sample and mostly female (~60%).
	T2D	93					
Klimontov, Myakina & Tyan (2016)[396]	T2D women	67	65	24-hour Holter ECG	24-hour CGM	Postprandial BGL reflected lower LF, but not HF. Eating caused a decrease in LF only.	Conditions of study did not reflect a regular daily routine.
Lutfi & Elhakeem (2016)[397]	Adults without diabetes	42	25.8	5-minute ECG	Fasting BGL	No. BGL positively correlated with HF, and inversely correlated with LF:HF.	Small sample size. ECG recordings were only 5-minutes long.
Rothberg et al. (2016)[383]	Adults without diabetes	31	27.9	10-minute ECG	Fasting and postprandial BGL	Yes, but only in T2D sample. Also, BGL inversely related to total power in T2D.	Small sample size. Did not adjust for kilojoule intake as a covariate in postprandial analysis.
	T1D	21	31.5				
	T2D	11	56.2				
Jarman et al. (2021)[389]	Adults without diabetes	25	27	10-minute ECG	Fasting and postprandial BGL	Yes, but only for postprandial BGL.	Small sample size. No diabetes sample

In Table 1.2, note the similarities between the studies by Rothberg and colleagues (2016) and Jarman and colleagues (2021). This is because the latter was a continuation of the former, conducted in the same research group: the Neuroscience Research Unit. In these studies, low frequency (LF) power, which represents sympathetic and parasympathetic nervous system activity,[398] and high frequency (HF) power, which reflects parasympathetic nervous system activity, were inversely associated with BGL. The low to high frequency ratio (LF:HF) is also mentioned in Table 1.2, and relates to sympathovagal balance. LF power, HF power, and LF:HF are three common measures of HRV used in the literature and in this thesis. The specific physiological correlates of these HRV measures will be discussed in more detail in later sections. At this point it is relevant only to understand that many different measures of HRV can be extrapolated from an ECG waveform or heart rate recording by mathematical equations, and that these HRV measures reflect autonomic activity.

In their 2016 paper, Rothberg and colleagues observed people with diabetes (n=33) and people without any chronic illness (n=31) before and after a meal and assessed fasting and postprandial HRV and BGL. They identified several associations between BGL and HRV parameters recorded in a fasting and a postprandial state, though these findings were only significant in the diabetes group.[383] The work of Jarman and colleagues (2021) continued this initial exploratory research by only studying people without any chronic illness or regular medications, and making improvements based on recommendations from previous literature. In this 2021 paper, n=25 individuals were observed before and after a meal, and several significant correlations were identified between HRV measures and BGL.[389] Kilojoule intake was controlled for in the statistical analysis to account for differences in meal composition, and the multiple linear regression analysis demonstrated that roughly 50% of the variance observed in postprandial BGL could be attributed to measures of HRV. This strongly suggests HRV measures may be clinically relevant in the prediction of BGL. To describe this in a real-world scenario, a simple 10-minute heart rate recording may be used to determine HRV measures, which in turn may provide an estimate or prediction of BGL around the same time. Combined with other routinely measured variables, such as age, body mass index, and blood pressure, HRV measures may reliably predict glycaemia. Future research should continue exploring the predictive quality of HRV measures, as well as the relationship between HRV and BGL.

The aims and hypotheses established in this research were based around a central thesis: that HRV measures can predict BGL, and thus HRV may be a viable non-invasive marker of BGL. This research may address some of the core concerns with current glucose monitoring. When developing these aims, the scope of a PhD candidature was considered as well as the specific needs of the main types of diabetes. Though T1D and T2D are similar in that they are

characterised by persistent hyperglycaemia related to insulin dysfunction, there are meaningful differences in their pathophysiology, epidemiology, treatment, and severity (Section 1.4). Importantly, CGM is more useful in T1D, and SMBG is a staple of T2D management. Considering this, two separate methodologies were designed for studies with distinct aims and hypothesis. The first of these was the UTS T2D Study, conducted on people with T2D. The second of these was conducted at Royal North Shore Hospital (RNSH) on people with T1D and is referred to in this thesis as the RNSH T1D Study. Prior to these two main studies, one initial, exploratory study was also conducted based on previous work published by Rothberg and colleagues (2016) and Jarman and colleagues (2021). This was known as the UTS Pilot Study. As such, this thesis is comprised of three separate studies, and the Methods, Results, and Discussion sections of each of these studies are compiled separately.

The UTS Pilot Study was a small exploratory study which investigated the relationship between HRV and BGL in people after they ate a meal and was conducted to provide preliminary data to assist with the design of larger subsequent studies. As such, it was also in part a feasibility study. The second study addressed a broader aim in people with T2D as well as people without and was known as the UTS T2D Study. This was launched after the UTS Pilot Study concluded, as the findings of this initial study, such as time to peak BGL, were used in the design of the UTS T2D Study. The importance of assessing BGL at specific time points and in specific metabolic states was discussed in Section 1.6.5. Additionally, it is recommended that people living with diabetes obtain glucose readings by SMBG before and/or after breakfast to monitor day-to-day variations in BGL data.[399] Due to the threat of glycaemic variability and its prevalence in postprandial states, it is also relevant for a novel technology to predict the change in BGL following a meal, as this may allow for adjustments in medication to intervene.[400, 401] In recognition of this, the methodology of the UTS T2D Study was designed around obtaining fasting and postprandial BGL, where appropriate. The third study focused on people with T1D. As discussed, people with T1D were studied separately to those with T2D due to differences in treatment and management of these distinct conditions, thus the need to dedicate a separate set of aims and studies for each of the main types of diabetes. This third study, the RNSH T1D Study, did not recruit non-diabetes participants for comparison, and reasons for this are provided in Section 4.1.1. The aims and hypotheses of these three studies are provided below, and this novel research may improve current standards in glucose monitoring and improve quality of care in diabetes.

1.12 Aims

1. UTS Pilot Study (Chapter 2) – Investigate changes in HRV measures and BGL over a two-hour period in a small pilot study on people without any chronic illness or regular medications.
2. UTS T2D Study (Chapter 3) – Investigate correlations between HRV measures and fasting and postprandial BGL in people with T2D and people without T2D.
3. RNSH T1D Study (Chapter 4) – Investigate acute changes in HRV measures preceding and during hypoglycaemia and hyperglycaemia events in T1D.

1.13 Hypotheses

1. UTS Pilot Study (Chapter 2) – HRV measures and BGL will change significantly between the start, middle, and end of a two-hour period.
2. UTS T2D Study (Chapter 3) – LF power, HF power, and total power will be significantly and negatively correlated with both fasting and postprandial BGL in both groups. LF:HF will be positively correlated with both fasting and postprandial BGL in both groups.
3. RNSH T1D Study (Chapter 4) – LF power, HF power, total power, LF:HF, normalised LF power, normalised HF power, RMSSD, and SDNN will increase significantly from euglycaemia to hypoglycaemia and from euglycaemia to hyperglycaemia.

Chapter 2. UTS Pilot Study

2.1 Methods

The aim of the Pilot Study was to investigate changes in HRV measures and BGL over a short period of time. More specifically, the Pilot Study aimed to determine the magnitude of changes in HRV and BGL in response to food intake, an important foundation for subsequent research, and was also in part a feasibility study. The research was based on previous work conducted in an Honours year,[389] in which n=25 individuals were assessed over a six-hour period. The Honours research presented novel findings on the acute associations between HRV measures and BGL across various timepoints over a six-hour period. In this, it was concluded that the timing of assessments was important in determining the strength of the association between HRV and BGL. As such, this was considered when developing the study design for the Pilot Study. A major difference between the Honours research and the UTS Pilot Study was the Pilot Study aimed to investigate whether changes in HRV and BGL would be significant across a shorter period of two hours, instead of six hours. This research is not novel, but it was an important precursor study, and the Methods are relevant to the second and third studies also conducted as part of this PhD.

2.1.1 Subjects

Participants were recruited from the Sydney Metropolitan region and were contacted about the study through local advertising, such as posters, social media, and word-of-mouth. Potential subjects were notified by email about the study protocol and exclusion criteria, and eligible participants were invited to attend the research laboratory at the University of Technology Sydney (UTS). The protocol was conducted with approval from the UTS Human Research Ethics Committee (HREC) (2014000110) and was developed to address the aims and hypotheses stated in Section 1.12 and Section 1.13, respectively. The sample consisted of individuals who were uninhibited by any chronic illness or regular medications, as verified by a questionnaire.[402, 403]

2.1.2 Exclusion Criteria

Prior to study commencement, participants were informed of the exclusion criteria by email, and were excluded from the study if, at present, they: were living with a chronic health

condition;[403] were taking regular medication, prescribed or otherwise;[402] had an acute illness;[146] smoked more than 10 cigarettes per day or consumed more than 10 standard alcoholic beverages per day, as required by UTS HREC; or were currently pregnant.[402] Additional criteria were included to adhere to guidelines set by UTS HREC (see Section 2.1.3). Participants were also required to be between the ages of 18 and 69. Eligible subjects were invited to meet with the researcher at the UTS City Campus after a caloric restriction of eight hours.[404] This meant that participants were required to abstain from food and drink (only water was permitted), as well as nicotine, medications, and alcohol for at least eight hours prior to study commencement. Participants who were unable to meet this restriction, such as if they needed to take pain killers for a headache, were to inform the researcher so that their appointment could be rescheduled for another day. As a final pre-study requirement, both the researcher and participant signed two copies of a consent form, and each party retained one copy.

2.1.3 Blood Pressure

Participants were screened for high blood pressure (BP) as per ethics protocol. BP was measured using an OMRON HEM-7000 (OMRON, Kyoto, Japan) automated BP monitor (Figure 2.1), after each participant had been allowed to rest in a seated position for five minutes, with a two-minute rest interval between each recording.[146] Due to the dynamic nature of BP, the mean of three left-arm BP recordings were used to provide better accuracy of the BP recording.[405] If the average of these three BP readings was < 140 mmHg systolic and < 90 mmHg diastolic, the participant was included in the study. If systolic BP was between 140 - 160 mmHg, or diastolic BP was between 90 - 100 mmHg, the subject was included in the study, but they were also advised to inform their general practitioner of their blood pressure recordings, if not already aware. Subjects with a systolic BP > 160 mmHg and/or diastolic BP > 100 mmHg were excluded from the study and offered to be escorted to the nearest medical centre. These were based on HREC requirements.

Figure 2.1 Example of participant with an automated blood pressure monitor.

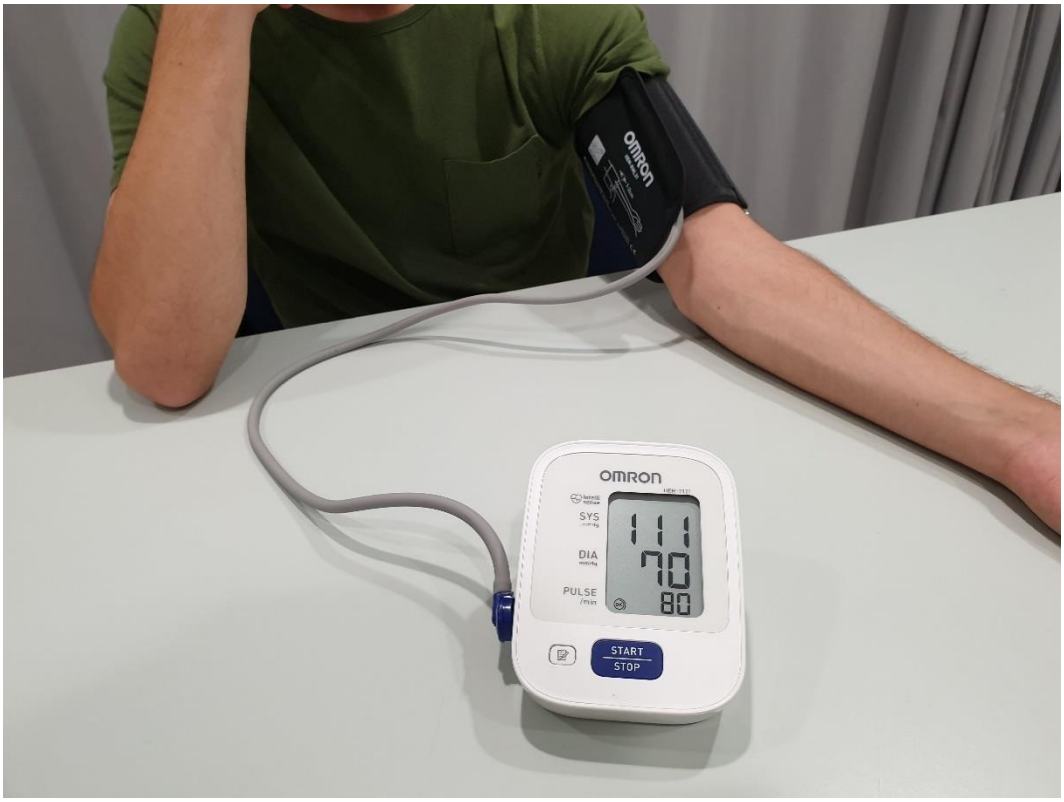

Figure 2.1 shows a participant with an OMRON HEM-7000 automated blood pressure monitor attached to their left arm by an inflatable cuff. Blood flow to the brachial artery was slowly constricted until arterial pressure was exceeded by the cuff pressure. This allowed the monitor to detect the maximum amount of force exerted by the heart on the brachial artery, which was an estimation of systolic blood pressure. In this case, it was given as 111 milligrams of mercury. The cuff then gradually deflated until the diastole of the cardiac cycle was detected, which is an estimation of diastolic blood pressure, or 70 milligrams of mercury. Heart rate is also provided by the monitor and was 80 beats per minute. Image used with consent from the participant.

2.1.4 Blood Glucose Assessment

Provided they were not excluded for high BP or for any other reason stated in Section 2.1.2, participants underwent an initial BGL assessment. Given that they had been asked to fast overnight or restrict their caloric intake to zero for at least eight hours prior, their metabolic state fulfilled the requirements for 'fasting'.[406] Fasting BGL was an approximation of baseline level, as it was not confounded by carbohydrate or caloric intake, which may vary considerably between individuals depending on their diet. The fasting assessment was undertaken at roughly 9:00am for each participant, as a way of diminishing the effect of circadian rhythms. These rhythms are responsible for natural fluctuation of physiological functions over the course of the day, including those related to the management of glycaemia.[407] As such, it was important to control for circadian rhythms by standardising the time at which each participant was assessed.

The ACCU-CHEK Performa II glucometer (Roche Diagnostics GmbH, Mannheim, Germany) was chosen for its accuracy to conduct the blood glucose assessment.[408] The subject's dominant hand was cleaned first, and then the disposable lancets were used to puncture the skin on the tip of the finger to produce a small sample of blood.[203] The blood glucose assessment is highly accurate, and capillary BGL assessed at the fingertip correlates strongly with arterial BGL.[199] An ACCU-CHEK Performa test strip (Roche Diagnostics GmbH, Manheim, Germany) was then applied to draw up the blood, and this was inserted into the glucometer to provide the measurement (Figure 2.2).

Figure 2.2 Example of a participant undergoing a blood glucose assessment.

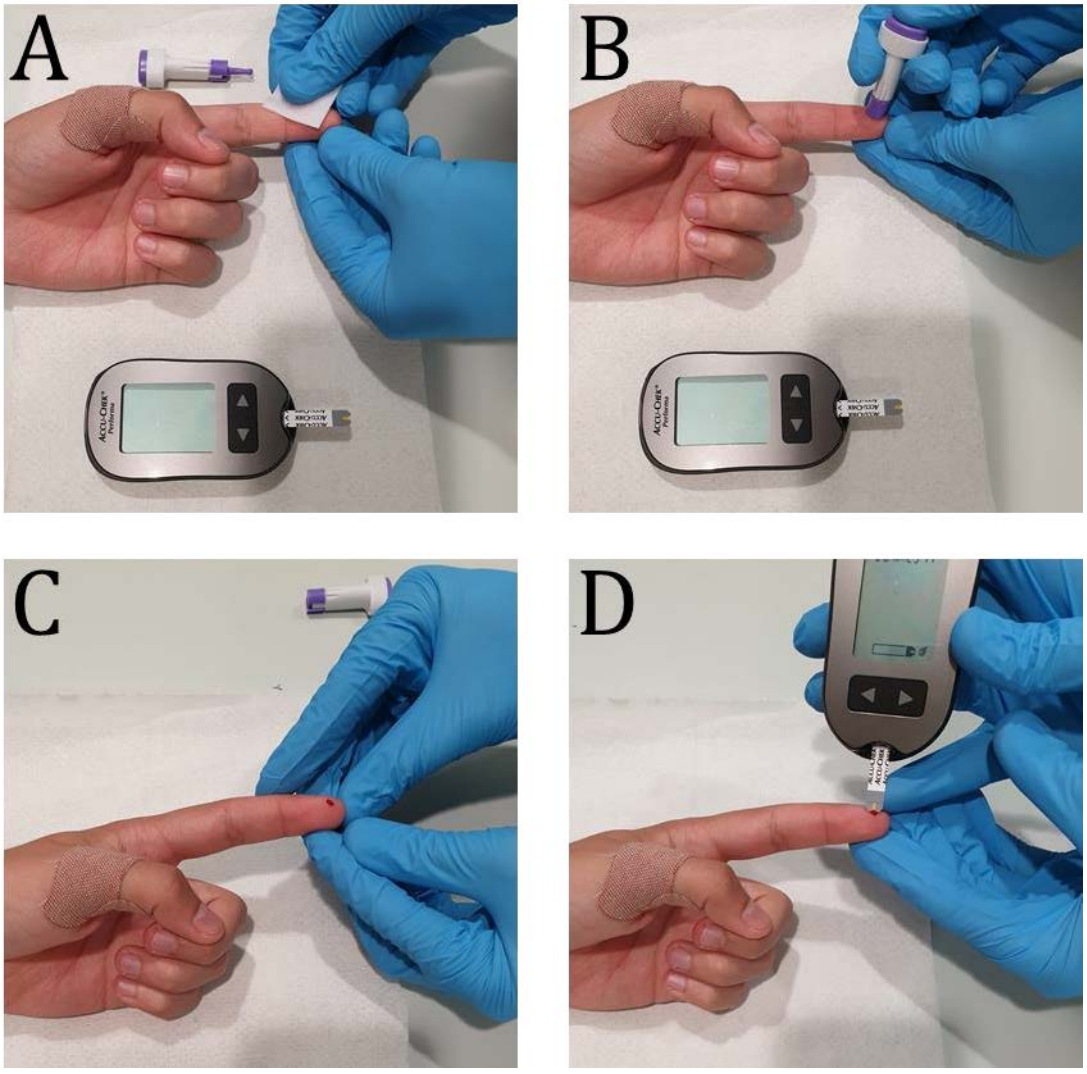

Figure 2.2 demonstrates how BGL was measured in this study. **A)** The participant's finger was cleaned and prepared using 70% isopropyl alcohol swabs. **B)** The disposable lancet was used to puncture the skin on the side of the tip of the finger. **C)** The subject's fingertip was squeezed gently to produce a small drop of blood. **D)** The test strip, inserted into the glucometer, measured the sample of blood. Following these four steps, the glucometer gave a reading of BGL in millimoles per litre. Biohazard material and sharps were disposed of appropriately. Image used with consent from the participant.

2.1.5 Heart Rate Variability

This section outlines the multi-step process used to obtain HRV data during this study. There are many reputable methods for calculating HRV, though nearly all of them involve collecting R-R interval data as a common step, followed by some form of computer processing to extrapolate HRV measures from the R-R interval data. For the purposes of the UTS Pilot Study, the philosophy was to utilise gold standards and common techniques where possible. The use of highly recognised techniques may facilitate the integration of this novel technology with clinical systems. ECG is considered the gold standard for collecting R-R interval data for HRV analysis.[409, 410] Though HRV can be recorded over 24-hours for additional clinical utility, it is important to develop this technology over short-term recordings, as this would be more convenient for diabetes screening and for SMBG. For example, a physician could perform a 10-minute ECG to predict diabetes risk, or a simple heart-rate monitor could be used by a person at home to predict current BGL. For this reason, 10-minute ECG recordings were suitable for the aims and scope of this PhD.

HRV measures may be expressed as units of time or frequency, and there are specific steps involved in extrapolating time-domain HRV measures compared to frequency-domain. Time-domain HRV directly reflects the variation in heart-rate over time, and is a measure of the intervals between successive regular cardiac cycles.[411] There are multiple types of time-domain HRV measures, and these are generally calculated from longer recordings, traditionally 24 hours or longer.[412] For these longer periods, more complex statistical time-domain measures can be extrapolated. For one time-domain HRV measure, this 24-hour recording provides the gold standard for stratification of cardiac morbidity and mortality risk.[358] As only short-term recordings were used in the UTS Pilot Study, time-domain measures were not utilised. However, frequency-domain HRV measures are a set of highly reputable variables which are favoured in this area of study as they reflect specific types of autonomic modulation.[383, 397] The specific neuroanatomical correlates of each frequency-domain measure will be outlined in this chapter.

2.1.5.1 Recording R-R Interval Data by Electrocardiogram

Subjects underwent a 10-minute ECG after resting in a seated position for 10 minutes to ensure their cardiac activity was not in an excited state. The ECG device was a three-lead FlexComp Infiniti (SA7550) encoder (Thought Technology Ltd., Montreal, Canada), which sampled electrical activity of the heart at a rate of 2,048 Hz.[413] Electrodes were placed according to the Einthoven configuration,[414] as shown in Figure 2.3. 10-minute recordings have better reproducibility and provide more accurate representations of autonomic activity in comparison

to other short-term HRV recordings, such as 5-minute or 2-minute recordings.[415] When the ECG was complete, the electrical waveform was downloaded from the device for further processing.

Figure 2.3 Einthoven electrode placement for the 10-minute electrocardiogram recording.

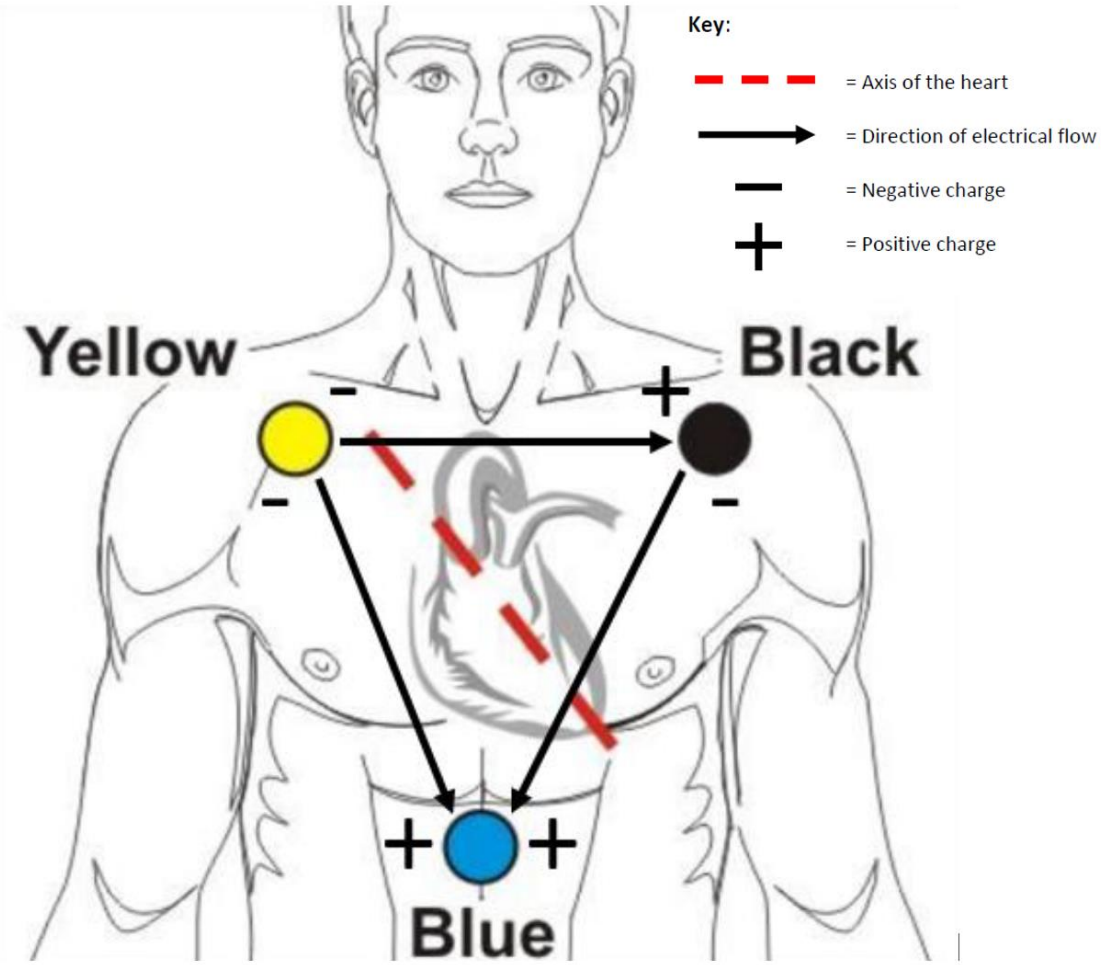

Figure 2.3 illustrates the reference positions for the Einthoven electrode placement. Electrical current flows from the negative electrodes (yellow) to the positive electrodes, as indicated by the arrows. The resulting potential is used to produce an ECG of the heart's electrical activity. Adapted from Combatalade (2010).

To derive the R-R intervals from the waveform, the 10-minute ECG waveform was processed using Kubios HRV Premium (ver. 3.3). This software applied a QRS detection algorithm to the waveform to detect the peaks in electrical activity apparent in each cardiac cycle. Each peak corresponded with a single heartbeat, which represents the main depolarisation of the heart as it contracts. On an ECG waveform, heartbeats are referred to as R-waves, and the distance in time between successive heartbeats is known as an R-R interval.[416] Once the R-R intervals for the 10-minute segment were determined, an artefact-detection algorithm was applied using Kubios HRV Premium to remove abnormal values or outliers. The automatic correction method

was used, which detected artefacts that were missed, extra or misaligned beat detections, as well as ectopic beats. For high-quality recordings, typically less than 1% of the R-waves detected will be flagged as artefacts, and they are usually related to the participant moving during the recording.[417] Finally, the R-R intervals were plotted on a time-series graph,[382] as shown in Figure 2.4. To clarify, the 'time-series' graph is not related to 'time-domain' HRV measures. As discussed, this study did not utilise time-domain measures. The time-series graph refers simply to how each R-R interval, measured in seconds, was plotted on a Y-axes with 'time of recording' on the X-axis.

Figure 2.4 Plotting the time-series graph from the electrocardiogram waveform.

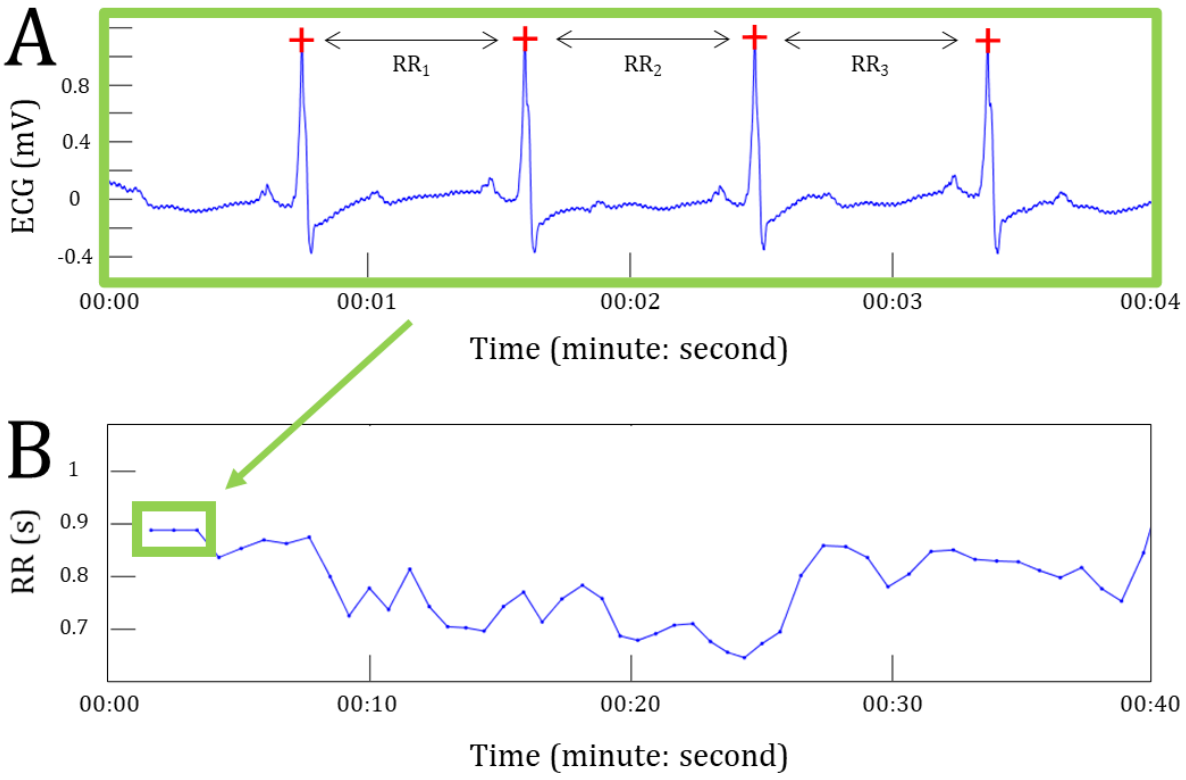

Figure 2.4 shows how the time-series graph was derived. The top graph **(A)** represents the first four seconds from a raw ECG trace. Using beat-detection algorithms in Kubios HRV Premium, R-waves were flagged by red plus symbols. The time, in seconds, between each R-wave was calculated as R-R intervals. The time-series graph presented in **(B)** shows every R-R interval for the first 40 seconds of the same ECG recording. The green box in **(B)** displays the three R-R intervals (RR_1, RR_2, RR_3) that were observed in **(A)**. As pictured, RR_1, RR_2, and RR_3 were roughly the same length of about 0.9 seconds. **+** = R-wave, **ECG** = Electrocardiogram, **mV** = Millivolt, **RR** = R-R interval, **S** = Seconds.

2.1.5.2 Extrapolation of Frequency-Domain Measures

Once the time-series graph was prepared, the data was ready for a non-parametric fast Fourier transformation, a highly-recognised means of calculating frequency-domain HRV measures.[146,

[313, 382] There are two common methods favoured by researchers in this area of study: autoregressive spectral analysis and fast Fourier transform. The measures extrapolated through these methods are not interchangeable, and investigations in people living with diabetes usually determine HRV measures by fast Fourier transform as the preferred method.[418] The time-series graph was converted to a power frequency spectrogram using Welch's method, which is a periodogram-based method to solve the discrete Fourier transform.[313, 419] An example spectrogram, derived from a participant's ECG waveform that was processed, is shown in Figure 2.5. Within the spectrogram, three frequency bands were categorised: very low frequency (VLF) power, which ranged from 0.00-0.040 Hz on the spectrogram; low frequency (LF) power, from 0.04-0.15 Hz; and high frequency (HF) power, from 0.15-0.40 Hz.[382, 420] The power of each of these bands, calculated from the area under the curve, is expressed in milliseconds squared (ms^2). For example, LF power for a participant at baseline may be 900 ms^2. This power is correlated with both sympathetic and parasympathetic nervous system activity, and changes in the magnitude of this power reflect changes in autonomic control.

Figure 2.5 Frequency spectrogram derived from fast Fourier transform.

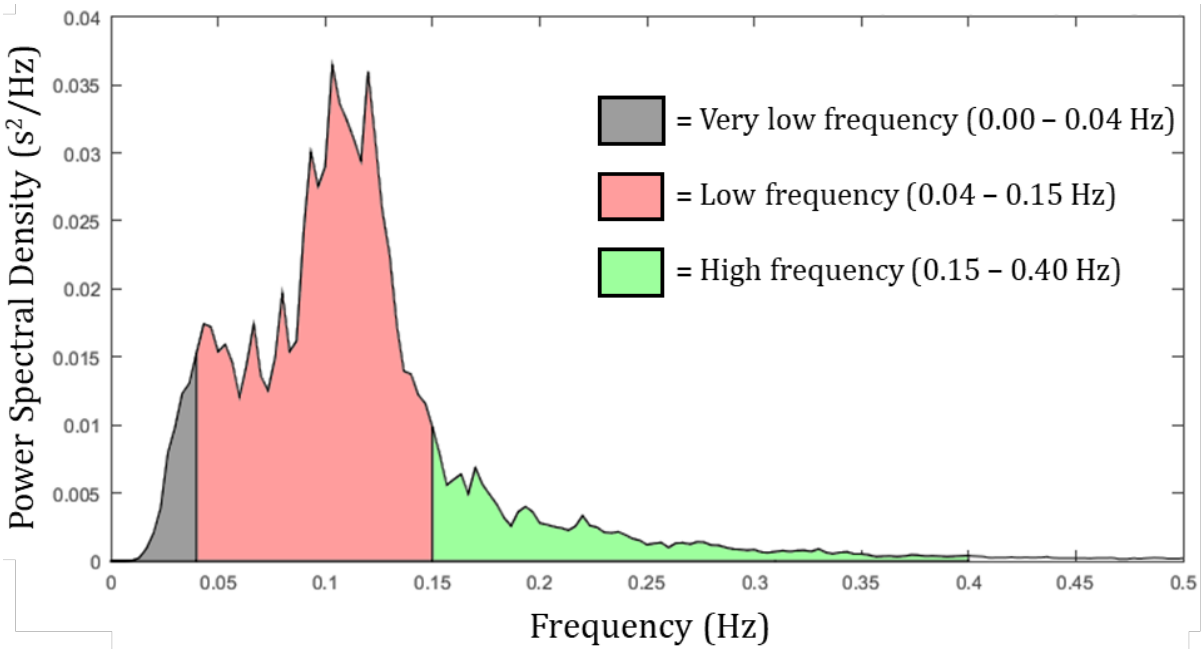

Figure 2.5 represents a frequency spectrogram obtained by fast Fourier transform. The power of each frequency is denoted by the area under the curve, and the units are in milliseconds squared. Low frequency typically has the greatest power, as shown in this example, though this has no noteworthy implications. The power of each measure is usually not compared with different measures, for example low frequency is not compared with high frequency. However, high frequency may be compared at post-intervention versus baseline to determine a change in autonomic activity, or compared between different sample groups to determine if autonomic function is different between them. **Hz** = Hertz, **s^2** = Seconds squared.

LF power is associated with sympathetic and parasympathetic nervous system activity, and individuals with higher levels of sympathetic activity, such as during stressful periods, would expect their LF power to increase above their baseline levels.[421] HF power correlates with parasympathetic nervous system activity and the activity of the vagus nerve, and thus predominates at rest.[422] Vagus nerve activity is sometimes referred to as vagal tone. Individuals would expect their HF power to be higher when at rest, and particularly during sleep.[421] The VLF band represents mostly background noise for short-term recordings, as 10-minutes of recording is not long enough per the Nyquist frequency, which is defined as half the average sampling frequency. For heart rate analysis, this is the mean heart rate. In addition to VLF, LF, and HF power, there were two other measures of HRV that were used in this study. The first of these was total power, which was calculated by measuring the area under the curve for the entire spectrogram from 0.00-0.40 Hz. As such, it can be thought of as the sum of VLF, LF, and HF, as the resulting power is identical to total power. The second of these was the low to high frequency ratio (LF:HF), which is calculated by dividing LF power by HF power. LF:HF represents sympathovagal balance, and increases in LF:HF over time indicate an increase in sympathetic activity relative to parasympathetic activity.[421] Due to short-term VLF power being largely composed of meaningless background noise, it was not used as an individual measure of HRV. As such, the four HRV measures were LF power, HF power, LF:HF, and total power. Of these, LF power, HF power, and total power were natural logarithm transformed as their distribution was highly skewed, though this is typical of HRV studies.[146, 423, 424] For a summary of the steps in Section 2.1.5.1 and 2.1.5.2, see Figure 2.6.

Figure 2.6 Flow chart for extrapolation of heart rate variability from electrocardiogram data.

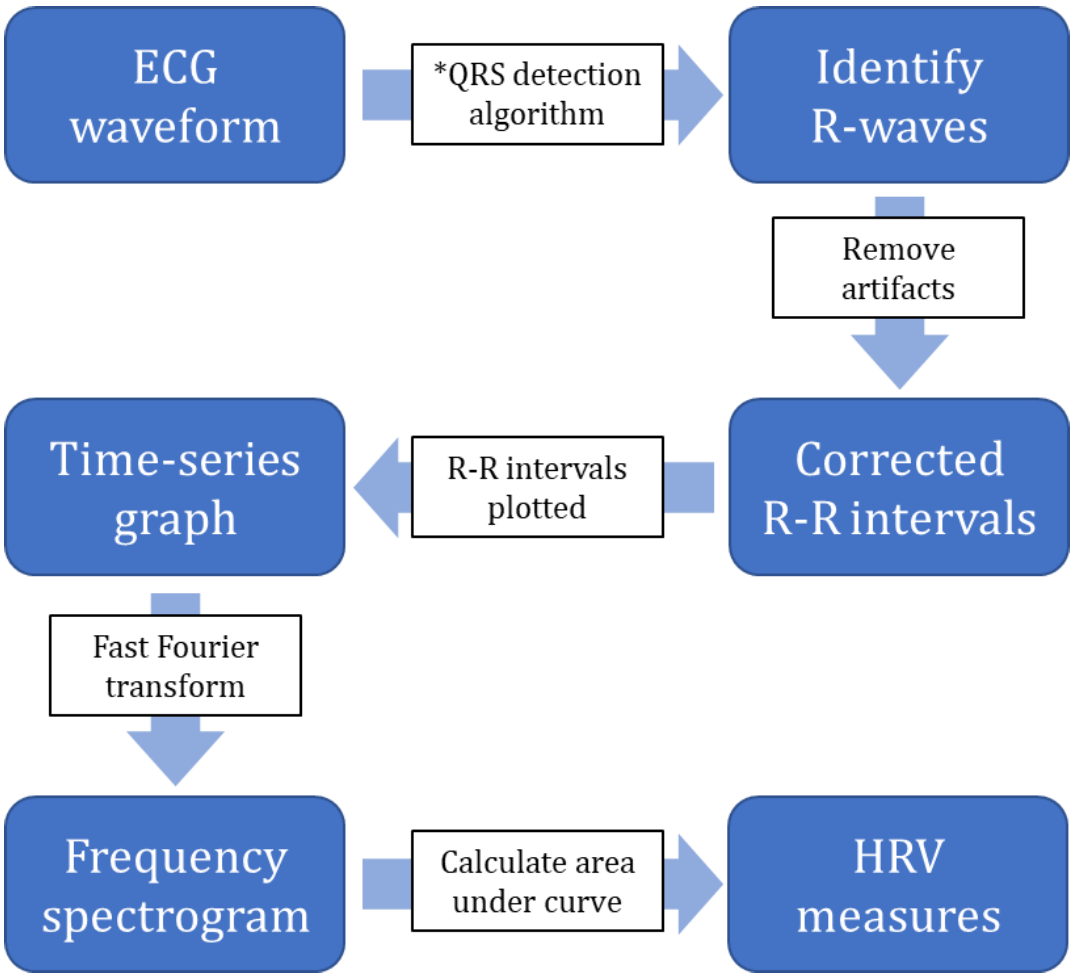

Figure 2.6 displays the step-by-step process by which HRV measures were obtained. The original ECG waveform was recorded over a 10-minute period. This data was downloaded from the device and then the following actions were performed using Kubios HRV Premium. The software was used to apply a QRS detection algorithm and an artefact removal algorithm to prepare a corrected waveform with each R-wave marked with a red plus symbol. A manual check was conducted to ensure that the algorithms had flagged each R-wave correctly, and then the R-R intervals were plotted on a time-series graph. Finally, the frequency spectrogram was produced by fast Fourier transform. The area under the curve for each frequency was measured, and these corresponded with each HRV measure, for example low frequency power and high frequency power. ***QRS** refers to the QRS complex, the central peak that can be observed on any regular ECG, and which correlates with ventricular depolarisation. **ECG** = Electrocardiogram, **HRV** = Heart rate variability.

2.1.6 Follow-up

As described previously, participants underwent a fasting BGL assessment and ECG after a caloric restriction of at least eight hours. This assessment can be referred to as the first or the 9:00am assessment. To satisfy the aims of this study, which intended to record changes in HRV

and BGL across a two-hour period, participants were asked to return to the laboratory for additional assessments. Given that the first assessment occurred at roughly 9:00am, the second assessment was scheduled for 10:00am and the third assessment was at 11:00am. These three times were agreeable with all participants. The selection of these times was arbitrary – the priority was to ensure that participants were assessed at roughly similar times of the day to remove the confounding effect of circadian rhythms. This methodology was expanded through future research.

2.1.6.1 Meal and Additional Assessments

As discussed in Section 1.6.4, BGL remains highly stable in the absence of caloric intake or certain drugs. Without some form of intervention, the BGL of participants in this study would have fluctuated very little from fasting levels, and there would have been few noteworthy observations over a two-hour period. As such, once fasting BGL was recorded in participants at 9:00am, they were asked to leave the laboratory and enjoy a meal of their choice. This allowed for the second assessment at 10:00am to capture an important change in BGL – that which naturally occurs after consuming a meal. Changes in HRV measures during this time were also observed. Though participants were expected to skip breakfast until after the study commenced, which may be considered an irregular meal schedule, research has shown that day-to-day changes in BGL are not significantly affected by consuming meals at irregular times.[425]

For the 10:00am assessment, participants underwent a second blood glucose assessment and a second 10-minute ECG recording. They were then instructed to leave the laboratory and return at 11:00am for the third blood glucose assessment and third ECG, and they were not permitted to eat during this break. For each of these follow-up assessments, BGL and HRV were assessed as described in Section 2.1.4 and 2.1.5, which is to say that the methodology remained constant regardless of what time of day the assessment related to. The main significant difference between the 9:00am, 10:00am, and 11:00am assessments was the metabolic state of participants, as this changed over the course of the day and with the introduction of caloric intake. However, all participants were required to undergo all three assessments at the specified times, as well as follow the instructions related to the meal.

2.1.6.2 Food Diary

To reduce participation burden, subjects were free to consume a regular meal between the 9:00am and 10:00am assessment, rather than undergo a standardised glucose load such as the

oral glucose tolerance test, which may cause nausea.[426] Research suggests that glucose peaks observed after a 2-hour glucose tolerance test are similar to glucose peaks that occur after a regular, standardized meal.[427] However, standardized meals do not reflect realistic daily routines, and this study aimed to observe fluctuations in HRV and BGL under loosely-routine circumstances. Therefore, participants were free to eat a meal of their own choosing. To account for differences in the composition of subject's chosen meals, which may have confounded their glucose profiles,[402] participants were instructed to report on all food and drink consumed between the 9:00am assessment and the 10:00am assessment. This quantitative information was converted by the researcher into kilojoule data, such that kilojoule intake could be used as a covariate in the statistical analysis.

The MyFitnessPal™ calorie counter search tool was used to estimate nutritional values for food and drink products consumed by participants in this study. Commonly, subjects consumed products from popular brands and chain restaurants, and accurate kilojoule data for these products was available from the MyFitnessPal™ website. For all other food items, including homemade products, kilojoule data was estimated using values uploaded by users of the MyFitnessPal™ website. Certain types of food are known to confound postprandial BGL; for example, some food increases BGL disproportionate to the serving of kilojoules in the food. Participants were asked to avoid any of the following common foods known to exaggerate or diminish glycaemia: cinnamon,[428] vinegar,[429] green tea,[430] and black tea.[431] Otherwise, subjects were not restricted in what or how much they could eat or drink, only that they had to return to the laboratory for the follow-up assessment at 10:00am (Figure 2.7).

Figure 2.7 Protocol summary for the UTS Pilot Study.

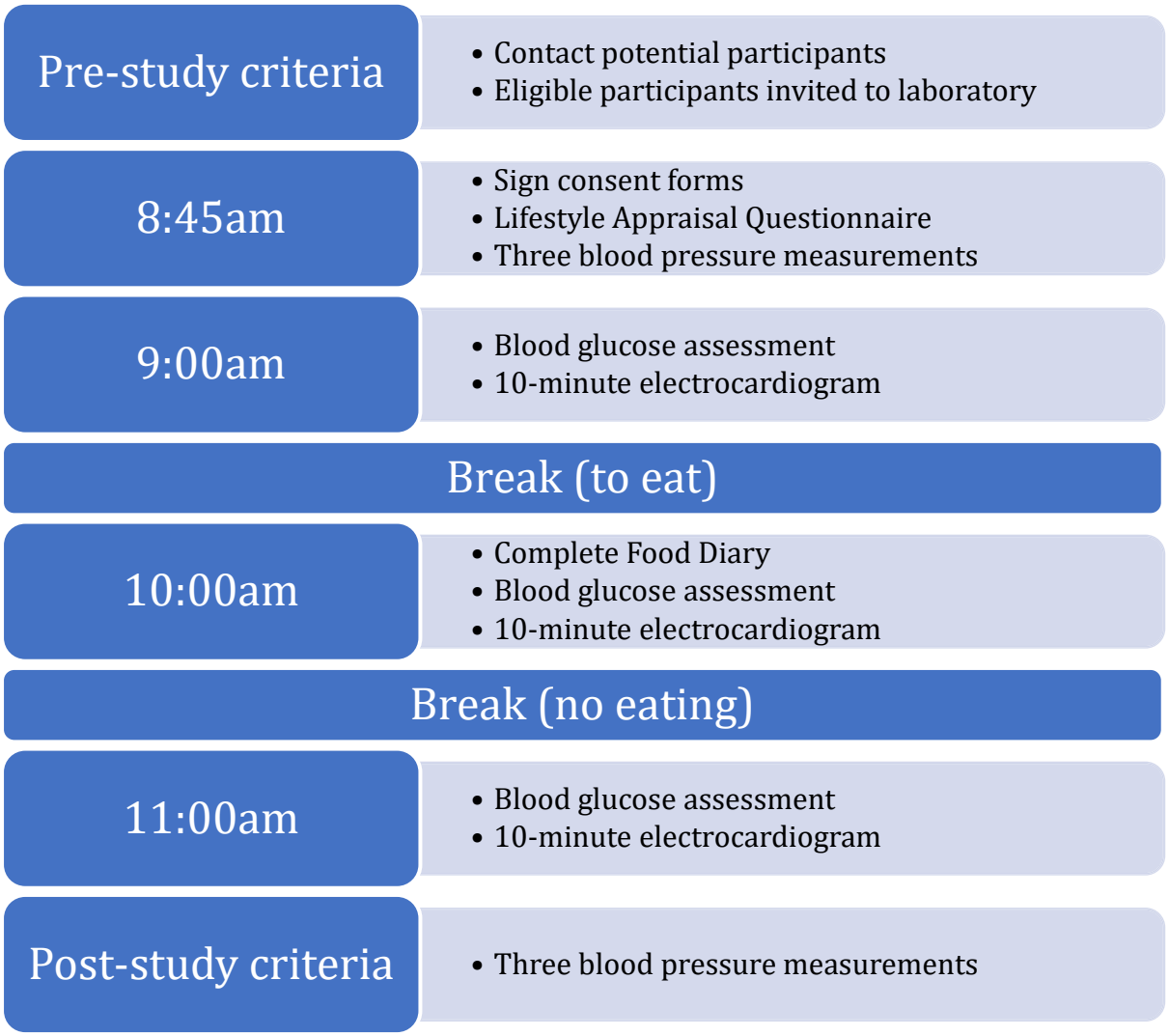

Figure 2.7 outlines the step-by-step process for each participant during the Pilot Study. Times given are approximate and were only used as rough guidelines for each participant. When subjects were contacted about the study, they were also informed of the study protocol and the exclusion criteria. Before the study could commence, participants had to refrain from eating or drinking (water was allowed) for at least eight hours prior. Drugs, including medications, nicotine, and alcohol, were also prohibited for this period. For the blood pressure recordings and ECG, participants were allowed to rest in a seated position for at least 10 minutes, to allow them to be in a resting state.

2.1.7 Statistical Analysis

Statistical analyses were conducted using SPSS version 22.0 (IBM SPSS Statistics, USA). Paired-sample t-tests (two-tailed) were used to identify whether BGL or HRV measures were significantly different between the 9:00am, 10:00am, and 11:00am assessments. These HRV measures were LF power, HF power, LF:HF, and total power. Statistical significance was set at $p < 0.05$. "A Power Primer" by J. Cohens was consulted to determine a sample size (n) which

would provide sufficient power for the study. For a comparison of means between 3 timepoints in a single group and a significance set to 0.05 and a large effect size of 0.5, a sample size of n=30 would provide a power level of 0.8, or an 80% probability of rejecting a false null hypothesis at the 5% significance level. As such, the recruitment goal was at least 30 people.

2.2 Results

The goal of the UTS Pilot Study was to recruit n=30 subjects and investigate changes in HRV measures and BGL between the start, middle, and end of a two-hour period. Concerns were raised during recruitment that the ECG data collected from participants was unfit for HRV analysis due to faulty equipment. Efforts were made to resolve the issue and to continue recruitment, however the study was eventually terminated early with n=14 participants assessed, which did not meet the sample power requirements. The total data set was 42 data points (n=14 participants with three assessments each), however, 17 of the 42 ECG recordings were unusable due to considerable levels of noise (Figure 2.8), and some of those that were usable had to be cut and shortened to reduce the effects of noise and artefacts. In terms of demographics, the age range of the sample was 19-55 years with a mean age of 28 ± 11 years. The mean body mass index was 24 ± 4 kg/m^2 with a sex breakdown of 50% male and 50% female. The mean kilojoule intake after the fasting assessment was 3700 ± 1700 kilojoules.

Figure 2.8 Effect of electrocardiogram noise on extrapolating heart rate variability measures.

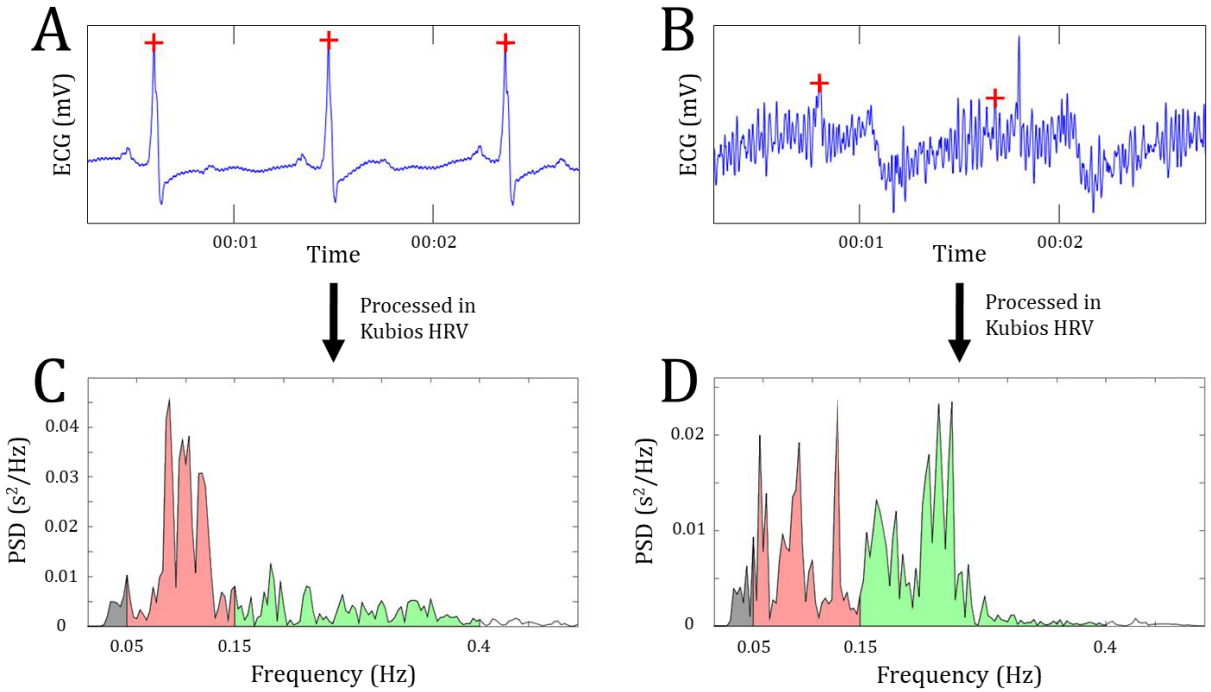

Figure 2.8 illustrates how poor ECG data can affect the determination of HRV measures. **(A)** A high-quality ECG trace recorded from a subject, with the R-waves (+) correctly identified by the software. **(B)** A low-quality ECG recorded from a subject that was age and sex-matched with the subject with the high-quality ECG. Note how, due to the 'noisy' ECG, it was difficult for the beat-detection software to identify the R-waves (+) which corresponded with each heartbeat. In this case, the noise was created from faulty equipment. **(C)** The full 10-minute recording of the high-quality ECG after it was processed into a power frequency spectrogram. **(D)** The low-quality ECG resulted in exaggerated high frequency power (green) and reduced low frequency power (pink) after processing. **ECG** = Electrocardiogram, **Hz** = Hertz, **mV** = Millivolt, **PSD** = Power spectral density, **RR** = R-R interval, **s²** = Second squared.

2.2.1 Glucose Level and Blood Pressure

Mean values for BGL and blood pressure recorded at each assessment are presented in Table 2.1. Paired sample t-tests (two-tailed) were used to determine whether these physiological variables changed significantly between each time point. BGL recorded at 9:00am was relatively uniform as participants were in a fasting state, and this was reflected in the relatively small standard deviation compared to BGL recorded in later assessments (Table 2.1). The increase in BGL from 9:00am to 10:00am was significant ($p<0.01$), as was the overall change in BGL from 9:00am to 11:00am ($p<0.01$) Though BGL appeared to decrease from 10:00am to 11:00am, the change was not significant. Other physiological variables assessed included heart rate, and systolic and diastolic blood pressure. There was no significant change in any of these three variables between 9:00am and 11:00am, the two points at which these were assessed. Figure

2.9 compares the means for each of these four variables at the three time points assessed and includes a visual representation of standard deviations.

Table 2.1 Blood glucose and blood pressure means and standard deviations recorded in the UTS Pilot Study sample (n=14). Blood glucose measurements were taken at the start, middle, and end of a two-hour period, at 9:00am, 10:00am, and 11:00am, respectively. Blood pressure and heart rate were recorded only at the start and conclusion of the study as per ethics requirement, and so data was provided for the 9:00am and 11:00am assessments only. ****** = p<0.01, **BGL** = Blood glucose level, **BP** = Blood pressure, **bpm** = Beats per minute, **mmHg** = Millimetres of mercury, **mmol/L** = Millimoles per litre.

				p		
Variable	9:00am	10:00am	11:00am	9:00am vs. 10:00am	10:00am vs. 11:00am	9:00am vs. 11:00am
BGL (mmol/L)	4.8 ± 0.3	6.1 ± 0.6	5.6 ± 0.8	<0.01**	0.32	<0.01**
Heart rate (bpm)	71 ± 13	-	70 ± 13	-	-	0.35
Systolic BP (mmHg)	114 ± 11	-	112 ± 9	-	-	0.42
Diastolic BP (mmHg)	73 ± 11	-	73 ± 8	-	-	0.72

Figure 2.9 Blood glucose, blood pressure, and heart rate data for the three assessments (n=14).

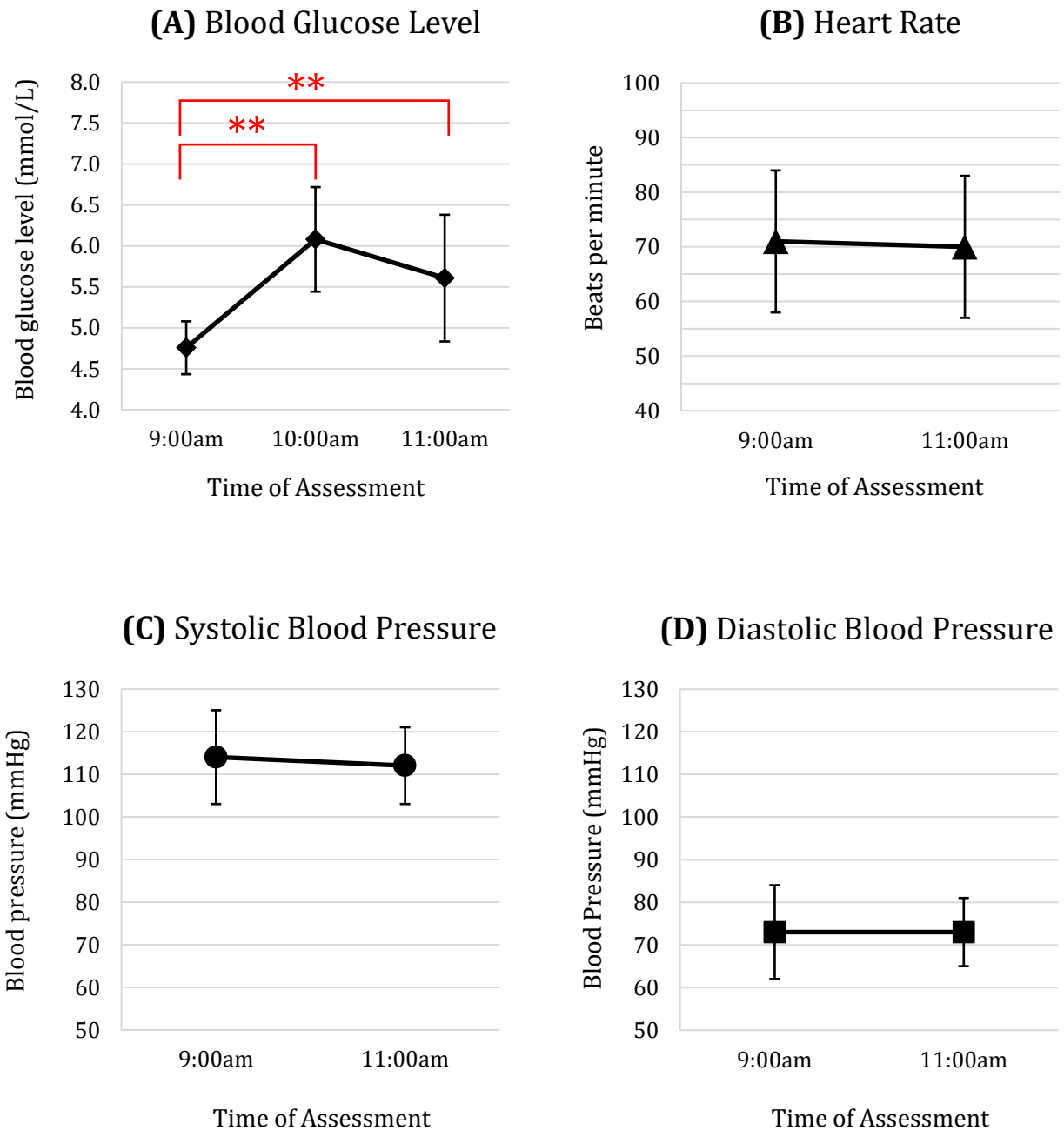

Figure 2.9 displays the means for the physiological variables that were assessed at each time point. Error bars represent the standard deviation for means of each variable. **(A)** The low to high frequency ratio showed large standard deviations across the 9:00am and 11:00am assessments and demonstrated no significant change. **(B)** Low frequency power was significantly different from the 9:00am assessment compared to 11:00am. **(C)** High frequency power had moderate standard deviations across all time points, and at 11:00am was significantly lower compared to 9:00am. **(D)** Total power decreased in the 11:00am assessment compared to 9:00am. ** = $p<0.01$, **mmHg** = Millimetres of mercury, **mmol/L** = Millimoles per litre, **ms²** = Milliseconds squared.

2.2.2 Heart Rate Variability Measures

ECG recordings were obtained in 10-minute epochs at each of the three assessments. In turn, these were transformed into HRV measures, reflecting autonomic activity at each of the three assessments. LF power, HF power, LF:HF, and total power were the HRV measures of interest in this study, and mean values are provided in Table 2.2. Note that the values provided here are natural log-transformed – raw HRV values are rarely presented in the literature as they are highly skewed. The only significant changes in these HRV measures were between 9:00am and 11:00am, as LF power ($p<0.001$), HF power ($p<0.05$), and total power ($p<0.01$) were all significantly lower in the 11:00am assessment compared to the 9:00am assessment. For a visual reference and comparison of these means and standard deviations, see Figure 2.10.

Table 2.2 Heart rate variability measures means and standard deviations recorded in the UTS Pilot Study sample (n=14). LF:HF is a ratio calculated by dividing LF power by HF power, and thus has no units. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **HF** = High frequency, **LF** = Low frequency, **LF:HF** = low to high frequency ratio, **ms²** = Milliseconds squared.

				p-value		
Variable	9:00am	10:00am	11:00am	9:00am vs. 10:00am	10:00am vs. 11:00am	9:00am vs. 11:00am
LF power (ms^2)	7.0 ± 0.9	7.0 ± 0.7	6.4 ± 0.9	0.80	0.60	<0.01***
HF power (ms^2)	5.8 ± 1.3	6.0 ± 1.1	5.0 ± 1.3	0.49	0.90	<0.05*
Total power (ms^2)	7.5 ± 1.0	7.4 ± 0.9	6.8 ± 0.9	0.94	0.69	<0.01**
LF:HF	4.8 ± 4.1	2.8 ± 1.7	5.3 ± 4.1	0.72	0.53	0.94

Figure 2.10 Change in the mean blood glucose level and mean low frequency, high frequency, and total power between the three time points (n=14).

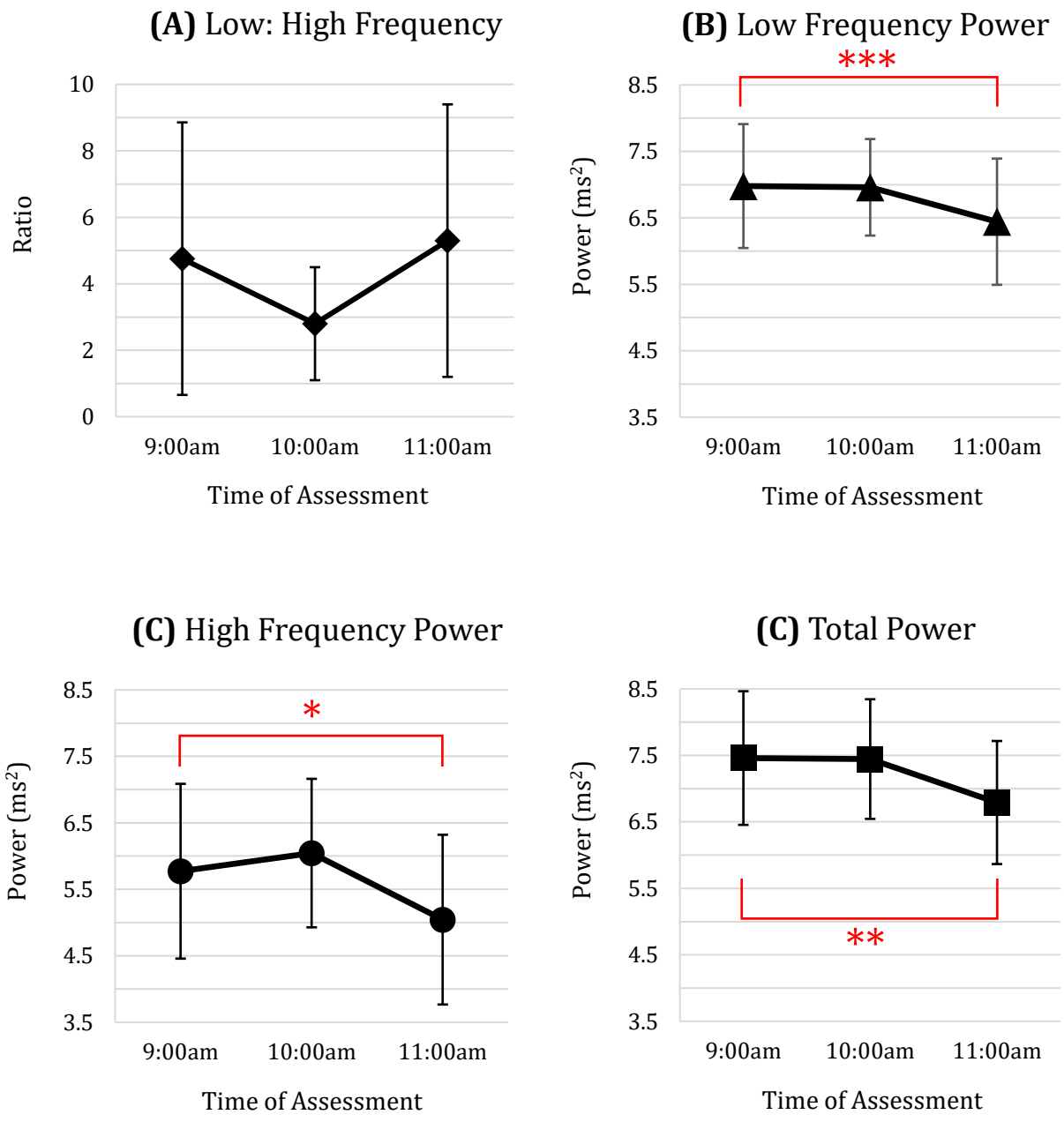

Figure 2.10 displays the means for the physiological variables that were assessed at each time point. Error bars represent the standard deviation for each mean. LF power, HF power, and total power were natural log transformed. **(A)** The low to high frequency ratio showed large standard deviations across the 9:00am and 11:00am assessments and demonstrated no significant change. **(B)** Low frequency power was significantly different from the 9:00am assessment compared to 11:00am. **(C)** High frequency power had moderate standard deviations across all time points, and at 11:00am was significantly lower compared to 9:00am. **(D)** Total power decreased in the 11:00am assessment compared to 9:00am. **ms²** = Milliseconds squared.

2.2.3 Summary of Results

Though the recruitment target was not met in this pilot study due to technical issues, statistical analysis was conducted on the data set to provide information for subsequent research. In this sample, BGL – as assessed by invasive blood glucose assessment – rose significantly ($p<0.01$) after the ingestion of a meal by an average of 1.3 mmol/L. BGL was also slow to return to baseline or fasting levels, as the difference between BGL from 9:00am to 11:00am was also significant ($p<0.01$). There was no change in heart rate or blood pressure between the start and end of the study, though certain HRV measures changed over the course of the two hours. LF power, HF power, and total power were all significantly lower at the end of the study (11:00am) compared to the start of the study (9:00am).

2.3 Discussion

The ability to estimate BGL in certain metabolic states, such as before and after meals, without using invasive measures represents an important milestone in diabetes research. A non-invasive marker of fasting or postprandial BGL may address the limitations of current glucose monitoring technologies. In this study, frequency-domain HRV measures and blood glucose concentrations were monitored in a small sample to determine how they fluctuate over a short period of time. Specifically, the aim was to determine the magnitude of changes in HRV and BGL in response to acute food intake. This study was conducted to obtain pilot data and to address a broader set of aims which relate to how HRV measures may be capable of predicting BGL in adults. Though the quality of data collected in this study was poor, there was meaningful information gathered which was implemented in other components of this PhD candidature. For example, this study investigated the dynamics of HRV measures and BGL from baseline to post-meal, and this information was considered when designing future studies. These will be expanded on in later chapters. The following sections will discuss results of the present study and present them in the context of the wider literature.

2.3.1 Dynamics of Heart Rate Variability

The values of the HRV measures observed in this sample of 14 participants were within expected ranges for subjects with no chronic illness or regular medications.[432] However, as with many physiological variables, there was considerable variation in the data set as indicated by the standard deviation values (Figure 2.10). This was expected, though it is important that the reasons for this variability be explored. Variability in HRV data may be attributed to several factors, such as sex, age, and general health of individuals.[433] Research by Yamasaki and colleagues (1996) showed that LF power peaks between 8:00am and 12:00pm in males and 12:00pm and 0:00am in females. To clarify, the average male would expect their LF power to be highest in the morning and the average female would expect their LF power to be highest any time between midday and midnight. Therefore, variability in HRV measures between males and females may be related to the timing of assessments. In this study, all participants were assessed at 9:00am, 10:00am, and 11:00am to attempt to control for circadian effects on HRV measures. However, circadian rhythms may be difficult to control for stringently as the literature suggests daily fluctuations in HRV measures are different between males and females. Future studies may consider normalizing the data by subtracting each follow-up assessment from the baseline. This may also control for the differences in baseline HRV between males and females. Additionally, though the mean age of the sample was relatively young (28 ± 11 years),

the presence of some older participants – as old as 55 years of age – may have contributed to the variability of the HRV data observed in the sample. This is because all measures of HRV decline with age.[434]

Interpretation of the findings of the present study requires an understanding of the different measures of HRV and their neurophysiological correlates. To reiterate, LF power is correlated with both sympathetic and parasympathetic nervous system activity, HF power is correlated with parasympathetic nervous system, total power represents total autonomic activity, and LF:HF is an index of sympathovagal balance, whereby increases in LF:HF indicate a shift toward sympathetic predominance. There is no specific way of determining sympathetic activity from HRV. In this study, mean HRV measures, including LF power, HF power, and total power, were all significantly lower at the end of the study (11:00am) compared to baseline levels (9:00am). When discussing these findings, it also important to consider that LF:HF did not change significantly, which indicates that there was no meaningful shift toward sympathetic or parasympathetic dominance. With this information, we can conclude that mean sympathetic and parasympathetic tone were reduced at 11:00am compared to 9:00am, as both LF power and HF power were significantly lower at 11:00am. This explanation is consistent with the findings that total power was also significantly lower at 11:00am, and that LF:HF did not change significantly. Whether this decline in LF power and HF power was related to the consumption of the meal or the change in time of day – which would likely be related to circadian fluctuations in HRV – is not a conclusion that can be made based on the data from this study. By chance, there were relatively more ECG recordings from the 10:00am assessments which were unusable compared to the 9:00am and 11:00am, and thus fewer data points were included in the statistical analysis. This contributed to a smaller standard deviation for LF:HF at the 10:00am assessment, and may have also contributed to LF:HF being misaligned from the values of LF power and HF power at 10:00am.

With reference to the literature, Klimontov, Myakina & Tyan (2016) measured morning fasting HRV as well as afternoon postprandial HRV in n=67 women with T2D and found LF power was significantly lower in the afternoon compared to the morning, and HF power was not significantly different.[396] In their paper, the authors argued that the reduction in LF power was due to either the consumption of the meal or the associated rise in BGL, but could not specify which of these was more important based on their analyses. Differences in the findings between Klimontov, Myakina & Tyan (2016) and the UTS Pilot Study may be due to differences in the sample groups, as women with T2D are physiologically distinct to people without diabetes in terms of autonomic activity and glucose management. In a study comparing fasting measures with postprandial measures Lu and colleagues (1999) showed that LF power does not change

after a meal, and HF power increases significantly.[435] In a sample of n=30 young subjects (mean age 21 ± 4 years), Soni and colleagues (2017) showed there was an increase in sympathetic activity and a decrease in parasympathetic activity two hours after a meal.[436] In this study, participants did not eat at exactly 9:00am, so though the study period was two-hours long, it was not technically two-hours since meal, so a comparison with the work of Soni and colleagues (2017) is not ideal. More recent literature agrees that sympathetic tone, as measured partly by LF power, increases after a meal, however blocking of these sympathetic pathways does not affect postprandial effects.[437] This may be because sympathetic activity during absorptive phase of digestion may not be related to postprandial BGL.

Three studies in this area of study on groups of people without diabetes provide conflicting results. In their study of n=68 adults (66% male; mean age 26 ± 4 years), Choi, Choi & Kim (2008) found that HF power and total power were significantly lower in the afternoon (1:30pm to 4:00pm) compared to the morning (8:30am to 11:00am), whilst LF power and LF:HF were unchanged over this time period.[438] However, Armstrong and colleagues (2011) reported no change in HF power from the morning to the afternoon in n=12 young adults aged 19-25.[439] Lipsitz and colleagues (1993) observed an increase in mean LF power after n=11 young adults (mean age 26 ± 5 years) consumed a mixed meal. Due to the lack of change in plasma norepinephrine, they concluded that this increase in LF power represented cardiac sympatho-excitation. Discrepancies in these findings may be due to differences in the age of samples between the studies, as well as sample sizes.

For LF:HF, two studies on people without diabetes or any other chronic illness demonstrated consistent results. Paolisso and colleagues (1997) showed that ingestion of glucose in subjects was associated with an increase in LF:HF, indicating an increase in sympathetic nervous system activity.[440] Tentolouris and colleagues (2003) demonstrated that LF:HF increased significantly in lean women (n=15, mean age 35 ± 11 years) in response to carbohydrate ingestion.[441] However, this study did not assess other traditional frequency-domain measures, including LF power, HF power, or total power. It is important to provide context for changes in sympathovagal balance, as indicated by LF:HF. As demonstrated in the present study, changes in LF power and HF power were shown to be consistent, as demonstrated by the lack of change in LF:HF and the decrease in total power. Though this is not a conclusive understanding of the HRV dynamics involved, as there are other factors which may have been involved, this study successfully demonstrated acute fluctuations in HRV measures in response to food intake. As outlined in Chapter 1, this is a novel area of research. It was not the aim of the UTS Pilot Study to conduct rigorous research and consolidate the current literature in this area, but rather to obtain real-world pilot data to use in the development of more rigorous studies.

2.3.2 Dynamics of Postprandial Blood Glucose

In this pilot study, mean BGL of the sample increased significantly ($p<0.01$) from 9:00am to 10:00am by an average of 1.3 mmol/L after the consumption of a regular meal. Though an increase in BGL was expected, the magnitude of this increase was difficult to anticipate. In a postprandial state, shortly after an individual has consumed food, glucose levels in the blood rise rapidly as nutrients are broken down in the gastrointestinal tract and absorbed into the blood. There is also an associated rise in circulating insulin, which aims to reduce the glycaemic spike following the meal,[442] though insulin levels were not assessed in this study. Most participants in this study finished their meal at 9:15am, and therefore the blood glucose assessment was performed roughly 45 minutes after the meal. Generally, the timing of this assessment should have captured the peak BGL of participants post-meal. However, the time required for glucose levels to peak after a meal, as well as the magnitude of that peak, are affected by a range of variables, including the composition of the meal and the health of the individual. These variables will be discussed in this section to determine if the results of the UTS Pilot Study are consistent with previous literature.

For meals composed entirely of carbohydrates, BGL increases rapidly for the first 30 minutes after consumption until it peaks, and then steadily declines until BGL returns to baseline levels 120-180 minutes after consumption.[443] However, according to Brand-Miller and colleagues (2009), this is not just true for carbohydrates. Analysis of a database of over 1000 foods showed that peak BGL occurs after 30 minutes for most meals consisting solely of one type of food, including milk, yoghurt, legumes, vegetables, soft drinks, or fruit.[444] This is generally irrespective of the glycaemic index of the food, for example white wheat bread has a higher index compared to rye bran bread, but both foods produce peak BGL 30 minutes after consumption.[443] The glycaemic index of a food is more accurately used to estimate the total change in BGL from fasting to postprandial that can be expected from eating the food, rather than the time taken to achieve that peak BGL.[445] This change is known as the incremental glucose peak, which can be obtained by subtracting the peak BGL after a meal from the baseline, or the fasting BGL. Esposito and colleagues (2008) contend that incremental glucose peak is a better marker of certain cardiovascular complications of diabetes compared to traditional markers, such as HbA_{1c} and fasting BGL.[446] This is because incremental glucose peak is a general measure of glucose variability, and higher levels of variability are associated with long-term cardiovascular disease. Incremental glucose peak is higher in homogenous meals, for example a meal like yoghurt which is composed entirely of dairy products, which means BGL will increase rapidly to a peak within 30 minutes and then decline relatively quickly.

For meals consisting of more than one type of food product, which represents most meals and thus are referred to as 'regular meals', peak BGL is achieved after 40-45 minutes.[447] Other studies, such as one conducted by Elizondo-Montemayor and colleagues (2015), show that BGL peaks between 30-45 minutes post-meal in subjects without diabetes (n=38),[448] though differences in methodology may contribute to these slight differences in timing. Based on this evidence, meals consisting of multiple food types take longer to produce peak BGL. This is because of the 'food matrix' phenomenon, which effectively slows the rate at which nutrients are absorbed across the gastrointestinal tract, such that glucose levels in the blood do not rise as quickly. This results in a 'flattening' of the glucose curve. The matrix effect is greater in mixed meals, for example mashed potatoes eaten alongside high-protein foods such as chicken breast or salad produce a lower but more sustained glycaemic response compared to mashed potatoes eaten on their own.[449] Adding different food types to a meal reduces the glycaemic response, which is a desirable outcome when considering that larger glucose fluctuations over time are associated with complications of cardiovascular disease, such as aortic stiffness.[450] Additionally, there are short-term benefits to glucose regulation. A study conducted by Nilsson and colleagues (2012) highlighted that low, but sustained BGL profiles produced by low glycaemic index carbohydrates enhance cognitive function.[451]

In summary, regular meals cause low, sustained increases in BGL. This low increase in BGL was observed in the UTS Pilot Study, recorded as a mean 1.3 mmol/L increase from 9:00am to 10:00am. Of note, there was no significant change observed in sympathetic or parasympathetic tone between 9:00am and 10:00am. The results of this study do not indicate a real-time relationship between HRV and BGL, as BGL increased without a measurable response in HRV measures. The change in HRV was delayed, indicated a delayed relationship. The lack of findings here may be due to the poor quality of ECG data. A more rigorous study design and statistical analysis with high quality data should follow-up this study to confirm. Also in this study, kilojoule intake was identified as a useful measure in this area of research, as it predicts the total glucose response, such as the magnitude of the BGL peak and the time for which postprandial BGL is sustained.[445] The glycaemic index of the meal and kilojoule value determines the height of the glycaemic response when it reaches its peak. This information, including the timing of assessments and the magnitude of the changes in BGL observed between assessments, was used to develop future studies that were also conducted as part of this PhD candidate. These will be described in later chapters.

2.3.3 Strengths and Limitations

The main purpose of this study was to provide early pilot data to assist with the design of future studies. To that end, a strength of this research was that it condensed and focused previous research, which was conducted in an Honours year, whilst allowing for preliminary analyses to be conducted on HRV measures and BGL. The six-hour methodology utilised in the Honours research was identified to be excessive, and the UTS Pilot Study successfully implemented a shorter study design of two hours whilst maintaining the same number of assessments, at 9:00am, 10:00am, and 11:00am, instead of 9:00am, 12:00pm, and 3:00pm. The 'trial-and-error' approach that was taken with this pilot study, as a prelude to larger, more robust experiments, allowed for significant limitations to be identified so they could be accounted for in the future studies. Though the poor quality of the data reduced the credibility of the results, there were several meaningful limitations identified in this study which were improved in larger, future studies.

Firstly, the use of a stationary ECG instead of a portable ECG was not ideal for this specific area of research. The ECG did not allow for long-term monitoring of HRV, as it required participants to be seated, and movement artefacts were common. This also made it difficult to observe HRV in participants whilst they ate – as participants would have been required to be seated for the entire two-hours of the study. Recording autonomic data during mealtime may have yielded noteworthy data. Future studies should consider using a Holter monitor, which is a smaller, lightweight ECG, or even a simple heart rate monitor to continuously record HRV in participants for longer periods of time. This would allow participants to leave the laboratory and eat a regular meal of their choosing whilst being continuously monitored.

Secondly, the timing of blood glucose assessments was not an accurate reflection of specific metabolic states in all cases. For the 9:00am assessment, the blood glucose assessment was an accurate representation of fasting or baseline BGL. This is because participants were required to abstain from caloric intake for eight hours prior to the 9:00am assessment. However, the second assessment at 10:00am did not necessarily equate with postprandial BGL. True postprandial BGL reflects the peak glucose level in a participant after a meal, which occurs 40-50 minutes after most regular meals. The second assessment was conducted at 10:00am for all participants, and this standardisation was to control for circadian rhythms, however this may have not been a stringent method of control given that HRV circadian rhythms are different between males and females. Due to differences in how long it took participants to leave the laboratory and finish their meal, this 10:00am assessment was not performed 40-50 minutes after many participants finished their meal, and thus was not a true reflection of postprandial BGL. As discussed in Section 1.6.5, there is a strong consensus in the literature that postprandial BGL is a

better predictor of diabetes complications such as cardiovascular disease.[191, 192, 195] Therefore, future studies of a longitudinal design should aim to assess both fasting and postprandial BGL, as the ability to predict BGL in their metabolic states may provide meaningful clinical data.

Another limitation of this study, as well as the following experiments conducted as part of this PhD, is that the exclusion criteria for daily alcoholic beverage consumption of no more than 10 standard drinks per day was substantially higher than the regular daily intake of 2 standard drinks per day. This exclusion criteria was based on requirements set by the UTS HREC, rather than based on examples set in the literature. This is a limitation since excessive alcohol consumption has been associated with autonomic neuropathy. Additionally, participants were free to leave the UTS laboratory between recordings, however this may have increased metabolic demand and reduced glycaemia, as well as caused changes in HRV. Future research should consider restricting the activity of participants whilst they are being monitored.

There were several other limitations of the UTS Pilot Study which were improved on in the later studies conducted as part of this PhD. As an exploratory study, the small sample size was a major weakness of the research, and the lack of a clinical sample, such as a diabetes sample, limited the clinical relevance of the findings. As the overall aim of this PhD candidature was to justify future research into the clinical applications of HRV measures in predicting BGL, it was important that future studies improved on the sample size and included clinical samples. Future studies should base their power calculations on the expected correlation coefficient (r-value) between the two variables of interest, which in this area of study would be BGL (e.g. fasting) and each HRV measure (e.g. total power). Improving on the sample size, as well as the overall data set, would allow for predictive models to be developed, which may better demonstrate the capabilities of HRV measures in estimating BGL.

Chapter 3. UTS T2D Study

3.1 Methods

Due to technological issues with data collection in the UTS Pilot Study, the UTS T2D Study attempted to expand on the aims and hypotheses developed in the Pilot Study through a more comprehensive study. In short, the Pilot Study investigated changes in HRV measures and BGL in n=14 participants over a two-hour period. The T2D Study aimed to explore the applications of HRV measures as non-invasive markers of BGL in people with T2D, who may benefit greatly from a non-invasive alternative to current SMBG methods. This was a core component of the thesis. Primarily, the aim was to achieve this by correlation analysis and by multiple regression analysis. It was hypothesised that the physiological association between HRV measures and BGL was strong enough to justify the clinical applications of HRV measures as a non-invasive estimate of glycaemia. It was also important to investigate whether HRV may have applications for people with optimal levels of blood glucose. Thus, the UTS T2D Study recruited a sample of people with T2D and a sample of people without any chronic illness. Associations between HRV measures and BGL were determined in different metabolic states, including fasting and postprandial, as the importance of these has been discussed.

Though the literature agrees that CGM is superior to intermittent glucose monitoring for glycaemic management in T2D, CGM systems are not indicated for T2D. There is insufficient evidence to suggest that the introduction of a reliable non-invasive method of CGM would replace intermittent glucose monitoring in T2D, as efficiency of current SMBG is already low. There is, however, strong evidence to support that introducing a non-invasive method of intermittent glucose monitoring would improve efficacy of SMBG, (Chapter 1). As such, the UTS T2D Study investigated whether HRV measures could be used to predict BGL measured by intermittent forms of SMBG, such as the 'finger spot' check most-commonly used in T2D. HRV measures may offer a non-invasive alternative to current SMBG techniques, which are universally invasive, and thus improve SMBG effectiveness.

3.1.1 Subjects

As with the Pilot Study, subjects were uninhibited by any chronic illness or regular medications, as verified by a questionnaire. Participants were deemed to be appropriate for the T2D group if they were currently living with T2D at the time of commencing the study and had been

diagnosed with the condition at least six months prior by a qualified medical practitioner. Subjects were recruited by word-of-mouth and social media, and eligible participants were invited to the researcher's laboratory at UTS to complete the protocol as approved by UTS HREC (approval number: 2014000110, then updated to ETH19-3676). In addition to standard recruitment procedures, this study was endorsed by Diabetes Australia, and an advertisement was featured on the "Take Part: Current research opportunities" section of their website.

3.1.2 Pre-study Requirements

For a detailed description of the pre-study requirements for subjects, please refer to Section 2.1.2, as they are the same as the UTS Pilot Study. In this study, requirements for participants in the T2D group were adjusted for the sake of practicality; for example, given the high comorbidity of T2D with other lifestyle-related diseases, particularly cardiovascular diseases such as hypertension, it was not practical to exclude participants in the T2D group if they had any other chronic illnesses or if they were taking any medications. However, participants were excluded if they had any history of cardiac autonomic neuropathy or if they were taking medications which affected cardiac function, such as β-blockers or tricyclic drugs.[452, 453] As with the subjects without T2D, people in the T2D sample group were required to be aged between 18-69 years and were ineligible to participate if they were currently pregnant, smoked more than 10 cigarettes per day, consumed more than 10 standard alcoholic beverages per day, or if their blood pressure (BP) was too high during the pre or post-study BP recordings (Section 2.1.3). This information was collected in the Lifestyle Appraisal Questionnaire (LAQ),[454] which provided a score of a person's lifestyle risk factors as well as a score of their perceived stress over the previous eight weeks. Subjects in the T2D group were asked to fast for at least eight hours, as with the group without T2D, but they were to continue their medications as best they could and prioritise any medical advice given to them by their doctor or diabetes educator.

3.1.3 Expansion of Previous Methodology

As with the Pilot Study, participants attended the UTS laboratory after an overnight fast or a caloric restriction of at least eight hours. They were required to sign consent forms and undergo pre-study blood pressure measurements, as described in Section 2.1.3. Provided they were not excluded by this point, they were then subject to a blood glucose assessment as described in Section 2.1.4. In the Pilot Study, issues with data collection stemmed from faulty ECG

equipment, and so this study expanded upon this original methodology by using alternative means of recording HRV.

3.1.4 Recording R-R Interval Data by Heart Rate Monitor

Though ECG is the gold-standard for determining HRV, it is not the only reputable method. ECG devices are generally expensive, bulky, and require many electrodes to be attached to the host. This may not represent an appealing alternative to current SMBG methods, even though it is non-invasive. The T2D Study investigated the potential of HRV as a non-invasive alternative to invasive procedures, and thus priority was given to lightweight, portable devices when selecting a device capable of determining HRV. This led to the selection of the BodyGuard 2 (Firstbeat Technologies, Finland) as illustrated in Figure 3.1. HRV measures obtained from portable devices have a small level of error compared to measures obtained from an ECG, however two recent reviews of the literature have concluded that this small error is acceptable given the superior convenience and efficiency of the portable devices.[455, 456]

Figure 3.1 Application of BodyGuard 2 heart rate monitor.

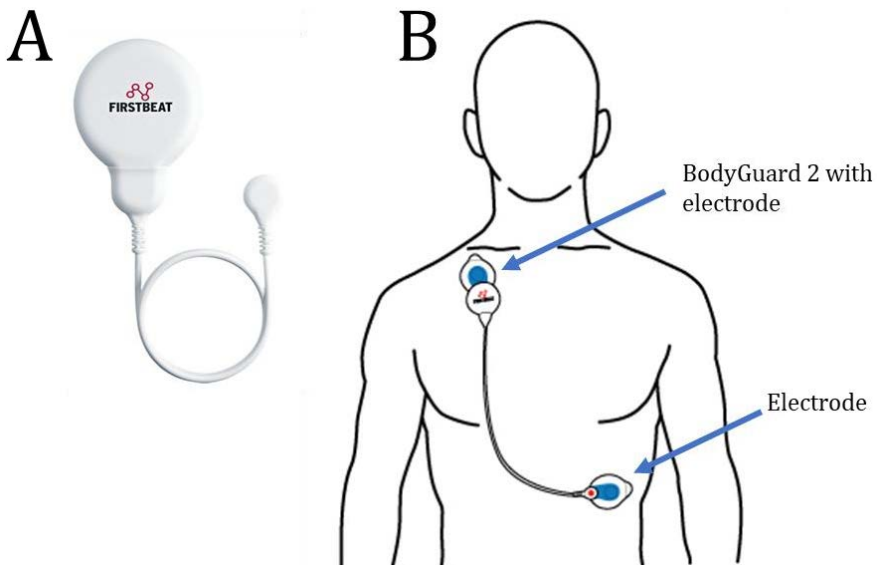

Figure 3.1 shows the device used to capture R-R interval data in this study. **(A)** The Firstbeat BodyGuard 2 device without electrodes attached. The large 'head' of the device could be removed, exposing a USB-A port which could connect to a computer for data download. **(B)** The head of the monitor attached below the collarbone on the right side of the body by an adhesive electrode. The tail-end of the device also attached to the surface of the skin by an electrode and was positioned on the left side of the body on the rib cage. Image taken and adapted from Firstbeat Technologies (2021).

Due to its portability, the BodyGuard 2 remained attached to participants for the entire duration of the study. It was attached to participants at two sites on their torso (Figure 3.1) after these

sites had been cleaned using isopropyl alcohol wipes. The device recorded continuously, even when outside of the laboratory, and this allowed for subject's HRV to be calculated at any point. After the device was returned from each participant at the conclusion of the study, the R-R interval data was downloaded onto a computer and processed using Kubios HRV Premium. Extrapolation of HRV measures from this point onward followed the exact same methodology as described in Section 2.1.5.2. The main difference between the Pilot Study and the T2D Study in regard to HRV determination was the method by which R-R interval data was obtained.

3.1.4.1 Selection of HRV Intervals to be Analysed

The BodyGuard 2 recorded R-R interval data continuously, and thus there were many options when it came to selecting which periods to analyse. It was also possible to analyse R-R data in periods longer than 10 minutes. However, it was important that all periods to be compared were of the same duration. This is because longer recordings, by their nature, show increased HRV, and thus statistical comparison between a 10-minute and a 20-minute epoch would be meaningless.[358] Consequently, the focus of the UTS T2D Study was to capture 10-minute intervals of HRV data that corresponded with noteworthy states during the study. The first of these states was 'fasting HRV' which was calculated from the 10 minutes of R-R data that immediately preceded the fasting BGL assessment. This state may also represent 'resting HRV', as participants were allowed to rest for 10 minutes prior to recording this 10-minute state. Fasting HRV was recorded to set a baseline, as it was an estimation of autonomic activity without the effects of caloric intake.

The second of these noteworthy states was 'prandial HRV', which corresponded with autonomic activity whilst participants were eating their meal. It was calculated by selecting 10 minutes of R-R interval data that overlapped with the eating period of each participant, then processing that into HRV measures. As with the Pilot Study, subjects in the UTS T2D Study were asked to consume a regular meal after their initial fasting assessment (see Section 2.1.6.1). However, instead of returning at a set time, such as 10:00am, participants were required to return 30 minutes after finishing their meal. They were also asked to report on the contents of their meal (see Section 2.1.6.2 for more details), as well as the times in which they started and finished their meal. In the Pilot Study, it was recognized that circadian rhythms were responsible for natural but significant variation in HRV and BGL (Section 2.1.4). This is true not just for people without diabetes, but also people with diabetes.[407] Therefore, all participants in this study were restricted to being assessed only in the morning, between the hours of 7:00am and 12:00pm.

3.1.5 Postprandial Assessment

There are two key stages of metabolism during which snapshot data of BGL is considered most useful. The first of these is fasting, when BGL is lowest, and the second is postprandial, when BGL peaks after a meal. One of the most desirable outcomes of new and upcoming measures of BGL is the ability to accurately estimate BGL during either of these two states (Section 1.11). A device capable of non-invasively estimating glycaemia in an individual in a fasting state as well as a postprandial state would represent an appealing alternative to current SMBG. Other authors have reinforced the importance of assessing both fasting and postprandial BGL when investigating the relationship of BGL with HRV measures.[457] As with the Pilot Study, fasting BGL in the UTS T2D Study was captured using a blood glucose assessment after participants undertook an overnight fast. When attempting to capture postprandial BGL, there were several factors that needed to be considered. True postprandial BGL is difficult to measure, as the blood glucose assessment is an intermittent measure of BGL and thus cannot track glycaemia continuously. Even readings from CGM systems lag behind true postprandial BGL as it changes rapidly after a meal. Another factor to consider in this study was that different participants chose meals of different compositions, as expected, however this affected the time required for BGL to reach its peak.

Based on evidence provided in the Discussion of the Pilot Study (Section 2.3), as well as evidence from the literature, it was expected that peak BGL would be achieved 45 minutes on average after participants ate a regular meal.[442] There are various factors which affect the overall shape of the glucose curve for different individuals, however, the main confounder in the UTS T2D Study was the nutritional content of the meal. Meals high in carbohydrates require less time to produce a peak glycaemic response. To accommodate for the fact that some participants in this study consumed meals higher or lower in carbohydrates than average, which would cause their BGL to peak earlier or later, respectively, all participants underwent a BGL assessment twice. The first was 40 minutes after eating, and the second was 50 minutes after eating, for a total of two blood glucose assessments 10 minutes apart (Figure 3.2). Both BGL values were recorded, but only the higher value was used in the analysis as it was a close approximation of peak postprandial BGL.

Figure 3.2 Timing of assessments in the UTS T2D Study.

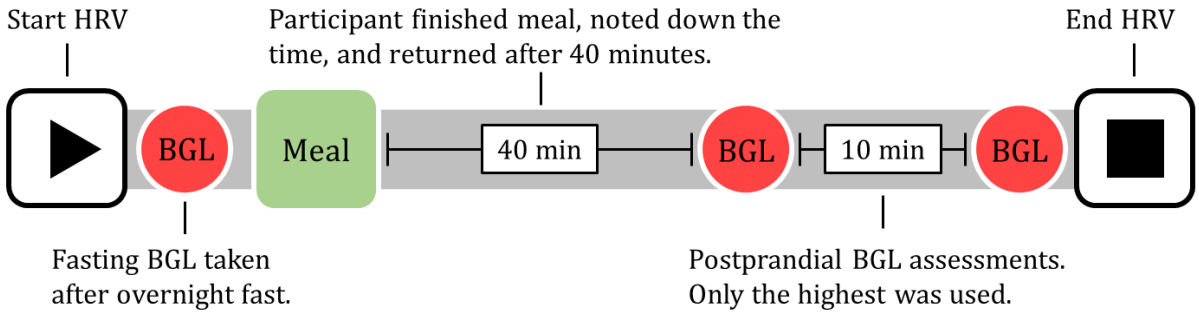

Figure 3.2 summarizes the timing of the three glucose assessments relative to the meal and the recording of heart rate. In short, recording of HRV began prior to the fasting BGL assessment, and ended only once the final postprandial assessment was measured. Following the fasting assessment, participants were asked to leave the laboratory and consume a meal of their choice nearby, as well as note down the time at which they finished the meal. Participants returned 40 minutes after this point for a second blood glucose assessment. A third assessment was conducted 10 minutes after the second, and the higher of these two was used as the postprandial BGL. **BGL** = Blood glucose level, **HRV** = Heart rate variability, **Min** = Minutes.

3.1.6 HbA_{1c} Assessment

HbA_{1c} level is considered an essential tool for monitoring diabetes, and people living with T2D routinely undergo assessments of HbA_{1c} as part of how they manage their condition. To properly determine whether HRV measures are predictive of glycaemia, HbA_{1c} levels were assessed in people with T2D to encourage further research into the value of HRV as a predictor of glycaemia. In the present study, HbA_{1c} levels were obtained from each T2D participant using the A1cNow+ Portable HbA_{1c} Test Kit (Point of Care Diagnostics Pty Ltd, North Rocks, Australia). These disposable devices measured HbA_{1c} levels from a blood sample, providing results within five minutes. Like the blood glucose assessment (Section 2.1.4), the HbA_{1c} assessment required only a small sample of blood from a fingerprick. This assessment was carried out at the same time as the fasting BGL assessment, because HbA_{1c} levels reflect only long-term glycaemia, they are not confounded by short-term metabolic states such as fasting.[458] HbA_{1c} levels of subjects without diabetes were not recorded because it was anticipated there would be little to no variation based on previous literature, and thus analysis would reveal little of importance.

3.1.7 Statistical Analysis

The statistical analysis investigated HRV and BGL in people with TD2 and people without T2D separately. Independent t-tests were performed in SPSS version 22.0 (IBM SPSS Statistics, USA)

to identify any significant ($p<0.05$) differences between the T2D group and the group without T2D in various categories. These included demographic features such as age, as well as physiological features such as blood pressure, BGL, and HRV measures. Independent t-tests were also used to compare these same sets of factors between men and women in each group. Primarily, this was to determine whether there was an appreciable effect of sex on the variables of interest, as this would require that sex be controlled for in the statistical analysis. For comparison of 'within-subjects' changes, such as comparing the change in BGL from fasting to postprandial levels in just people without diabetes, paired-sample t-tests were used.

Furthermore, the aim of the analysis was to explore the potential for HRV measures to predict glycaemia in both groups. The predictor variables were largely measures of HRV, including LF power, HF power, total power, and LF:HF. For a detailed description of how these measures were obtained, see Section 2.1.5.2. Additional predictor measures were age, BMI, and kilojoule intake. The dependent variables that the present study sought to predict were fasting BGL, postprandial BGL, and, in the group with diabetes, also HbA_{1c}. Correlation analysis was conducted on BGL data and HRV measures to explore potential relationships between the variables. Partial Pearson's correlations were used in all instances, controlling for age and BMI. Additionally, kilojoule intake was applied as a covariate for postprandial BGL, which may have been confounded by the variable meals of participants, but not for fasting BGL correlations, as participants had not eaten yet for this assessment. In instances where multiple HRV measures were correlated with a single dependent variable, such as fasting BGL or postprandial BGL, a multiple linear regression was carried out to identify the strongest predictor variables and to establish a regression model demonstrating the proportion of the outcome variable (BGL) which can be explained by the predictor variables (HRV measures). Regression models, a basic form of machine learning, perform reasonably well compared to advanced machine learning models in terms of predicting glycaemic events in diabetes.[336] However, it is important that the model is implemented in real-time, as this provides clinical significance.

To ensure that the assumptions of the ANOVA were not violated, the data set was checked for outliers, normal distribution, linear relationships, homoscedasticity, and multicollinearity. Normal Q-Q plots of the studentized residuals were used to check that the data points were linearly organised, which confirmed normal distribution. Linear relationships between the dependent variable (BGL) and independent variables (HRV measures) were confirmed by scatter plots and are shown in the Results. Homoscedasticity was assumed by checking that scatterplots of the residuals were equally distributed and did not follow any pattern or skewed distribution. To ensure the independent variables were not highly correlated with each other, which may affect the interpretation of the multiple regression model, the variables were

checked for multicollinearity in SPSS. Variance inflation factor values were checked to ensure they were between 1-10, indicating no multicollinearity.

To determine a sample size (n) which would provide sufficient power for the study, J. Cohen's "A Power Primer" was consulted. For a correlation analysis between two independent groups, which included a T2D group and a group of subjects without T2D, with a significance (α) set to 0.05 and a large effect size (r) of 0.5, a sample size of n=30 in each group would provide a power level of 0.8, or an 80% probability of rejecting a false null hypothesis at the 5% significance level. As such, the recruitment goal was at least 30 people with and 30 people without T2D.

3.2 Results

The UTS T2D Study investigated the relationship between frequency-domain HRV measures and glycaemia in a sample of people with T2D as well as a sample of people without any chronic illness. Recruitment was facilitated through social media advertising as well as with assistance from Diabetes Australia. For this study, recruitment of n=30 participants in each group was not possible due to COVID-19 restrictions implemented by the Australian Federal Government and NSW State Government after 21st March 2020, with repeated restrictions imposed between 2020 and 2021. As such, the UTS T2D study was concluded early after n= 27 people with T2D and n=29 subjects without T2D were recruited.

3.2.1 Demographic Differences: Non-Diabetes vs. Diabetes

Notable demographic data, including mean age and BMI of each sample, is presented in Table 3.1. Additionally, mean scores are provided for the LAQ Part 1 (scales higher with lifestyle risk factors, such as poor diet) and LAQ Part 2 (assesses the effect of stress on a person's life). Independent t-tests established whether there were any significant differences ($p<0.05$) in these variables between the two samples. Additional information was collected from the T2D sample, for example the mean HbA_{1c} level was 6.9 ± 1.4% and the median level was 6.6%. The mean duration of diabetes was 10 ± 8 years. 52% of the T2D sample reported that they knew of at least one first-degree relative with T2D, 44% knew of at least one second-degree relative with T2D, and 26% knew of at least one first-degree and one second-degree relative with T2D.

Table 3.1 Means and standard deviations for demographic differences between the group without diabetes (n=29) and the group with diabetes (n=27). Data is presented as mean ± standard deviation. BMI was calculated as weight (in kilograms) divided by height (in metres) squared. The LAQ Part 1 was scored separately from Part 2, and mean scores are provided in this table. ******* = p<0.001, **BMI** = Body mass index, **kg/m²** = Kilogram per square metre, **LAQ** = Lifestyle Appraisal Questionnaire.

Variable	Non-diabetes group	Type 2 diabetes	p-value
n	29	27	-
Age (years)	33 ± 13	51 ± 10	<0.001***
BMI (kg/m²)	24 ± 3	33 ± 6	<0.001***
LAQ Part 1	10 ± 8	20 ± 6	<0.001***
LAQ Part 2	18 ± 13	22 ± 10	0.24

From Table 3.1, it can be inferred that individuals in the T2D sample were approximately 18 years older on average (p<0.001) and had higher BMI (p<0.001) compared to individuals in the group without diabetes. As such, correlation analyses may need to control for these demographic differences. People with T2D also scored higher in the LAQ Part 1 (p<0.001), and though mean LAQ Part 2 scores were also higher in the T2D sample, the difference compared to the group without diabetes was not significant.

3.2.2 Physiological Differences: Non-Diabetes vs. Diabetes

Mean values for physiological data, including blood pressure, BGL, and HRV measures, are presented in Table 3.2. Blood pressure was averaged from pre-study and post-study values. Kilojoule intake was determined using a food diary which participants completed after eating a regular meal. Independent t-tests were conducted to determine whether there were any significant differences in these physiological measures between the two sample groups.

Table 3.2 Means and standard deviations for physiological differences between the group without diabetes (n=29) and group with diabetes (n=27). Data is presented as mean ± standard deviation. Fasting BGL was recorded after a fast of at least eight hours and postprandial BGL was recorded 40-50 minutes after a meal. Fasting HRV was determined using the first 10-minutes of the recording. LF power, HF power, and total power are natural log transformed. ***** = p<0.05, ****** = p<0.01, ******* = p<0.001, **BGL** = Blood glucose level, **BP** = Blood pressure, **HF** = High frequency, **HRV** = Heart rate variability, **kJ** = Kilojoule, **LF** = Low frequency, **LF:HF** = low to high frequency ratio, **mmHg** = Millimetres of mercury, **mmol/L** = Millimoles per litre, **ms²** = Milliseconds squared.

Variable	Non-diabetes group	Type 2 diabetes	p-value
BP (mmHg)			
Systolic	115 ± 10	124 ± 12	<0.01**
Diastolic	74 ± 7	83 ± 8	<0.001***
BGL (mmol/L)			
Fasting	4.9 ± 0.5	8.1 ± 2.2	<0.001***
Postprandial	6.4 ± 1.1	11.2 ± 3.7	<0.001***
Change in BGL	1.4 ± 1.0	3.1 ± 2.4	<0.01**
Kilojoule intake (kJ)	2280 ± 1537	1600 ± 878	0.05*
Fasting HRV (ms^2)			
LF power	6.9 ± 0.7	5.7 ± 1.2	<0.001***
HF power	5.8 ± 0.8	4.3 ± 1.4	<0.001***
Total power	7.6 ± 0.6	6.3 ± 1.1	<0.001***
LF:HF	3.9 ± 2.6	5.9 ± 4.1	0.04*

Included in Table 3.2 were a considerable number of significant differences between the two different groups. Systolic (p<0.01) and diastolic (p<0.001) blood pressure were both 9 mmHg higher on average in the T2D sample compared to the sample without diabetes. This difference is highlighted in Figure 3.3

Figure 3.3 Comparison of systolic and diastolic blood pressure between participants without diabetes (n=29) and diabetes participants (n=27).

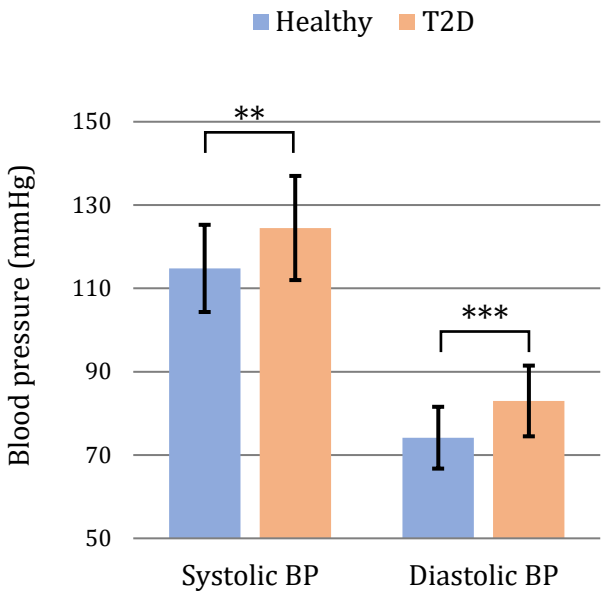

Figure 3.3 provides blood pressure means with error bars as standard deviations. Blood pressure was averaged from the three pre-study and three post-study recordings, all of which were conducted after five minutes at rest. ** = p<0.01, *** = p<0.001, **BP** = Blood pressure, **mmHg** = Millimetres of mercury, **T2D** = Type 2 diabetes.

Table 3.2 also presented BGL data. Compared to the group without T2D, BGL was higher in the T2D group in both the fasting (p<0.001) and postprandial (p<0.001) state. In addition to this, paired t-tests determined that BGL increased significantly from fasting to postprandial in the group without diabetes (p<0.001), and in the T2D group (p<0.001). These results are visualized in Figure 3.4. There was also greater variability in BGL for the T2D sample, as indicated by the larger standard deviations compared to the sample without diabetes. There was a significantly higher change in BGL across the T2D group (p<0.01). During the meal, T2D participants consumed almost 700 kilojoules less on average than their counterparts without diabetes.

Figure 3.4 Comparison of blood glucose level and kilojoule intake between the group without diabetes (n=29) and the diabetes group (n=27).

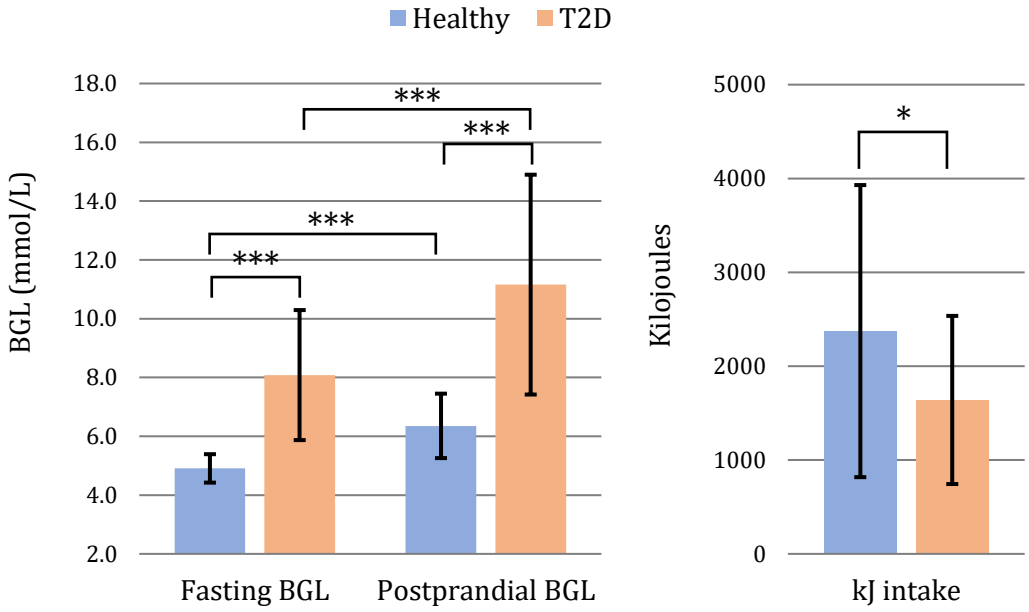

Figure 3.4 compares the means of fasting and postprandial BGL with error bars as standard deviations. BGL was compared at fasting vs. postprandial, as well as non-diabetes vs. diabetes. Paired t-tests compared fasting BGL with postprandial BGL in the non-diabetes group and compared fasting BGL with postprandial BGL in the T2D group. Independent t-tests compared fasting BGL between the two samples and then compared postprandial BGL between the two samples. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **BGL** = Blood glucose level, **kJ** = Kilojoule, **mmol/L** = Millimoles per litre, **T2D** = Type 2 diabetes.

Finally, Table 3.2 also presented HRV data from the fasting state, which represents baseline HRV. Though not shown, prandial HRV showed the same differences between non-T2D and T2D samples. For simplicity, only fasting HRV was used to compare baseline HRV between the groups. It is important to remember that, though it is easy to compare different HRV measures because some share the same units (ms^2), this is not an appropriate analysis. Generally, LF power is high than HF power, just as systolic blood pressure is usually higher than diastolic blood pressure. This is not a meaningful comparison. Independent t-tests were only conducted to determine if HRV measures were significantly different between the group with diabetes and the group without. All measures were significantly different between the two groups. People with T2D had lower LF power ($p<0.001$), HF power ($p<0.001$), and total power ($p<0.001$) but higher LF:HF ($p<0.05$). These differences are highlighted in Figure 3.5.

Figure 3.5 Comparison of heart rate variability measures between non-diabetes (n=29) and diabetes groups (n=27).

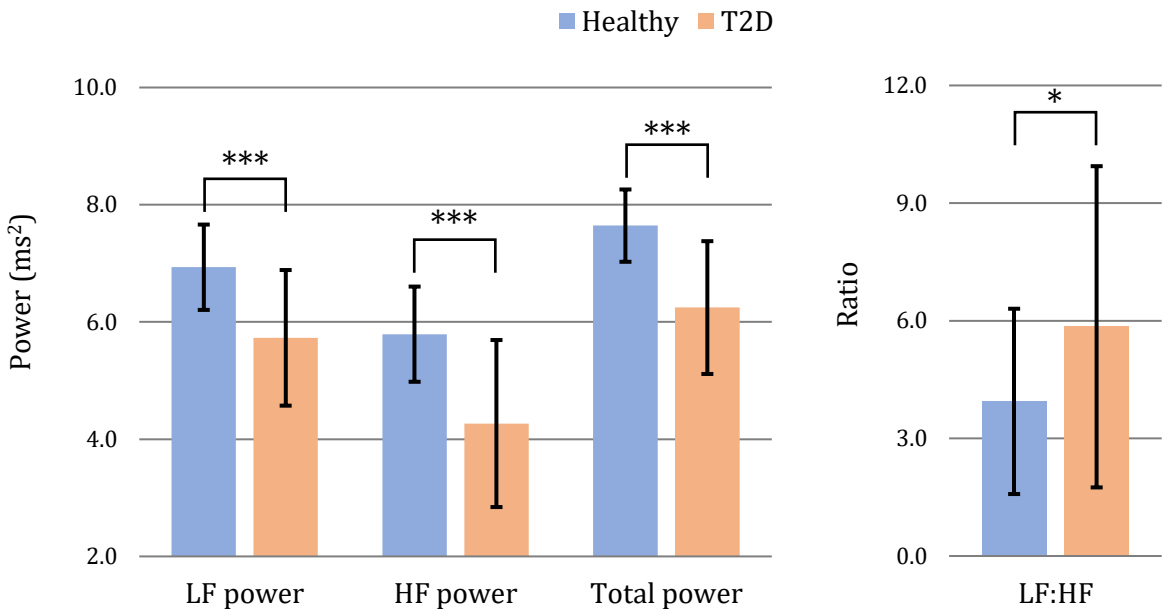

Figure 3.5 presents the means of the different HRV measures with error bars as standard deviations. HRV measures were from the fasting state and were determined using the first 10-minutes of the recording. * = $p<0.05$, *** = $p<0.001$, **HF** = High frequency, **LF** = Low frequency, **ms²** = Milliseconds squared, **T2D** = Type 2 diabetes.

3.2.2.1 Males vs. Females

The sample size was sufficiently large to compare differences in males (n=15) and females without diabetes (n=14), as well as T2D males (n=13) and T2D females (n=14). The primary goal was to establish whether there were significant differences between men and women in the samples, as this would have indicated that sex had an appreciable confounding effect on the data and should be controlled for in the statistical analysis. Mean age and BMI, as well as kilojoule intake, are compared in Table 3.3 for the sample without diabetes as well as the T2D sample. Mean LAQ scores were also compared between males and females, but no significant differences were observed. No significant differences were observed between age of males and age of females, or BMI of males and BMI of females, in either sample. Within the group without diabetes, males consumed over 1000 kilojoules on average than their female counterparts ($p = 0.04$).

Table 3.3 Demographic differences between males (n=15) and females (n=14) without diabetes, as well as males (n=13) and females with diabetes (n=14). Data is presented as mean ± standard deviation. BMI was calculated as weight (in kilograms) divided by height (in metres) squared. Kilojoule intake was determined using a food diary which participants completed after eating a regular meal. ***** = $p<0.05$, **BMI** = Body mass index, **kg/m²** = Kilogram per square metre, **kJ** = Kilojoule, **T2D** = Type 2 diabetes.

	Non-diabetes			**T2D**		
Variable	Males	Females	p-value	Males	Females	p-value
n	15	14	-	13	14	-
Age (years)	32 ± 14	33 ± 11	0.95	49 ± 12	51 ± 10	0.70
BMI (kg/m²)	25 ± 4	24 ± 3	0.43	31 ± 6	34 ± 7	0.27
kJ intake	2900 ± 1900	1700 ± 800	0.04*	1800 ± 1100	1500 ± 600	0.37

Physiological data from each sample was also separated into males vs. females, including systolic and diastolic blood pressure, fasting and postprandial BGL, and HRV measures (Table 3.4). The four main HRV measures that were investigated in this study were LF power, HF power, total power, and LF:HF. Only HRV measures from the fasting state are presented in Table 3.4. There were no significant differences in systolic or diastolic blood pressure between males and females for either sample. To demonstrate that differences in physiological variables between diabetes and non-diabetes groups were consistent for both males and females, additional independent t-tests were used to compare physiological data in males between the two samples as well as females between the two samples. The results from these analyses are presented in Figure 3.6. It was determined that males in the T2D sample had higher diastolic blood pressure than their non-diabetes counterparts ($p<0.05$). Compared to females in the non-diabetes sample, T2D females had both higher systolic ($p<0.05$) and diastolic ($p<0.01$) blood pressure. Statistical analysis shown in Table 3.4 only compares men and women from the same group, either T2D or non-diabetes. Statistical comparison of men without diabetes compared with men with diabetes, for example, was conducted but is only presented in figures.

Table 3.4 Physiological differences between males (n=15) and females (n=14) without diabetes, as well as males (n=13) and females with diabetes (n=14). Data is presented as mean ± standard deviation. Systolic and diastolic blood pressure values were averaged from pre-study and post-study values. Change in BGL = 'postprandial' subtracted by 'fasting' BGL. Fasting HRV was determined using the first 10 minutes of the recording. **BGL** = Blood glucose level, **BP** = Blood pressure, **HF** = High frequency, **HRV** = Heart rate variability, **LF** = Low frequency, **LF:HF** = low to high frequency ratio, **mmHg** = Millimetres of mercury, **mmol/L** = Millimoles per litre, **ms²** = Milliseconds squared.

	Non-diabetes			**T2D**		
Variable	Males	Females	p-value	Males	Females	p-value
n	15	14	-	13	14	-
BP* (mmHg)						
Systolic	116 ±11	113 ± 11	0.50	125 ± 13	124 ± 12	0.74
Diastolic	74 ± 8	74 ± 7	0.91	81 ± 8	85 ± 8	0.32
BGL (mmol/L)						
Fasting	5.1 ± 0.5	4.7 ± 0.4	0.05	8.0 ± 2.0	8.2 ± 2.4	0.83
Postprandial	6.5 ± 1.1	6.2 ± 1.1	0.60	10.7 ± 3.0	11.6 ± 4.4	0.58
Change	1.4 ± 1.1	1.5 ± 1.0	0.74	2.7 ± 2.4	3.4 ± 2.5	0.51
Fasting HRV (ms^2)						
LF power	6.7 ± 0.7	7.1 ± 0.7	0.16	5.9 ± 1.2	5.6 ± 1.2	0.53
HF power	5.7 ± 0.7	5.8 ± 0.9	0.78	4.3 ± 1.6	4.2 ± 1.3	0.84
Total power	7.5 ± 0.5	7.8 ± 0.6	0.11	6.2 ± 1.2	6.3 ± 1.1	0.96
LF:HF	3.6 ± 2.6	4.3 ± 2.2	0.48	5.9 ± 4.3	5.8 ± 4.1	0.92

Figure 3.6 Differences in blood pressure between males (n=15) and females (n=14) without diabetes as well as males (n=13) and females with diabetes (n=14).

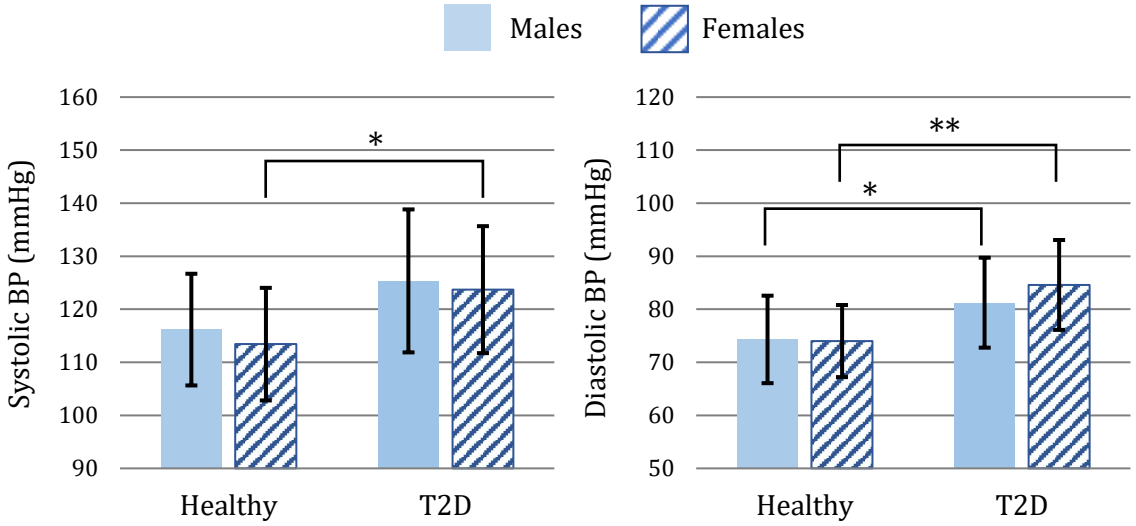

Figure 3.6 presents the means of blood pressure with error bars as standard deviations. Systolic and diastolic blood pressure values were averaged from pre-study and post-study values. Systolic and diastolic values are reported at different levels on the same scale, as diastolic blood pressure is generally lower than systolic. * = $p<0.05$, ** = $p<0.01$, **BP** = Blood pressure, **mmHg** = Millimetres of mercury, **T2D** = Type 2 diabetes.

Returning to Table 3.4, fasting BGL was not significantly different in males compared to females without diabetes, or between males and females with T2D. Neither postprandial BGL nor the change in BGL from fasting to postprandial was significantly different between the sexes in either sample. Though not indicated in the table, independent t-tests confirmed that fasting ($p<0.001$) and postprandial ($p<0.001$) BGL were both higher in T2D males ($p<0.001$) and T2D females ($p<0.001$) compared to their non-diabetes counterparts. These results are shown in Figure 3.7

Figure 3.7 Differences in blood glucose level between males (n=15) and females (n=14) without diabetes, as well as males (n=13) and females with diabetes (n=14).

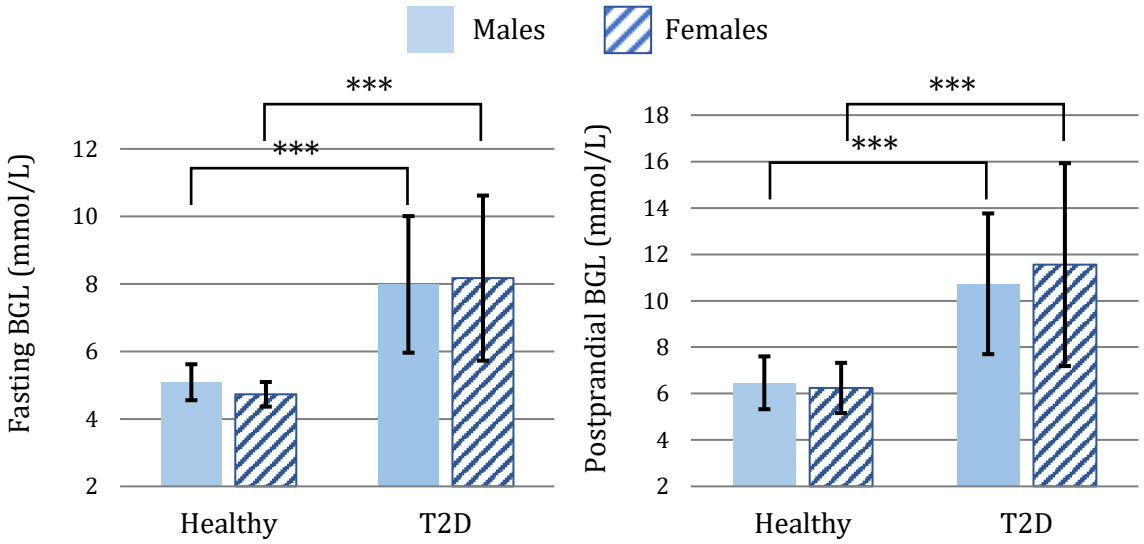

Figure 3.7 visualises the means of fasting and postprandial BGL with error bars as standard deviations. Fasting BGL was recorded after a caloric restriction of at least eight hours and postprandial BGL was recorded 40-50 minutes after eating a meal. * = $p<0.05$, *** = $p<0.001$, **BGL** = Blood glucose level, **mmol/L** = Millimoles per litre, **T2D** = Type 2 diabetes.

Statistical analysis was used to explore differences in each measure between males and females. Though different HRV measures can appear similar in units, it is not appropriate to compare them, as this would be analogous to comparing differences between systolic and diastolic blood pressure. Differences in their values are due to the intrinsic nature of each measure. In Table 3.4 it was shown that there were no significant differences in any HRV measure between males and females in the sample without diabetes, and none in the T2D sample. These analyses are visualised in Figure 3.8 for the sample without diabetes and Figure 3.9 for the T2D sample.

Figure 3.8 Differences in heart rate variability measures between males (n=15) and females (n=14) without diabetes.

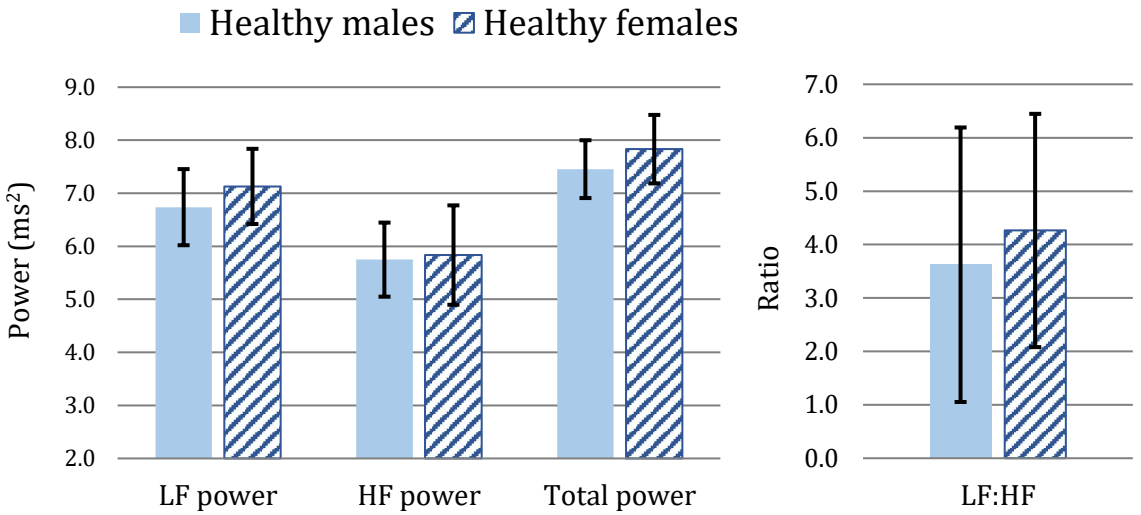

Figure 3.8 presents differences in HRV measures between males and females without diabetes with error bars as standard deviations. HRV measures are all from the fasting state, which was determined using the first 10 minutes of the recording. There are significant differences in power level, but this is implicit in their nature. **BGL** = Blood glucose level, **HF** = High frequency, **LF** = Low frequency, **ms²** = Milliseconds squared.

Figure 3.9 Differences in heart rate variability measures between males with diabetes (n=13) and females with diabetes (n=14).

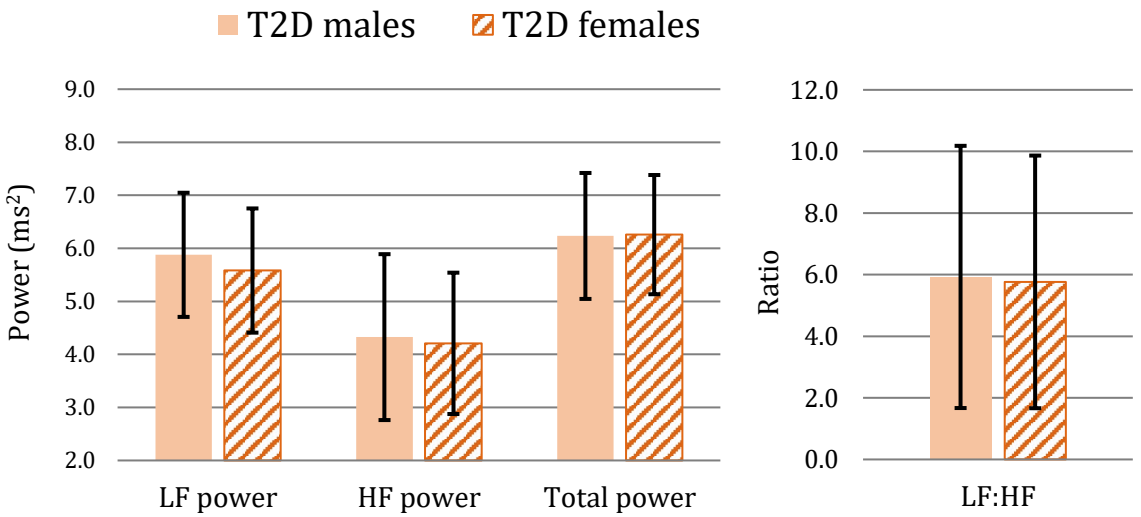

Figure 3.9 presents differences in HRV measures between T2D males and females with error bars as standard deviations. HRV measures are all from the fasting state, which was determined using the first 10 minutes of the recording. **BGL** = Blood glucose level, **HF** = High frequency, **LF** = Low frequency, **ms²** = Milliseconds squared, **T2D** = Type 2 diabetes.

3.2.3 Correlation Analysis

The primary aim of the correlation analysis was to investigate associations between HRV measures and BGL in each sample as a basis for developing regression models, which are a basic form of machine learning. Before this analysis could be performed, it was important to determine if any confounding variables existed in the data set, as partial Pearson's correlation allows for multiple variables to be included as covariates to account for their confounding effect. Based on the literature and the scope of this PhD candidature, the main confounders of concern for this data set were sex, kilojoule intake, age, and BMI. In Section 3.2.2.1, it was demonstrated that there were no significant differences between men and women for either sample, with the exception that kilojoule intake was lower in women compared to men. As such, sex was not controlled for in the partial correlation analysis. Kilojoule intake was identified as a confounder and was included as a covariate for any correlation analysis involving postprandial BGL, for otherwise there would be no manner of controlling for the different meal composition of participants. As subjects had not yet eaten for the fasting assessment, kilojoule intake was not included as a covariate for any analysis involving fasting BGL.

Several methods were used to determine the confounding effect of age and BMI on the data. These two were correlated against the variables of interest, which were fasting BGL, postprandial BGL, and each of the four HRV measures from the fasting state as well as the prandial state. For the group without diabetes, participant age was correlated with fasting BGL ($r = 0.46$; $p = 0.02$) but not postprandial BGL. Age was also associated with prandial LF power ($r = -0.47$; $p = 0.02$), prandial HF power ($r = -0.46$; $p = 0.02$), and prandial total power ($r = -0.43$; $p = 0.03$), but not prandial LF:HF. Age was also not related to any of the four HRV measures from the fasting state. Participant BMI was significantly correlated with fasting BGL ($r = 0.47$; $p = 0.01$) and postprandial BGL ($r = 0.41$; $p = 0.03$). As with age, BMI was significantly correlated with prandial LF power ($r = -0.55$; $p < 0.01$), prandial HF power ($r = -0.40$; $p = 0.04$), and prandial total power ($r = -0.49$; $p = 0.01$), but not prandial LF:HF or any of the four HRV measures from the fasting state. As for the T2D group, age and BMI weren't associated with any of the variables of interest, which included fasting and postprandial BGL, and each of the four HRV measures from the fasting state as well as the prandial state. Though these findings indicated that age and BMI were confounders in only the sample without diabetes, age and BMI were included as covariates in the analysis of both the sample without diabetes and the T2D sample for consistency. A review of the literature also indicated that controlling for age and BMI in the analysis was appropriate, as most studies agree they are strongly related and can confound the statistical analysis.[459, 460] It is important that the analysis of the present study

investigates whether HRV measures predict BGL even when accounting for age and BMI, as well as kilojoule intake.

3.2.3.1 Partial Correlations

Partial Pearson's correlations were used to investigate associations between HRV and BGL in the two groups separately, as correlations may be stronger or weaker in T2D compared to people without diabetes. HRV measures from the fasting state, including LF power, HF power, total power, and LF:HF, were correlated against both fasting and postprandial BGL for the group without diabetes. This analysis was repeated using data from the T2D group (Table 3.5). Then, the same four HRV measures were extrapolated from the prandial state – when participants were eating – and correlated against postprandial BGL, but not fasting BGL. This was because the prandial state comes after fasting, and there is little value in predicting BGL from a previous metabolic state using HRV. As with the fasting HRV measures, this analysis was conducted for both the group with and the group without T2D separately (Table 3.5). Also, kilojoule intake was not included as a covariate for HbA_{1c}, as recent food intake has no effect on HbA_{1c} levels. HbA_{1c} was not significantly associated with any HRV measure when controlling for age and BMI, though HbA_{1c} was significantly associated with fasting BGL ($r = 0.63$; $p < 0.01$) and postprandial BGL ($r = 0.60$; $p < 0.01$) in the diabetes group. As HbA_{1c} was not recorded in participants without diabetes, there was no correlation analysis to perform in this group.

Table 3.5 Partial Pearson's correlations between heart rate variability measures and blood glucose level, accounting for age, body mass index, and kilojoule intake as covariates in the group without diabetes (n=29) and the diabetes group (n=27). Data is presented as mean ± standard deviation. The missing r and p values for fasting blood glucose level are because these analyses do not fit the scope of this PhD. It was only relevant to investigate whether heart rate variability measures could predict current of future blood glucose. ***** = p<0.05, ****** = p<0.01, **BGL** = Blood glucose level, **BMI** = Body mass index, **HF** = High frequency, **HRV** = Heart rate variability, **LF** = Low frequency, **LF:HF** = low to high frequency ratio, **p** = p-value, **r** = correlation coefficient.

Variable	Non-diabetes		Type 2 diabetes	
	Fasting BGL	Postprandial BGL	Fasting BGL	Postprandial BGL
Fasting HRV				
LF power				
r	0.10	-0.13	-0.63	-0.46
p	0.64	0.55	<0.01**	<0.05*
HF power				
r	0.04	-0.31	-0.57	-0.33
p	0.84	0.15	<0.01**	0.17
Total power				
r	-0.09	-0.43	-0.66	-0.51
p	0.69	0.04*	<0.01**	0.03*
LF:HF				
r	-0.03	0.16	0.35	0.08
p	0.89	0.47	0.14	0.75
Prandial HRV				
LF power				
r	-	-0.33	-	-0.45
p	-	0.13	-	0.05
HF power				
r	-	-0.39	-	-0.43
p	-	0.07	-	0.07
Total power				
r	-	-0.61	-	-0.48
p	-	<0.01**	-	0.04*
LF:HF				
r	-	0.47	-	0.24
p	-	0.02*	-	0.31

From Table 3.5 it can be inferred that fasting BGL was not significantly associated with any of the fasting HRV measures in the group without diabetes. However, postprandial BGL was correlated with fasting total power (r = -0.43; p = 0.04), prandial total power (r = -0.61; p <

0.01), and prandial LF:HF (r = 0.47; p = 0.02) in the group without diabetes. The scatter plots for these significant correlations are presented in Figure 3.10.

Figure 3.10 Scatter plots for postprandial blood glucose level and significantly correlated heart rate variability measures in the sample without diabetes (n=29).

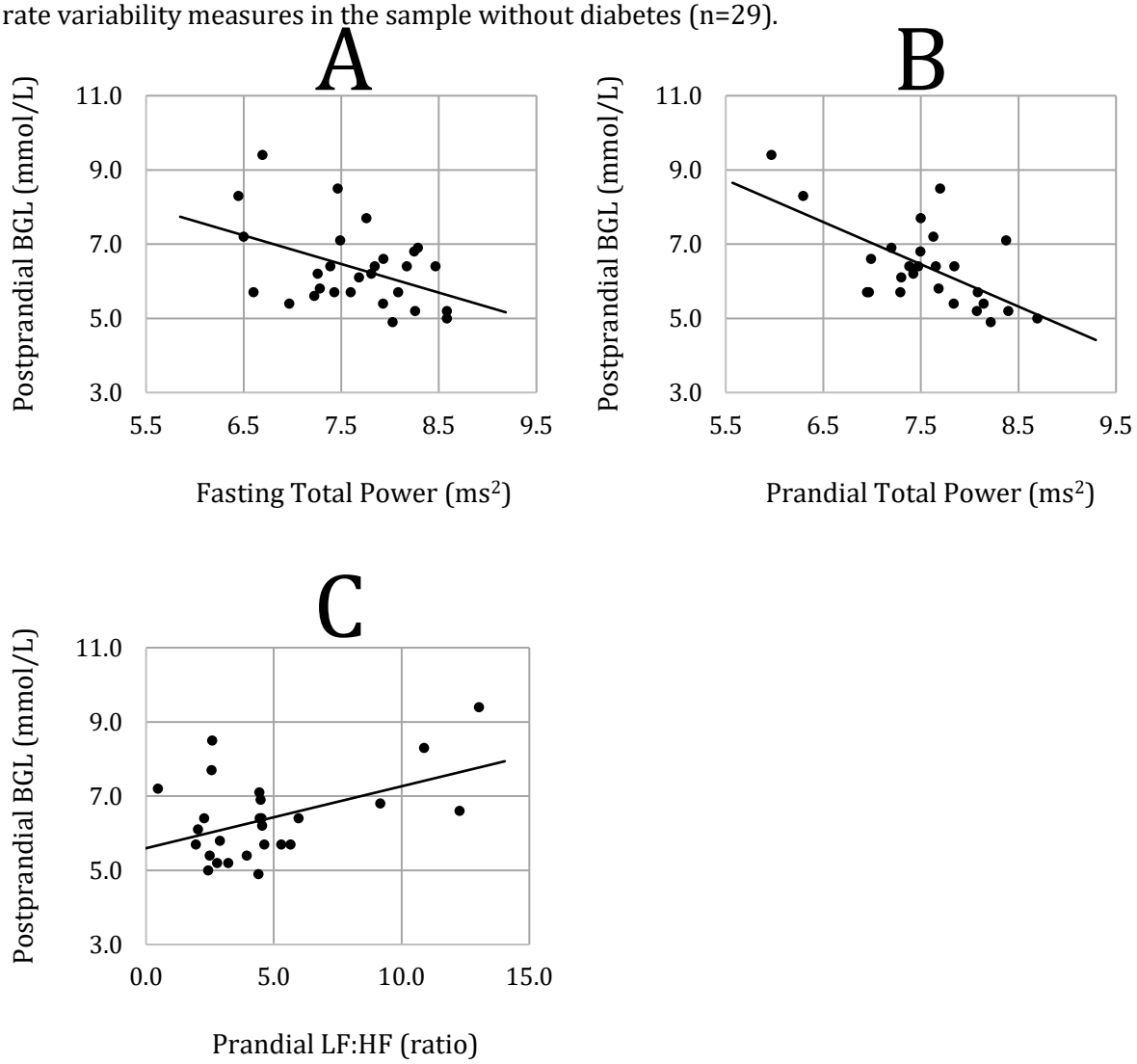

Figure 3.10 describes the relationship between each HRV measure and the dependant variable, postprandial BGL, using scatter plots. **(A)** Fasting total power was negatively associated with postprandial BGL, with a correlation coefficient (r) of -0.43 after controlling for covariates. **(B)** Prandial total power was also negatively correlated with BGL, with a correlation coefficient (r) of -0.61. **(C)** Prandial LF:HF had a positive relationship with BGL, with an r-value of 0.47. **BGL** = Blood glucose level, **LF:HF** = low to high frequency ratio, **mmol/L** = Millimoles per litre, **ms²** = Milliseconds squared.

Also in Table 3.5, several significant correlations were also identified in the T2D sample. Fasting BGL was negatively associated with fasting LF power (r = -0.63; p < 0.01), HF power (-0.57; p = 0.02), and total power (r = -0.66; p < 0.01). The relationship between each of these is described further by scatter plots in Figure 3.11. For postprandial BGL in the T2D sample, the HRV

measures which were significantly correlated were fasting LF power ($r = -0.46$; $p < 0.05$), fasting total power ($r = -0.51$; $p = 0.03$) and prandial total power ($r = -0.48$; $p = 0.04$). Scatter plots for these correlations are shown in Figure 3.12.

Figure 3.11 Scatter plots for fasting blood glucose level and significantly correlated heart rate variability measures in the diabetes sample (n=27).

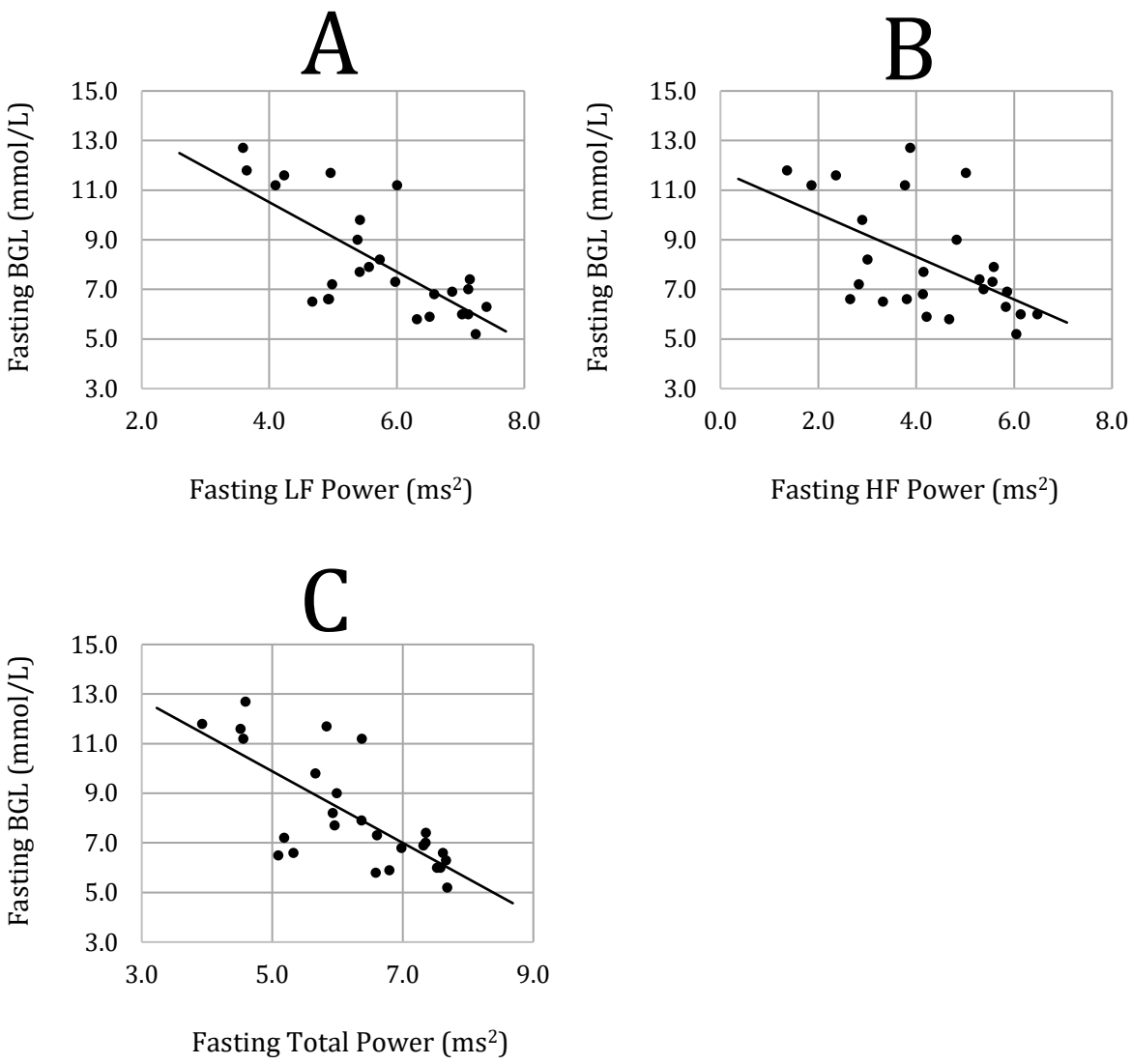

Figure 3.11 displays the relationship between each HRV measure and fasting BGL using scatter plots. **(A)** Higher fasting BGL was associated with lower fasting LF power. R was equal to -0.63 after controlling for covariates. **(B)** People with higher fasting BGL tended to have lower HF power, with an r-value of -0.57. **(C)** Fasting total power was also negatively correlated with fasting BGL. This was the strongest relationship ($r = -0.66$). **BGL** = Blood glucose level, **mmol/L** = Millimoles per litre, **ms²** = Milliseconds squared.

Figure 3.12 Scatter plots for postprandial blood glucose level and significantly correlated heart rate variability measures in the diabetes sample (n=27)

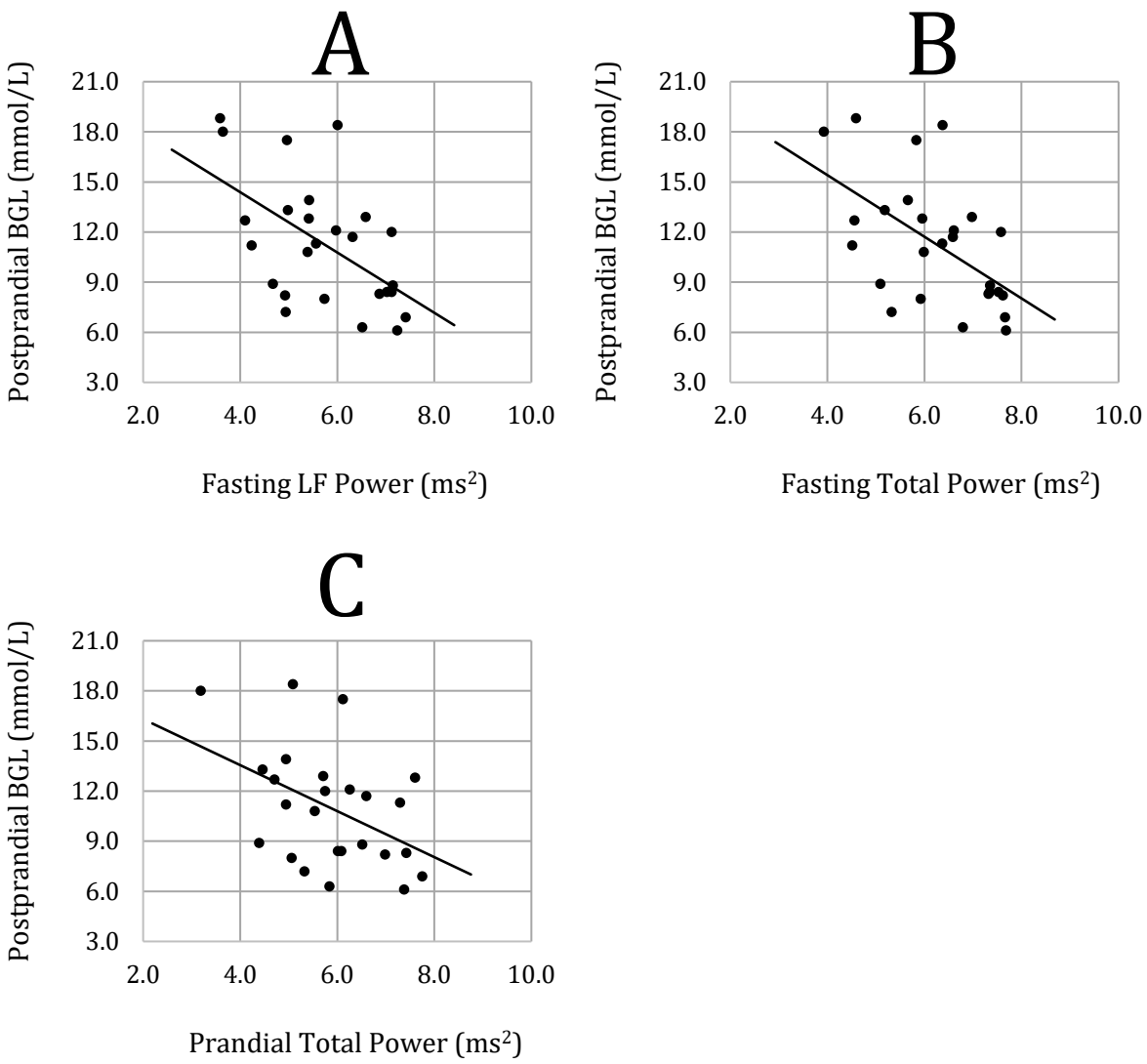

Figure 3.12 shows the relationship between the HRV measures observed in fasting and eating diabetes participants and postprandial levels of blood glucose. **(A)** Lower levels of low frequency power tended to be associated with higher levels of blood glucose (r = -0.46). **(B)** Total power measured in a fasting state was associated with levels of blood glucose after a meal (r = -0.51). **(C)** Postprandial BGL was also associated with total power measured in a prandial state (r = -0.48). **BGL** = Blood glucose level, **mmol/L** = Millimoles per litre, **ms²** = Milliseconds squared.

3.2.4 Multiple Linear Regression

Given that there were three HRV measures that were significantly correlated with postprandial BGL in the sample without diabetes, a multiple linear regression was performed (Table 3.6). Fasting total power, prandial total power, and prandial LF:HF were entered into the model. All predictors were retained by the model, but none were statistically significant.

Table 3.6 Regression analysis for postprandial blood glucose and the significantly correlated heart rate variability measures in the group without diabetes (n=29). 'B' and 'Standard Error' are unstandardized coefficients as the variables remain on their respective scales, for example total power scale is represented in units of milliseconds squared and blood glucose level is represented in units of millimoles per liter. 'Beta' are standardized coefficients as the variables have been adjusted to fit on the same scale. **BGL** = Blood glucose level, **LF:HF** = low to high frequency ratio.

Regression summary for dependent variable: postprandial BGL

$R = 0.66$; $R^2 = 0.44$; Adjusted $R^2 = 0.36$; $F(3,22) = 5.67$

$p = 0.005$, Standard Error of the Estimate = 0.90

Variable	B	Standard Error	Beta	t	p
(Constant)	14.36	3.11		4.61	0.00
Fasting total power	-0.39	0.36	-0.22	-1.10	0.28
Prandial total power	-0.70	0.46	-0.38	-1.53	0.14
Prandial LF:HF	0.07	0.08	0.19	0.90	0.38

A second regression was conducted in the diabetes sample for fasting BGL and the three significantly correlated HRV measures. Fasting LF, fasting HF, and fasting total power were entered into the model. All predictors were retained by the model, but none were statistically significant (Table 3.7).

Table 3.7 Regression analysis for fasting blood glucose and the significantly correlated heart rate variability measures in the diabetes group (n=27). 'B' and 'Standard Error' are unstandardized coefficients as the variables remain on their respective scales, for example total power scale is represented in units of milliseconds squared and blood glucose level is represented in units of millimoles per liter. 'Beta' are standardized coefficients as the variables have been adjusted to fit on the same scale. **BGL** = Blood glucose level, **HF** = High Frequency, **LF** = Low frequency.

Regression summary for dependent variable: fasting BGL

$R = 0.74$; $R^2 = 0.55$; Adjusted $R^2 = 0.49$; $F(3,22) = 9.09$

$p < 0.001$, Standard Error of the Estimate = 1.61

Variable	B	Standard Error	Beta	t	p
(Constant)	17.41	1.92		9.09	0.00
Fasting LF	-0.82	0.71	-0.42	-1.16	0.26
Fasting HF	0.21	0.38	0.13	0.54	0.59
Fasting total power	-0.88	0.69	-0.44	-1.27	0.22

For postprandial BGL in the diabetes sample, there were significant associations with three of the eight HRV measures that were investigated. Therefore, a linear regression was performed using fasting LF power, fasting total power, and prandial total power as the predictor inputs, and postprandial BGL was entered as the predicted outcome Table 3.8. The three predictor variables were retained by the model, but none were statistically significant within the model.

Table 3.8 Regression analysis for postprandial blood glucose and the significantly correlated heart rate variability measures in the diabetes group (n=27).'B' and 'Standard Error' are unstandardized coefficients as the variables remain on their respective scales, for example total power scale is represented in units of milliseconds squared and blood glucose level is represented in units of millimoles per liter. 'Beta' are standardized coefficients as the variables have been adjusted to fit on the same scale. **BGL** = Blood glucose level, **LF** = Low frequency.

Regression summary for dependent variable: postprandial BGL

$R = 0.52$; $R^2 = 0.27$; Adjusted $R^2 = 0.16$; $F(3,21) = 2.550$

$p = 0.083$, Standard Error of the Estimate = 3.22

Variable	B	Standard Error	Beta	t	p
(Constant)	21.61	3.91		5.53	0.00
Fasting LF	-0.62	1.40	-0.19	-0.44	0.66
Fasting total power	-0.54	1.65	-0.17	-0.39	0.75
Prandial total power	-0.62	0.89	-0.20	-0.69	0.50

3.2.5 Summary of Results

In this study, n= 27 people with T2D and n=29 people without diabetes were recruited and assessed. Individuals in the T2D sample were approximately 18 years older on average ($p<0.001$) and had higher BMI ($p<0.001$) compared to individuals in the sample without diabetes. People with T2D also tended to score higher on the LAQ Part 1 ($p<0.001$), which is associated with lifestyle risk factors such as poor diet and low physical activity. There were many significant differences between the two groups in terms of blood pressure, BGL, and measures of HRV. Comparisons were also made between the sexes in each sample, and the statistical analysis revealed there were no meaningful differences between men and women in terms of demographics or physiology, though women without diabetes did consume an average of 1200 kilojoules less than their male counterparts in this study.

Multiple correlation analyses were performed, using age and BMI as covariates as well as kilojoule intake where appropriate. Scatter plots and correlation coefficients determined that there was a general inverse correlation between BGL and HRV measures, for both samples. Where two or more measures of HRV were significantly correlated with BGL, a multiple linear regression was performed. From the multiple linear regression analysis in Table 3.6, it was determined that HRV measures, including fasting total power, prandial total power, and prandial LF:HF, accounted for 44% of the variance in postprandial BGL observed in the sample without diabetes ($R^2=0.44$). In Table 3.7, the regression analysis revealed that HRV measures obtained from a fasting state alone, including LF power, HF power, and total power, accounted for 55% of the variation in the dependent variable: fasting BGL in T2D ($R^2=0.55$). The third multiple linear regression analysis revealed that fasting LF and total power and prandial total power accounted for 27% of the variance in postprandial BGL in the diabetes group (Table 3.8). This concludes the statistical analysis and presentation of results. The following chapter will interpret these results and discuss them in the context of the literature.

3.3 Discussion

The UTS T2D Study aimed to add to the growing body of literature which has suggested HRV measures may be valuable in glucose prediction. The results of this research may invite further interest in the applications of HRV in diabetes management and may justify further research into this topic. In this section, the results of the present study will be discussed in the context of the current literature, and the core strengths and weaknesses of the study will be described, with suggestions for future research. In summary, there was an inverse correlation between BGL and HRV measures in general, for both the non-diabetes sample and the T2D sample. The multiple linear regressions indicated that HRV measures, including fasting total power, prandial total power, and prandial LF:HF, accounted for 44% of the variance in postprandial BGL observed in the sample without diabetes. Further, HRV measures obtained from a fasting state alone, including LF power, HF power, and total power, accounted for 55% of the variation in the dependent variable: fasting BGL in T2D. Finally, fasting LF and total power and prandial total power accounted for 27% of the variance in postprandial BGL in the diabetes group.

3.3.1 Sample Comparison

A roughly equal number of people without diabetes (n=29) and people with T2D (n=27) were recruited for this study. Due to the fact the people with T2D tend to be older and have a higher BMI compared to people who live without any chronic illness, it was expected that there would be meaningful differences between the samples in terms of demographics and physiology. Individuals with T2D were 18 years older on average and had higher BMI compared to their non-diabetes counterparts. A higher BMI in the T2D group was anticipated, as obesity is a major risk factor of T2D and is highly comorbid.[43] This was reinforced by the scores of the lifestyle questionnaire. The T2D group scored higher on the LAQ Part 1, indicating their lifestyles were associated with more risk factors, including modifiable risk factors such as poor diet and low physical activity, as well as non-modifiable risk factors such as family history of chronic illness. The higher age in the T2D group was also anticipated. However, there was a discrepancy between the mean age of the T2D group in this study compared to samples published in the literature. Though the median age of T2D onset is decreasing, with childhood obesity becoming more prevalent worldwide, T2D at present predominately affects older individuals, and the risk of developing T2D increases with age. A large-scale epidemiological study of Australians with T2D (n=743,709) conducted by Huo and colleagues (2018) indicated that the mean age was 65 years old.[461] Nanayakkara and colleagues (2018) reported a similar mean of 63 years of age in their sample of n=3,419 Australians with T2D,[462] as did Knowles and colleagues (2020) who

recruited n=59 Australian participants with T2D with an average age of 61 years old.[463] In this study, the mean age of T2D participants was 51 ± 10 years of age, which is 14 years younger than the large cohort of Huo and colleagues (2018). Evidently, the characteristics of the participants recruited in the UTS T2D Study do not accurately represent those of the wider Australian T2D population, as they were much younger on average. However, a lower HbA_{1c} and younger age of the T2D group is a desirable trait when investigating diagnostic capabilities of HRV, as the influence of autonomic neuropathy on BGL and HRV measures is lower in these groups.

In terms of physiology, there were major differences between the T2D group and the group without diabetes that were related to HRV measures, blood pressure, and BGL. LF power, HF power, and total power were all significantly lower on average in the T2D sample, and LF:HF – which is calculated differently to the other three measures, as it is a ratio of LF to HF power – was significantly higher in the T2D sample. This result was expected, as there is substantial literature showing that autonomic activity, as measured by HRV, declines with age and with diabetes duration (see Section 1.10.1). BGL was also significantly higher in the T2D group, though this was also expected as this is the defining characteristic of this clinical sample, and the higher blood pressure was also anticipated as blood pressure increases with age and hypertension is highly comorbid with T2D.[66] These physiological differences between the samples are consistent with findings of previous literature, and analysis of these differences was conducted only to provide evidence that the diabetes participants and participants without diabetes recruited in this study were representative of their respective populations. In addition to this, the values for HRV measures, blood pressure, and BGL were found to be within the expected ranges for the participants without diabetes,[432, 464] and this was also true for the T2D participants.[375, 465] However, HbA_{1c} was assessed in T2D participants as an added measure of their glycaemic status. The mean HbA_{1c} for the sample was 6.9 ± 1.4%, and 16 out of the total 27 participants (59%) presented with an HbA_{1c} level below 7.0%. Based on large-scale epidemiological data, only 50% of Australians with T2D maintain their HbA1c levels below the recommended level of 7.0%.[104] Therefore, there is a discrepancy of about 9% between this sample and the wider Australian diabetes population in terms of glycaemic management. This may be related to this sample being relatively young compared to the wider Australian T2D population, and glycaemic management tends to be better in younger people.[466]

3.3.1.1 Sex Comparison

Statistical analysis showed no significant difference between men and women for any demographic variable or for any of the physiological variables of interest, including HRV measures and BGL. A meta-analysis of 172 studies (n=63,612, 50% male) has demonstrated that LF power, total power, and LF:HF are lower, and HF power is greater in women compared to men.[467] This suggests that autonomic control of the female heart is characterized by higher vagal, or parasympathetic activity, and the male heart is characterized by relatively higher sympathetic dominance. These sex differences are also generally greater with increasing age.[467] This may explain why sex differences were unclear in the present study, as the sample did not include a wide range of age groups, and was mostly people 25-40 years old. The small sample size may also have been unable to detect the effect of sex on HRV measures in the statistical analysis, due to effect size.

In terms of sex differences in glycaemia, Kautzky-Willer and colleagues (2012) contend that fasting and postprandial BGL tend to be lower in women (n=611) with optimal glycaemia compared to men (n=361).[468] In their study, the female sample was younger, and this may have contributed to their statistical findings. However, a broader view of the literature suggests there is a consensus that women without diabetes have lower fasting BGL, even though evidence for sex differences in postprandial BGL may be less conclusive. For example, research conducted by Faerch and colleagues (2010) in a sample of n=6,006 people without chronic illness showed that women have lower fasting BGL, but higher postprandial BGL two-hours after a meal.[469] According to the authors, these sex differences in postprandial BGL were explained by height and fasting BGL, but differences in fasting BGL were not explained by height. In the UTS T2D Study, glucose levels were comparable between men and women. Mean fasting BGL in women was 4.7 ± 0.4 compared to 5.1 ± 0.5 in men. Though this was difference was not significant, it was close to significance ($p=0.05$) and increasing the sample size may have provided a significant p-value, aligning the results with that of previous literature.

There were also no physiological differences when comparing men and women in each sample, for example no significant differences between T2D males and T2D females. The only exception was women without diabetes consumed an average of 1200 kilojoules less than their male counterparts, and research from various countries suggests this is normal.[470-472] Where relevant, kilojoule intake was included as a covariate in the correlation analysis, and this accounted for the differences in meal composition between individuals, including the difference in average kilojoule intake between men and women.

3.3.2 Correlation Analysis and Confounders

Age and BMI were significantly correlated with measures of HRV and BGL. This was only true for the sample without diabetes – age and BMI were not related to any variables of interest in the T2D group. Additionally, kilojoule intake was not correlated with any variable of interest. However, it was decided that kilojoule intake would be included as a covariate for statistical analyses of both groups, to control for differences in meal composition between participants. Partial Pearson's correlation analyses were performed, using age, BMI, and kilojoule intake as covariates. In instances where at least two different HRV measures were significantly correlated with a single BGL measure, a multiple linear regression was conducted. This included postprandial BGL in the sample without diabetes, fasting BGL in the diabetes group, and postprandial BGL in the diabetes group.

3.3.3 Heart Rate Variability Associations with Fasting Blood Glucose Level

In the UTS Pilot Study, the aim was to investigate acute fluctuations in HRV measures and BGL, which involved statistical comparison of these variables between three time points. In the UTS T2D Study, the aim was to investigate associations between HRV measures and BGL to demonstrate the predictive applications of HRV measures. As such, differences between fasting and postprandial HRV measures were not investigated, as this was investigated in the Pilot Study. The main statistical analysis of the present study involved correlation analyses of the four HRV measures and fasting and postprandial BGL. In cases where at least two HRV measures were significantly correlated with a single BGL variable, whether it was fasting or postprandial, a multiple regression analysis was conducted to further explore the predictive value of the HRV measures. As discussed in Section 1.9.1.1, assessments of autonomic activity may provide meaningful predictions of BGL fluctuations, or even indicate if current BGL is high or low. The intention of this section is to discuss how the findings of this study add to these emerging applications of HRV and to compare these results with previous literature. Firstly, this section will explore the observed associations between HRV measures and fasting BGL in people without diabetes.

3.3.3.1 Non-Diabetes Sample

The results of the UTS T2D Study indicate no significant correlation between HRV and fasting BGL in people without diabetes, and this is consistent with similar small-scale studies. However, this does not align with the findings of larger studies in this area. A previous study conducted by

Rothberg and colleagues (2016) observed no significant associations between frequency-domain HRV measures and fasting BGL in people without diabetes.[383] The follow-up to this study, conducted by Jarman and colleagues (2021) in an Honours year, also found no significant relationship between HRV and fasting BGL in people without diabetes.[389] These studies were similar in design, and recruited relatively small numbers of participants. In larger studies, such as one conducted by Singh and colleagues (2000) on n=1,179 adults (mean age 47.4 years), fasting BGL was inversely correlated with LF power, HF power, and LF:HF.[392] However, in this study, the data from the n=1,179 participants was pooled with n=56 adults with impaired fasting glucose and n=84 people with diabetes for the statistical analysis. Including participants with suboptimal ranges of BGL may have strengthened the correlation analysis. Two other large-scale studies report similar findings but were also different in their approach to the analysis. For example, Jarczok and colleagues (2013) concluded that both LF power and HF power were inversely related to fasting BGL,[395] however their sample of n=2,441 adults was predominately male (75%), and there are significant differences in autonomic activity between men and women, as discussed. The dominance of male subjects may have skewed the results. Additionally, Stein and colleagues (2007) found that fasting BGL was negatively related to LF power and HF power in adults (n=1089),[394] however their sample was mostly elderly men (mean age 72 years). Though there were limitations to the designs of these studies, as well as differences in how they approached methodology and analysis, it is likely that HRV measures are significantly associated with fasting BGL in people without chronic illness. As stated, the results of the UTS T2D Study did not align with the findings of larger studies in this area. This may be due to the relatively younger sample that was recruited in the UTS T2D Study as well as the decision to not pool the participants without diabetes with the diabetes participants in the statistical analysis.

3.3.3.2 Diabetes Sample

In the UTS T2D Study, fasting BGL was negatively associated with fasting LF power ($r = -0.63$, $p < 0.01$), HF power ($r = -0.57$, $p < 0.01$), and total power ($r = -0.66$, $p < 0.01$). Though Rothberg and colleagues did not report any significant correlations between HRV and fasting BGL in people without chronic illness, they identified numerous correlations in people with diabetes.[383] In their pooled analysis of T1D and T2D participants (n=32), fasting BGL was correlated with HF power ($r = -0.46$, $p = 0.01$), LF:HF ($r = 0.50$, $p < 0.01$), and total power ($r = -0.40$, $p = 0.02$). Fasting BGL was not significantly associated with LF power ($r = -0.29$, $p = 0.12$). In an analysis of just their n=11 participants with T2D, fasting BGL was only significantly correlated with HF power ($r = -0.64$, $p = 0.04$) and LF:HF ($r = 0.78$, $p = 0.01$).[383] Early research on vagal tone in

diabetes, conducted by Liao and colleagues (1995), demonstrated that HF power was significantly lower in people with higher fasting BGL, compared to those with lower fasting BGL.[473] There is substantial research in the area of fasting BGL in people with T2D, as it is consistently implemented as a baseline for comparing BGL. This inverse relationship between fasting BGL and HRV measures has been confirmed by numerous studies,[326, 394] and a meta-analysis published by Benichou and colleagues (2018) indicates this correlation is consistent across many clinical studies.[474] There are two notable discrepancies between the results of Rothberg and colleagues and the UTS T2D Study. Firstly, Rothberg and colleagues (2016) did not observe any significant association between LF power and fasting BGL in their diabetes sample; and secondly, the UTS T2D Study did not observe any relationship of significance between LF:HF and fasting BGL. These are both likely due to low sample power, as the small sample size of the UTS T2D Study and the study conducted by Rothberg and colleagues (2016) may have restricted certain statistical analyses from indicating significance. It is clear from the literature that LF power and LF:HF should be significantly related to fasting BGL.

As more than one predictor variables were significantly associated with fasting BGL in the diabetes group, a multiple regression analysis was performed. Fasting LF power, HF power, and total power accounted for 55% of the variation in fasting BGL in the T2D group (R^2 = 0.55). Rothberg and colleagues (2016) demonstrated that fasting HRV measures, including LF:HF and total power, predicted 31% of the variance in fasting BGL in diabetes subjects (R^2 = 0.31).[383] Discrepancies in this R-squared value may be related to sample differences, as the Rothberg study pooled participants with T1D (n=21) and T2D (n=11) into a single diabetes group for the regression analysis, and there are meaningful neurophysiological differences between T1D and T2D.

3.3.4 Heart Rate Variability Associations with Postprandial Blood Glucose Level

Currently, there is less interest in the applications of HRV measures in predicting postprandial BGL compared to fasting BGL. This may be related to methodology constraints, as assessing participants in a postprandial state requires more resources and time organisation compared to assessing them in a fasting state. However, there is evidence that postprandial BGL may be equal in importance to fasting BGL in monitoring complications of diabetes, as well as general monitoring of diabetes progression and health. As discussed in Section 1.11, the ability to predict fasting or postprandial BGL using non-invasive means represents an important goal in diabetes research. Therefore, the UTS T2D Study also investigated associations between HRV measures and postprandial BGL, despite the constraints this added to the methodology.

Additionally, the UTS T2D Study was the first study to date to investigate associations between prandial HRV measures and postprandial BGL. Regarding prandial HRV and its predictive qualities, the findings presented in this thesis are novel, as most studies in this area have investigated associations between fasting HRV and BGL. The notion of predicting postprandial BGL using autonomic markers recorded whilst are eating is novel. This is important to consider for the following sections where these findings are discussed.

3.3.4.1 Non-Diabetes Sample

In the UTS T2D Study, there were fewer predictor variables that were significantly correlated with postprandial BGL, and so these three HRV measures accounted for less variance in postprandial BGL. As discussed, the notion that postprandial BGL may be predicted by HRV measures assessed whilst eating, which reflect preprandial autonomic activity, is novel. This study is the first the date to acknowledge these findings and builds on previous literature which indicated the value of HRV measures. Based on previous work conducted in this research unit and published by Jarman and colleagues (2021), postprandial BGL is negatively associated with LF power ($r = -0.62$, $p < 0.01$) and total power ($r = 0.57$, $p < 0.01$) in subjects without diabetes (n=25).[389] In this paper, HRV measures explained 52% of the variance in postprandial BGL. However, these five predictor HRV measures were recorded in different metabolic states, not just fasting. For example, LF power and total power from the fasting assessment, combined with LF power, HF power, and total from the postprandial assessment, accounted for 52% of the variance in postprandial BGL. This may explain why the UTS T2D Study (n=29), which assessed a similar number of participants, demonstrated that fasting total power, prandial total power, and prandial LF:HF accounted for 44% of the variance in postprandial BGL in the sample without diabetes, compared to 52% in the previous study.

3.3.4.2 Diabetes Sample

In the UTS T2D Study, fasting HRV measures were significantly and negatively associated with postprandial BGL, including LF power ($r = -0.46$, $p < 0.05$) and total power ($r = -0.51$, $p = 0.03$). Additionally, total power from the prandial assessment was also negatively correlated with postprandial BGL ($r = -0.48$, $p = 0.04$). Combined, these three HRV measures accounted for 27% of the variance in postprandial BGL in the diabetes group. Contrary to the findings of Rothberg and colleagues (2016), there was no significant correlation with LF:HF. This may be because

LF:HF was only correlated with BGL in the pooled diabetes group in the Rothberg study, and by comparison, there were no T1D participants in the diabetes group for the UTS T2D Study. Additionally, Rothberg and colleagues recorded HRV measures in a fasting state, but not a prandial state.

There is little research on the associations of postprandial BGL and HRV measures, as most studies focus on fasting BGL or focus on the changes in HRV measures after a meal. Myakina, Klimontov & Safarov (2015) monitored HRV and glucose levels in T2D subjects (n=70) for 24 hours by continuous CGM and ECG. Postprandial LF:HF was inversely related with daytime mean BGL ($r = -0.29$, $p < 0.05$) and peak BGL ($r = -0.33$, $p < 0.05$),[475] though these glucose measures are not the same as the measures used in the UTS T2D Study. In their 2016 paper, Rothberg and colleagues demonstrated that postprandial BGL was significantly associated with LF:HF ($r = 0.44$, $p = 0.01$) in their pooled diabetes sample (n=32), and in their sample of just n=11 T2D participants, postprandial BGL was associated with HF power ($r = -0.64$, $p = 0.04$).[383] The authors concluded that the physiological differences between T1D and T2D were cause for the differences in these correlation analyses. To reiterate, where prandial HRV measures were demonstrated to be associated with postprandial BGL, these findings are novel.

3.3.5 General Relationship of Blood Glucose Level and Autonomic Activity

The literature has provided substantial evidence that there is an association between HRV and BGL. There are consistent findings in the literature that when these two variables are recorded in a large group of people at a single time-point, they are significantly and inversely correlated. This is true even when HRV and BGL are assessed in different states of metabolism, and there are several factors which contribute to this correlation. Physical inactivity and a sedentary lifestyle are key risk factors for T2D and impaired fasting BGL,[476, 477] and lack of exercise is associated with lower HRV measures and poorer autonomic tone.[478] Therefore, in cross-sectional studies, people with lower HRV measures tend to have higher BGL, and physical fitness is a key confounder as it predicts both high BGL and low HRV.[479] Another key factor involved is that chronically high BGL, a landmark of diabetes, leads to neurodegeneration of the nerves of the autonomic nervous system and gradual loss of autonomic tone. As discussed in Section 1.5.2.3, autonomic neuropathy is a long-term complication of diabetes. Consequently, in groups of people with chronically high BGL, there is a tendency for autonomic neuropathy to be more prevalent compared to groups with lower or optimal ranges of BGL, and thus HRV measures are lower in people with higher BGL. However, these factors only explain why HRV and BGL are related when comparing groups of people with significantly different lifestyles and

with significantly different levels of blood glucose. This does not explain what was observed in the UTS T2D Study. HRV measures and BGL were still correlated in groups of people without diabetes, where differences in physical fitness are smaller and the effects of autonomic neuropathy are likely non-existent. These major confounders were accounted for in the UTS T2D Study. As such, the next section will explore the physiological and neural foundations of the relationship between HRV measures and BGL, as a basis for understanding why HRV and BGL are related even in specific groups of people.

3.3.5.1 Autonomic Response to Food Intake

The role of the autonomic nervous system in the regulation of glucose and fat metabolism is not well understood,[129] however it is recognised that autonomic functions are important for the metabolism of food and drink into glucose, and that some autonomic functions may precede changes in BGL. The physiological principle behind this involves insulin. Following the consumption of a meal, the absorption of nutrients into the blood via the gastrointestinal tract is associated with a small increase in plasma insulin, even before there is a rise in blood glucose.[390] This is part of the pre-absorptive, or cephalic phase. Activation of the ANS during both the pre-absorptive and absorptive phases of insulin is important in determining postprandial insulin activity.[480] As such, changes in HRV may precede the release of insulin.[480] This is relevant because insulin enacts a stimulatory effect on the sympathetic nervous system,[481, 482] which can be measured by LF power. This may explain why various studies, including the UTS T2D Study, show that LF power is inversely proportional to BGL in subjects without diabetes with optimal autonomic function.[392, 394, 395]

3.3.6 Strengths and Limitations

The results of this study indicate that HRV measures may estimate BGL in people without diabetes or people with diabetes. However, this conclusion does not have broad applications. For example, due to the study design, it cannot be concluded based on this research whether an individual could predict their BGL at any point in the day based on their HRV data. It may only be possible to estimate postprandial BGL, and even then, this may require HRV data to be recorded at a specific time point. Many researchers hope to demonstrate that autonomic activity, as measured by HRV, may predict BGL throughout the day, allowing for a continuous and non-invasive measure of BGL in real-time. However, this has yet to be demonstrated as a superior alternative to current glucose monitoring, and HRV measures continue to lack clinical relevance in this specific area. Regardless, this thesis presents novel findings. These findings

alone do not justify the use of HRV in clinical settings, such as estimating BGL in people with diabetes. However, these findings add to the growing evidence that HRV may have clinical applications in diabetes, as well as present novel findings that had yet to be provided in the literature. The scope of this PhD thesis was only to demonstrate that BGL may be estimated in certain important metabolic states, such as fasting or postprandial, using simple non-invasive technology and gold standard HRV processing techniques. The aims and hypotheses were sufficiently addressed through the methodology of this study, and it is important that the scope of this findings and the limitations of the study be considered.

In Section 1.9, the potential for machine learning to improve current glucose monitoring devices was discussed. In this study, regression analysis was used, which is a basic form of machine learning. The use of linear regression in this PhD research represents a lack of risk prediction. In clinical studies, linear regression often shows no significant results, and future research in this area should consider more rigorous statistical methods instead. However, these is some contention on this in the literature. Some authors contend that, in terms of predicting BGL, classic autoregression techniques perform worse than machine learning models, including machine-learning-based regression models and deep learning models.[331, 483] However, in their review article, Xie and Wang (2020) observed no significant advantage between the two types of method in predicting BGL.[484] There may be a bias in how authors promote the value of their predictive models. Rodríguez-Rodríguez and colleagues (2019) suggest that accurate glucose prediction can only be achieved by monitoring glucose levels over a short period of time and using a low sampling frequency.[485] However, they believe that this makes wearable sensors ideal for glucose prediction as they have limited requirements for hardware. The UTS T2D Study was also more interested in how machine learning can be integrated with SMBG, a topic which has comparatively little attention in the literature, potentially because the nature of SMBG makes it difficult to collect the large number of data points required to train a predictive model.[486]

A limitation of the UTS T2D Study was that certain confounders were not controlled for due to the scope of the study. As with the first experiment, the exclusion criteria for daily alcoholic beverage consumption of no more than 10 standard drinks per day was substantially higher than the regular daily intake of 2 standard drinks per day. This is a limitation since excessive alcohol consumption has been associated with autonomic neuropathy. Future studies should consider excluding subjects with a duration of diabetes (time since date of diagnosis) over 5 years, since longer duration is correlated with autonomic neuropathy and a more delayed prandial response. Since participants were not assessed specifically for autonomic neuropathy, an exclusion criteria could have been added for the T2D group excluding subjects with any

known peripheral neuropathy or previous foot ulcer as signs of existing neuropathy. Autonomic neuropathy causes significant changes in HRV, even when it is not related to diabetes progression. In this study, participants did not undergo a physical examination or screening prior to the study, which may have been useful in identifying autonomic neuropathy that participants were unaware of. Instead, participants were asked to provide details about any current comorbid conditions and any current medications, including dosage. Certain medications were prohibited and were grounds for exclusion from the study if participants were currently taking them (see Section 3.1.2), however most medications were allowed in the study to allow for real-world data to be recorded. Some studies suggests that subjects with T2D should abstain from medications that affect BGL or cardiovascular function prior to study participation, including antihypertensives, as they are confounders.[146, 419] Since time to peak prandial glucose is delayed in people with suboptimal glycaemia, this study design could have also benefited from excluding T2D subjects with HbA_{1c} over 7.5%. According to a review paper, there are several studies which indicate BP is related to HRV measures, and on a minute-to-minute basis this relationship is due to the autonomic nervous system exercising homeostatic control over the cardiorespiratory cycle.[487] For example, increased metabolic demand due to walking up a staircase may be associated with both a change in BP and HRV measures. Future studies should attempt to control for BP, such as in the statistical analysis or by restricting the movement of subjects during the study assessments, so that any correlations identified between HRV measures and BGL may be less confounded by BP, which may influence the changes in HRV.

It was not within the scope of this study to control for every major confounder variable, as it was not the aim to demonstrate that changes in BGL are strictly related to autonomic activity. The aim was to investigate whether the relationship between HRV measures and BGL was sufficient for the development of a regression model, which may in future be used to predict BGL through non-invasive means. An algorithm or model that predicts BGL using HRV measures may need to consider the effect of medications on physiological data rather than ignore it or attempt to remove that effect from a sample entirely. As with most clinical samples, there were various comorbid conditions and medications involved which would have affected levels of HRV and BGL observed in the present study. However, this is reflective of a real-world scenario. People living with diagnosed diabetes are likely to be medicated and have multiple comorbid conditions. As such, only basic variables such as age, BMI, kilojoule intake, and sex were controlled for in this study as these can be easily entered into a device for BGL prediction, and a real-world application of HRV would ideally incorporate these variables for added accuracy.

Participants were free to eat whatever they wanted to, as it was the intention of the study design to allow participants to resume their routine as close as possible, however there is only so much that statistics can do to control for these differences in the analysis. In this study, kilojoule intake was included as a covariate in the statistical analysis, and participants were advised to avoid certain foods such as tea and cinnamon which have known anti-glycaemic properties. However, this is an imperfect measure of controlling for significant differences in participant's meals, and a robust study design should ultimately attempt to standardize these meals, if meals are part of the study design. Though it was the intention of this study to observe subjects whilst they consumed meals of their choice, which would be considered 'regular' by their own standards, the differences in meal portions between subjects was not controlled for. Only the kilojoule intake of the meals was accounted for, and differences in the distention of subject's stomachs, due to consuming meals that may have been relatively larger compared to the group average, may have confounded autonomic responses since greater stomach distention would lead to greater sympathetic nerve activity.[488] Future studies should aim to standardise meal portions. Participants were also free to leave the UTS laboratory between recordings, which would reflect a regular routine as even office workers would expect some levels of movement. However, even low levels of activity, such as walking for a few minutes, increase metabolic demand and may lead to reduced glycaemia as well as changes in HRV. Future research should consider restricting the activity of participants whilst they are being monitored.

One of the strengths of the methodology was the implementation of two BGL assessments for the determination of postprandial BGL. CGM could not be used in this study due to resourcing issues, as CGM is not standard of care for most people with T2D, and the research unit lacked the proper ethics approval and qualified staff to administer a CGM system to either healthy subjects or subjects with T2D. Without CGM, it is difficult to capture the peak level of blood glucose after a meal, and even with CGM, observed recordings may lag behind true glucose levels. In this study, BGL was assessed by invasive blood glucose assessment in participants without diabetes 40 minutes and 50 minutes after eating, and the higher of these two values was used to provide an accurate assessment of postprandial BGL. The timing of the BGL assessments was the same with T2D participants, as peak BGL was expected to be achieved at roughly the same time in both groups. This was important for consistency, as it can be concluded that HRV measures may estimate BGL at roughly the same time in individuals regardless of whether they have diabetes or not. However, according to the literature, the glycaemic response to food may be complicated by the presence of metabolic diseases. In the case of T2D, this effect is difficult to quantify. Kanaley and colleagues (n=42) contend that BGL peaks later in people with T2D compared to people without diabetes, usually at least 60 minutes

after food ingestion.[419] However, research conducted by Esposito and colleagues (n=644) showed that BGL peaks 40 minutes after consuming a mixed meal in cases of T2D, which is the same time observed in groups without diabetes.[446] Given that Esposito and colleagues sampled a much larger group and from a wider demographic, and that the effect of T2D on the postprandial response is difficult to quantify, it may be accepted that BGL peaks after a meal at roughly the same time in people with and people with T2D. Even in cases where the presence of diabetes may have affected metabolic function, it is possible that the difference in time required for BGL to peak was accounted for by the use of two postprandial BGL assessments, one after the other. Future studies may consider the use of CGM to capture true postprandial BGL, as the time to peak postprandial BGL may be delayed in people with T2D and other comorbid conditions. From this study, it can be concluded that HRV measures may predict postprandial BGL in T2D or people without T2D 40-50 minutes after eating.

Chapter 4. RNSH T1D Study

4.1 Methods

T1D and T2D are more different than they are similar (Section 1.4), and glucose monitoring technology should be tailored to the needs of each group separately. As such, the RNSH T1D Study investigated how HRV measures may be used in T1D as a non-invasive alternative to current glucose monitoring. Because people with T1D are dependent on exogenous insulin to survive, daily glucose levels fluctuate greatly, and this increases the incidence of glucose excursions – events where BGL deviates greatly from baseline levels. Glucose excursions in the high range may cause mild symptoms such as thirst and fatigue in the short-term, and complications arise in the long-term if these excursions go unmanaged. Glucose excursions in the low range are usually referred to as hypoglycaemia, and these may quickly become life-threatening. In particular, the detection and early intervention of hypoglycaemia is a higher priority in cases of T1D because it is more common compared to T2D. As such, the specific aim of this study was to observe changes in HRV during and preceding glycaemic excursions, to justify the use of HRV measures as markers of suboptimal BGL.

4.1.1 Subjects

According to the Global HAT Study (n=27,585), hypoglycaemic events occur more often in people with T1D (83.0%) compared to T2D (46.5%). Rates of any hypoglycaemic event and any severe event in T1D were 73.3 and 4.9 events per patient-year, respectively. For people with T2D, occurrence of hypoglycaemia was 19.3 per year for any event and 2.5 per year for severe events.[86] There was a concern that this rate of hypoglycaemia in T1D would be difficult to capture without long periods of recording or observation. To improve the number of observed glycaemic excursions in this study, recruitment prioritised those who were at higher risk of experiencing glycaemic excursions. Many of these individuals had undergone recent changes in their medication or were experiencing problems with their CGM systems, resulting in more frequent glucose fluctuations. It was important that individuals experienced glycaemic excursions whilst they were being monitoring so that the relevant data could be collected.

Recruitment of people with T1D started at RNSH in collaboration with the Department of Endocrinology, through endocrinologists and study coordinators working at the hospital. Approval was gained from both UTS HREC (2014000110) as well as the Northern Sydney Local

Health District HREC (LNR/14/HAWKE/174) (Site Specific Assessment reference: LNRSSA/16/HAWKE/8). The researcher attended the hospital Department of Endocrinology to recruit people with T1D. As with the UTS T2D Study, it was difficult to exclude diabetes participants if they were also living with any other lifestyle-related diseases or taking any medications (Section 3.1.2). In particular, people with T1D were not excluded for cardiac autonomic neuropathy, as they were in the previous study, as it does not confound changes in HRV during glycaemic excursions, and these were the main states of interests in this study.[489] However, some selection criteria were applied based on recommendations from previous literature. For one, subjects were free of clinically-apparent coronary heart disease or congestive heart failure.[490] They were also required to be aged between 18 and 69 years old, and were excluded if they were currently pregnant, smoked more than 10 cigarettes per day, consumed more than 10 standard alcoholic beverages per day, or if their BP was too high during the pre or post-study BP recordings (Section 2.1.3). Because glycaemic excursions occur very rarely in people without chronic illness, it was anticipated that little to no meaningful data would be captured by including them in the study. Thus, there was no control group recruited for the RNSH T1D Study. Instead, there was a focus on comparing changes in HRV during glycaemic excursions with individual's baseline HRV measures. An investigation of the relationship between HRV and BGL in individuals without chronic illness was provided in the UTS T2D Study, as appropriate.

4.1.2 Pre-study Requirements

Provided they suited the eligibility criteria, participants were invited to RNSH to discuss the study with the researcher and their endocrinologist. Subjects were not expected to fast or abstain from any medications prior to the study due to concerns this would interfere with their glycaemic health, and this was a greater concern in this study compared to the UTS T2D Study. Participants were asked to continue taking their regular medications and prioritise any medical advice given to them by their doctor or diabetes educator. Participants were advised they could withdraw from the study at any point without reason and were asked to sign two copies of a consent form, one of which they retained. Following this, BP was recorded, and the BP requirements were checked based on UTS HREC guidelines (Section 2.1.3).

4.1.3 Continuous Glucose Monitoring

All subjects who participated in this study were required to use some form of CGM, as this is the gold-standard for collecting data on glycaemic excursions. The devices used in this study were the Medtronic Guardian Connect, which is a CGM system which connects with the insulin pumps such as the MiniMed 640G and 670G (Medtronic Australasia Pty Ltd, Macquarie Park, Australia), and the Dexcom G4 and G5 (Dexcom, Inc., San Diego, Unites States of America). Subjects using the FreeStyle Libre Flash Glucose Monitoring System (Abbott Medical Australia Pty Ltd, Macquarie Park, Australia) were also included in this study, as this device is capable of continuously recording glucose levels, though the data is provided retrospectively and not in real-time, as with the Dexcom and Medtronic systems. All devices were owned by the participants themselves and were operating before the start of the study. Participants were advised to continue operating their devices for the duration of the study, including calibrating the CGM systems with blood glucose assessments where necessary. All subjects knew to calibrate their own systems using their personal lancet devices and glucose test strips and were instructed to continue as per their standard of care. The CGM systems used by participants were made available to the researcher when the participants returned to the hospital so that the glucose data could be downloaded. HbA_{1c} levels were estimated by the CGM systems based on weekly fluctuations in glycaemia.

4.1.4 Recording of R-R Interval Data by Heart Rate Monitor

Due to the unpredictable nature of glycaemic excursions and their infrequent timing, short-term observations of T1D participants were unlikely to capture sufficient samples, even in high-risk cases. Therefore, participants were required to undergo CGM and heart rate recording for a minimum of 24 hours to ensure a reasonable chance of recording HRV during glycaemic excursions. The device used for R-R recording was the FirstBeat BodyGuard 2, and a detailed description of how this was attached to participants can be found in Section 3.1.4. Wearable single-lead devices are now commonly-used for continuous HRV monitoring in outpatient settings.[491] The BodyGuard 2 was attached at the hospital and participants then returned to their regular schedules and work routines whilst being mindful that the device was to remain attached. Instructions were provided on how to remove and reattach the monitor using fresh electrodes provided, so that participants could shower and maintain proper hygiene as usual. Participants were advised that they were to wear the device for as long as comfortable up to a maximum of 72 hours, as additional fresh electrodes would not be provided after this point. Participants only needed to return to the hospital within one week to return the data and device

to the researcher. The data was then downloaded and used with Kubios HRV Premium. For details on how HRV measures were extrapolated from the raw R-R interval data, see Section 2.1.5.1 and 2.1.5.2 (UTS T2D Study: Methods).

4.1.5 Meals, Medications, and Physical Activity Diary

Autonomic activity and glycaemia are affected by a range of day-to-day activities and events. To gather important information about participant's regular routines, which they were encouraged to continue,[491] each subject was required to provide details regarding any meals they ate, medications they took, or any physical activity they undertook. Participants were instructed on how to complete the diary and were provided with some examples, such as "1:30pm chicken sandwich with cheese and mayonnaise" and "8:00-8:15am walked to the train station". Mealtimes were useful for identify periods when participant's glucose levels were likely to spike, and physical activity times were useful for identifying periods of HRV data to be excluded for analysis because of the confounding effect of exercise on HRV.

4.1.6 Selection of Glycaemic Excursions and R-R Interval Data

Between 24-72 hours of continuous R-R interval data and glucose data were collected from each participant in this study. Glucose levels are typically measured every five minutes by CGM systems per standardized reporting.[492] However, it should be noted that the data downloaded by the glucose monitors for this study only provided a glucose reading every 15 minutes, and each glucose data point was timestamped. This provided a reference point for determining the exact time at which glucose excursions occurred. For example, if a participant's BGL dropped below a certain value, it was possible to determine the exact time during the day at which that occurred. Additionally, each R-wave recorded by the heart rate monitors was timestamped. This allowed for HRV epochs to be calculated in the 10 minutes before, during, and after the start every glycaemic excursion, and thus it was possible to observe changes in HRV during a hypoglycaemia or hyperglycaemia event. Noteworthy changes in certain HRV measures may serve as an early indicator of glycaemic excursions.

However, care had to be taken when determining an appropriate threshold for defining these excursions. Although BGL < 4.0 mmol/L and BGL > 7.7 are the most widely used definitions of hypoglycaemia and hyperglycaemia, respectively, these do not represent thresholds for clinically significant glycaemic excursions. In practice, symptoms do not necessarily arise when BGL exceeds either of these thresholds,[493, 494] even though this does not occur under regular

physiological conditions.[495] For example, when hypoglycaemia episodes are defined as BGL < 4.0 mmol/L, less than 40% of those episodes are symptomatic. When hypoglycaemia is defined by a more clinically relevant threshold, such as < 3.0 mmol/L, roughly 70% of subjects experience autonomic symptoms, and only 30% do not.[494] This is relevant to this study because in Section 1.11: Basis for Research, it was established that HRV measures may be predictive of hypoglycaemic or hyperglycaemia due to the distinct autonomic response which occurs during glycaemic excursions. If this response is absent from most of the glycaemic events that are recorded, then any observed changes in HRV are unlikely to be related to the glycaemic excursion. Therefore, it was important to utilise a more stringent and clinically relevant definition of hypoglycaemia when selecting excursions to analyse.

Based on recommendations from multiple clinical trials, hypoglycaemia was defined as BGL < 3.0 mmol/L and hyperglycaemia was defined as BGL ≥ 12.0 mmol/L.[496-498] In addition to this, for a hypoglycaemic event to be included for analysis in this study, it could not be followed by BGL returning to 4.5 mmol/L or more within 10 minutes.[499] Glycaemic excursions were only selected for analysis if the entire event was captured by both the glucose monitor and the heart rate monitor. For example, for some participants there was over two weeks of glucose data to be downloaded from the glucose monitors, but only 48 hours of R-R interval data from the heart rate monitors. Thus, only glucose data that overlapped with the 48 hours of R-R interval data was relevant to be analysed.

4.1.7 Measures of Heart Rate Variability

The measures of HRV used in this study included low frequency (LF) power, high frequency (HF) power, the low to high frequency ratio (LF:HF), total power, and normalised low frequency (LFnu). LFnu is more strongly correlated with sympathetic nervous system activity compared to LF power,[500] though ultimately both sympathetic and parasympathetic activity are reflected in LF power and LFnu. LFnu is calculated from the formula:

$$LFnu = 100 \times \left(\frac{LF}{LF + HF}\right)$$

Where the derived value is a percentage, or proportion of LF power to HF power. This is calculated differently to LF:HF, which is simply LF power divided by HF power. The high frequency counterpart to LFnu, known as normalised high frequency power (HFnu), is calculated as follows:

$$HFnu = 100 \times \left(\frac{HF}{LF + HF}\right)$$

As such, the sum of LFnu and HFnu will always be 100%, as they are algebraic inverses. Because of this, statistical analysis involving LFnu and HFnu produces identical, but inverse values.[501] For example, correlating BGL against LFnu and then against HFnu will produce the same p-value and correlation coefficients, however the sign of the 'r' coefficient will be reversed, as these variables are inverses. To avoid redundancies, only LFnu and not HFnu will be referred to in the statistical analysis.

Additionally, time-domain measures of HRV were used in this study as long-term recordings were available due to continuous, at-home heart rate recording. These included the root mean square of successive RR interval differences (RMSSD), and the standard deviation of NN intervals (SDNN). RMSSD is closely associated with HF power and reflects parasympathetic control of HRV. SDNN measures sympathetic and parasympathetic activity on HRV, and is closely related to LF power.[502]

4.1.8 Statistical Analysis

For a preliminary analysis, correlations between BGL and various measures of HRV measures were investigated in only the first participant of this study. These correlation analyses, combined with scatter plots, was performed in SPSS version 22.0 (IBM SPSS Statistics, USA) to investigate a possible trend in HRV measures when BGL was in the low range or in the high range. Given the large number of data points collected over the 24 hours for each participant, there were no concerns about sample power when using Pearson's correlations to identify significant ($p<0.05$) correlations between glucose level and HRV in a single participant. This statistical analysis aimed to continue previous research and the results are provided only for a comprehensive analysis of the relationship between HRV and BGL. This preliminary analysis was not important for the main aim of this research.

The main analysis in this study aimed to identify changes in HRV measures during a hypoglycaemic event, as stated in the aims and hypotheses. Non-overlapping HRV epochs were calculated from 10-minute samples, and these were sorted into three independent groups. These were 'Pre-hypo, 'Hypo, and 'Post-hypo, which refer to 10-minute epochs recorded 10 minutes prior to the start of the hypoglycaemic event, 10 minutes during, and 10 minutes after, respectively. These three groups represent a longitudinal approach, as the same set of HRV measures were calculated from the same sample at three different time points. Longitudinal studies typically have higher statistical power compared to cross-sectional studies.[503] As such, a one-way analysis of variance (ANOVA) with repeated measures was used to determine any significant ($p<0.05$) differences between the related means: Pre-hypo, Hypo, and Post-hypo HRV

measures. A significant difference ($p<0.05$) would indicate a change in HRV over the course of the hypoglycaemia excursion. To ensure that the assumptions of the repeated measures ANOVA were not violated, the data set was checked for outliers, normal distribution, and sphericity. Shapiro-Wilk Test, appropriate for small sample sizes ($n<50$), was used to confirm that variables were normally distributed. Normal Q-Q plots showed that the data points were linearly organised, which also confirmed normal distribution. Mauchly's test of sphericity was used to indicate any violations of the assumption of sphericity in the data and a Greenhouse-Geisser correction was applied in cases where the assumption of sphericity was violated.[504] These steps were repeated for the hyperglycaemic events that were observed, and the HRV means were labelled as 'Pre-hyper', 'Hyper', and 'Post-hyper'.

With reference to Cohen's "A Power Primer", the minimum sample size (n) required to achieve 0.8 power for this study was determined. For an ANOVA comparing the means of three related groups (Pre-hypo, Hypo, and Post-hypo), with a significance (α) of 0.05 and a large effect size ($r=0.5$), it was necessary to observe at least $n=21$ hypoglycaemia and $n=21$ hyperglycaemia events to provide sufficient power.[505] It was estimated that it would be necessary to recruit 15 participants with T1D to satisfy these requirements.

4.2 Results

The RNSH T1D Study aimed to investigate the activity of HRV measures during glycaemic excursions, including hypoglycaemia and hyperglycaemia. Both time and frequency-domain measures of HRV were investigated, as data was collected over a 24-hour period. For this study, 15 participants with T1D were recruited, with a sex breakdown of 4 males and 11 females. Participants were aged between 20-61 years. The average duration of the heart rate recordings was 30 hours, with most participants opting to begin recording between 10:00am and 12:30pm. Additional sample information is reported in Table 4.1. HbA_{1c} was measured by the CGM systems using weekly estimations of BGL, and the mean was 6.9%. Duration of diabetes for each subject was not recorded by the researchers in error and due to logistical problems created by the COVID-19 infection control measures implemented in March 2020.

Table 4.1 Means and standard deviations for demographic and physiological features of the sample (n=15). Data is presented as mean ± standard deviation. Body mass index was calculated from weight (in kilograms) divided by height (in metres) squared. Blood pressure was determined using the average of three left-arm recordings. HbA_{1c} was estimated by the CGM systems. **BMI** = Body mass index, **BP** = Blood pressure, **kg/m²** = Kilogram per square metre, **mmHg** = Millimetres of mercury, **mmol/L** = Millimoles per litre.

Variable	Mean ± standard deviation
Age (years)	36 ± 16
BMI (kg/m^2)	25 ± 3
Systolic BP (mmHg)	127 ± 14
Diastolic BP (mmHg)	77 ± 7
Estimated HbA_{1c} (%)	6.9 ± 0.9

4.2.1 Preliminary Correlation Analysis

A preliminary correlation analysis was conducted on the first participant recruited in this study to investigate a potential relationship between HRV measures and BGL as the two variables fluctuated over a 24-hour period. A Pearson's correlation determined there was no significant association between BGL and any HRV measure, including LF power, HF power, total power, LF:HF, LFnu, HFnu, RMSSD, SDNN, or even mean heart rate. Scatter plots of these variables of interest as they fluctuated over the course of the 24-hour monitoring period were equally distributed (Figure 4.1). This preliminary analysis was not important to the aim of this study. However, the results of this analysis are shown here to provide some noteworthy information.

Figure 4.1 Scatter plots of glucose level versus various heart rate variability measures for Participant 01 (n=96).

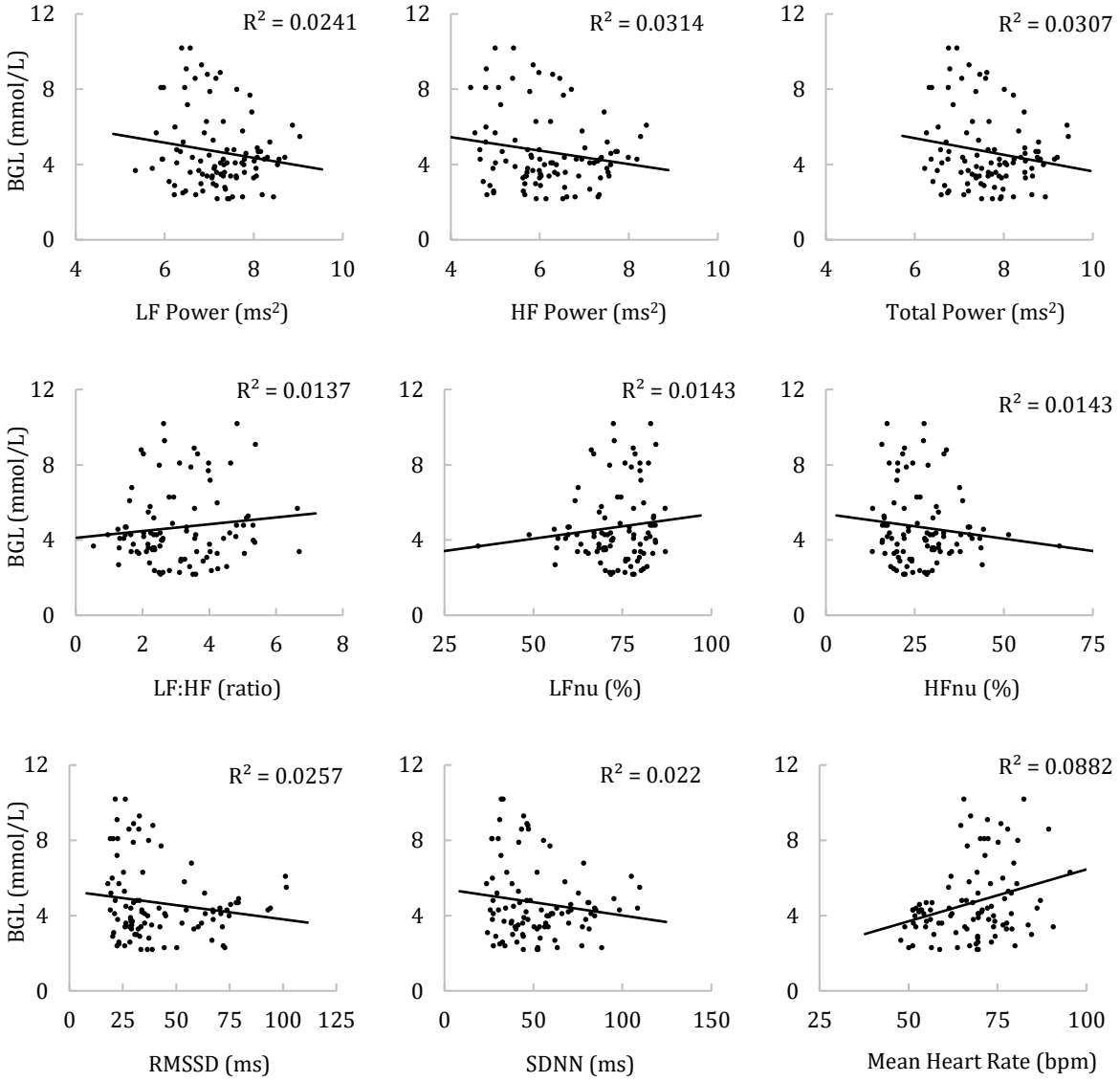

Figure 4.1 visualises the relationship between HRV measures and BGL as variables fluctuated in a single participant over the course of the study period. Each independent variable (x-axis) was plotted against the dependant variable, BGL, on scatter plots with a linear trendline representing the R^2 value. HRV measures were calculated from 15-minute epochs and were matched with each glucose data point measured at the same time. Glucose levels were recorded once every 15 minutes. Over 24-hours, this provided n=96 data points. All values were taken from a single participant. Note that the plots of LFnu and HFnu are symmetrical and the R^2 values are identical, as they are algebraic inverses. **BGL** = Blood glucose level, **bpm** = Beats per minute, **HF** = High frequency, **HFnu** = Normalised high frequency, **LF** = Low frequency, **LF:HF** = low to high frequency ratio, **LFnu** = Normalised low frequency, **mmol/L** = Millimoles per litre, **ms** = Milliseconds **ms²** = Milliseconds squared, **RMSSD** = Root mean square of successive RR interval differences, **SDNN** = Standard deviation of NN intervals.

4.2.2 Hypoglycaemia

A review of the literature was used to establish a threshold for defining hypoglycaemia, which was BGL < 3.0 mmol/L. Based on this criterion, n=22 hypoglycaemia events were observed over the course of 450 hours of subject activity, which satisfied the requirements of the power analysis. For each instance of hypoglycaemia, three 10-minute epochs of R-R interval data were processed into HRV measures, providing information on HRV at the following points: 10 minutes prior to the event ('Pre-hypo'), at the start of the event ('Hypo'), and 10 minutes after the event ('Post-hypo'). Initially, trends in HRV measures were investigated before and after some of these hypoglycaemia events by visual inspection of line graphs. Three of these line graphs are shown in Figure 4.2. These show HRV activity 30 minutes before and after three instances of hypoglycaemia, each occurring in a different participant. As there were no obvious trends seen in this initial analysis, this was followed up with a statistical analysis (n=22).

Figure 4.2 Change in heart rate variability before and after hypoglycaemia in three events.

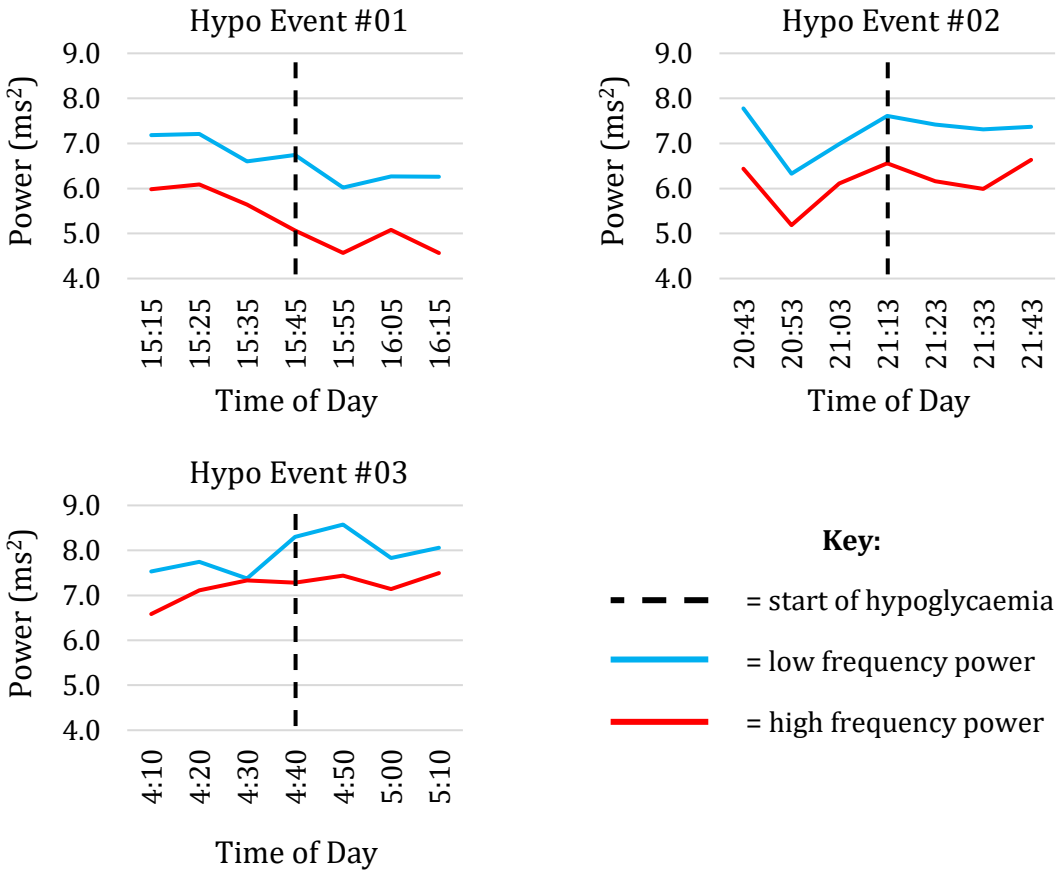

Figure 4.2 shows heart rate variability before and after three different hypoglycaemia events experienced by three different participants. For each individual figure, n=1. The black dotted line indicates the start of the hypoglycaemia, measured as blood glucose < 3.0 mmol/L, and HRV data is presented 30 minutes before and after the start of the event. As seen in Hypo Event #01, the event started at 3:45pm. **Hypo** = Hypoglycaemia, **ms²** = Milliseconds squared.

For the statistical analysis, the means of seven HRV measures were calculated for three HRV epochs – Pre-hypo, Hypo, and Post-hypo – from the n=22 instances of hypoglycaemia. These HRV measures were LF power, HF power, total power, LF:HF, normalised LF power, SDNN, and RMSSD. A one-way ANOVA with repeated measures determined if there were any significant differences between mean Pre-hypo, mean Hypo, and mean Post-hypo for each of the seven HRV measures (Table 4.2). Shapiro-Wilk test confirmed that all variables analysed were normally distributed ($p>0.05$) at all levels, and normal Q-Q plots were inspected manually to ensure data points were linearly organised, which also confirmed normal distribution. As with the previous studies, and as is common in HRV studies, LF power, HF power, and total power were natural log transformed to ensure normal distribution, as they are typically skewed without log transformation.[146, 423, 424]

Table 4.2 Mean heart rate variability measures (n=22) at 10 minutes prior, during, and 10 minutes after hypoglycaemia.All the measures presented in this table are frequency-domain, except for SDNN and RMSSD, which are time-domain. The analysis of variance determined there was no significance difference between any of the repeated measures for all variables. **ANOVA** = Analysis of variance, **HF** = High frequency, **Hypo** = Hypoglycaemia, **LF** = Low frequency, **LF:HF** = low to high frequency ratio, **LFnu** = Normalised low frequency power, **ms** = Milliseconds, **ms²** = Milliseconds squared, **NS** = Non-significant.

Variable	Pre-hypo	Hypo	Post-hypo	ANOVA
n	22	22	22	-
LF power (ms^2)	6.2 ± 1.4	6.2 ± 1.2	6.1 ± 1.2	NS
HF power (ms^2)	4.9 ± 1.5	4.9 ± 1.5	4.9 ± 1.6	NS
Total power (ms^2)	6.6 ± 1.4	6.6 ± 1.2	6.6 ± 1.2	NS
LF:HF	4.4 ± 2.9	4.7 ± 3.4	4.5 ± 3.3	NS
LFnu (%)	76 ± 11	77 ± 12	75 ± 14	NS
SDNN (ms)	35 ± 20	33 ± 16	34 ± 19	NS
RMSSD (ms)	27 ± 23	23 ± 16	25 ± 19	NS

A graphical comparison of the first three frequency-domain HRV measures, including mean LF power, HF power, and total power, is presented in Figure 4.3. Mauchly's test of sphericity showed no violation of the assumption of sphericity for the LF power data set, $\chi^2(2) = 0.649$, $p = 0.72$, or the HF power data set, $\chi^2(2) = 1.733$, $p = 0.42$. or the total power data set, $\chi^2(2) = 0.635$, $p = 0.73$. As such, Greenhouse-Geisser correction was not applied for any of the following one-way ANOVA with repeated measures. The ANOVA revealed that there was no significant

difference between the three time points for LF power ($F(2, 42) = 0.023$, $p = 0.98$), HF power ($F(2, 42) = 0.091$, $p = 0.91$), or total power ($F(2, 42) = 0.001$, $p = 1.00$).

Figure 4.3 Comparison of mean low frequency, high frequency, and total power at three time points during hypoglycaemia (n=22).

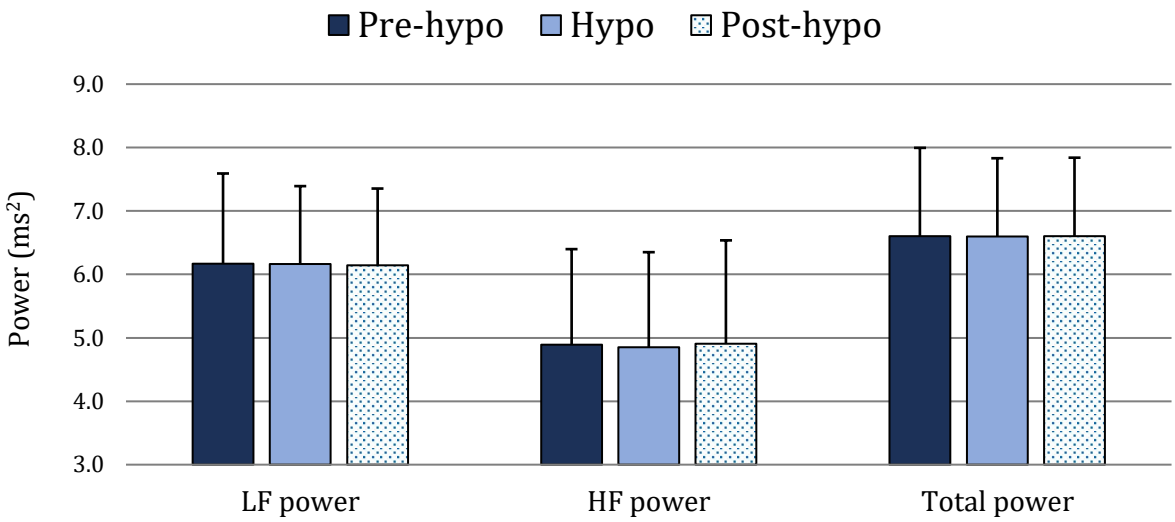

Figure 4.3 shows how frequency-domain measures compare before, during, and after hypoglycaemia. Data is presented as means with error bars as standard deviations. Though LF power, HF power, and total power are presented on the same axis, it is not appropriate to compare values between different measures of heart rate variability. Only differences between the three time points for each measure were investigated. **HF** = High frequency, **LF** = Low frequency, **ms²** = Milliseconds squared.

A visual representation of the other two frequency-domain HRV measures, LF:HF and normalised LF power, is shown in Figure 4.4. According to Mauchly's test, there was no violation of sphericity for the LF:HF data set, $\chi^2(2) = 0.306$, $p = 0.86$, or the normalised LF power data set, $\chi^2(2) = 1.709$, $p = 0.43$. As such, a Greenhouse-Geisser correction was not applied. A repeated measures ANOVA revealed that mean LF:HF was not significantly different across the three time points ($F(2, 42) = 0.148$, $p = 0.86$), and neither was mean normalised LF power ($F(2, 42) = 0.316$, $p = 0.73$).

Figure 4.4 Comparison of mean low to high frequency ratio and normalised low frequency power at three time points during hypoglycaemia (n=22).

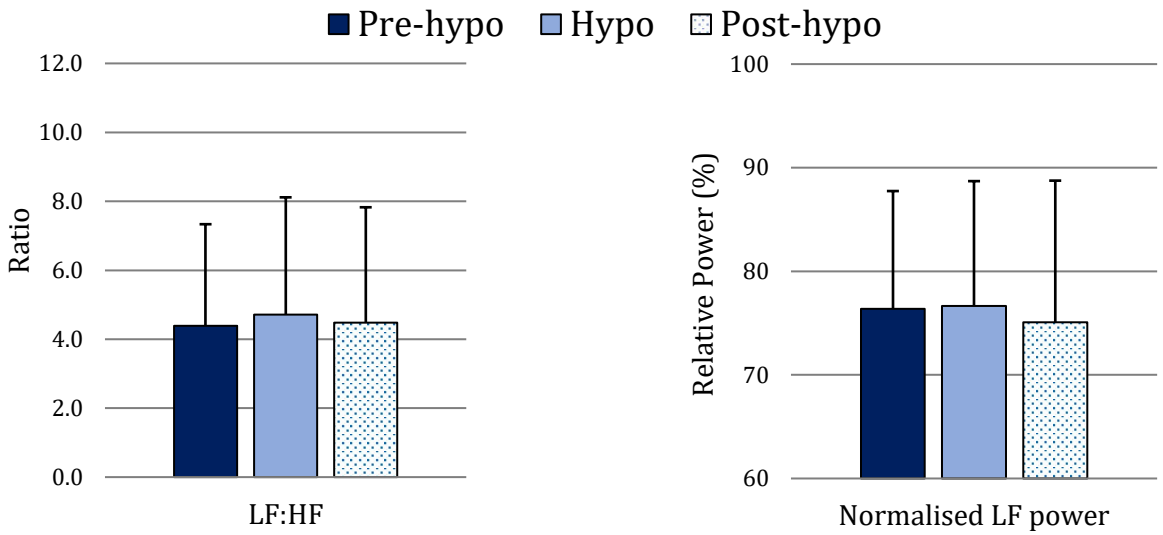

Figure 4.4 illustrates the change in LF:HF and normalised LF power before, during, and after hypoglycaemia. Errors bars represent standard deviations, and each bar indicates a mean value. LF:HF is represented without units, as it is a ratio of LF to HF power. Normalised LF power is presented as a percentage as it indicates the power of LF relative to HF. **LF** = Low frequency, **LF:HF** = low to high frequency ratio.

For the time-domain measures, including SDNN and RMSSD, means and standard deviations are compared in Figure 4.5. Mauchly's test of sphericity showed no violation of the assumption of sphericity for SDNN, $\chi^2(2) = 3.252$, $p = 0.20$, and so a one-way repeated measures ANOVA was conducted without Greenhouse-Geisser correction. The ANOVA determined that mean SDNN was not significantly different between the three time points ($F(2, 42) = 0.438$, $p = 0.65$). There was a violation of the assumption of sphericity for RMSSD, $\chi^2(2) = 22.031$, $p < 0.05$, and so a repeated measures ANOVA was conducted with Greenhouse-Geisser correction. The Greenhouse-Geisser correction affects the degrees of freedom applied in the analysis, but not the partitioning of Sum of Squares of the F-statistic. This statistical analysis demonstrated that mean RMSSD was not significantly different ($F(1.199, 25.185) = 0.741$, $p = 0.42$).

Figure 4.5 Comparison of time-domain measures at three time points during hypogylcaemia (n=22).

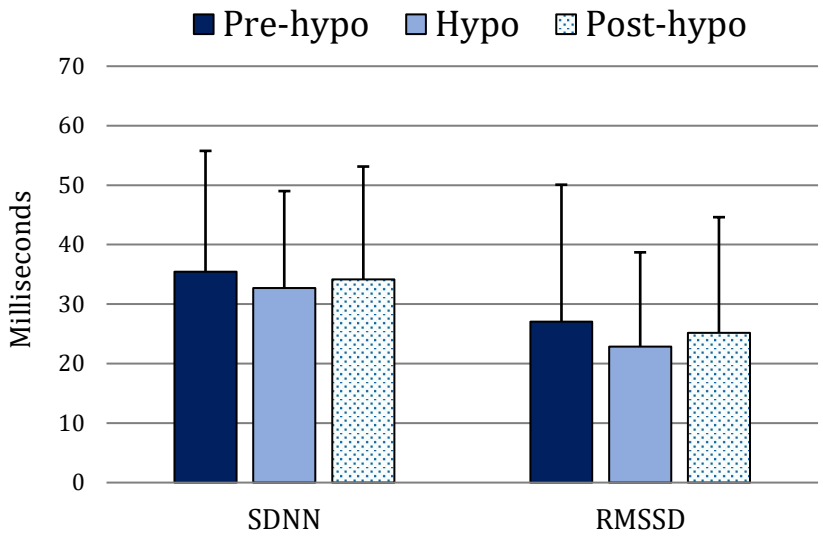

Figure 4.5 visualises differences in mean SDNN and RMSSD before, during, and after hypoglycaemia. Data is presented as means with error bars as standard deviations. Time-domain measures of HRV such as these are measured in milliseconds. As with frequency-domain measures, differences between mean SDDN and mean RMSSD were not investigated. **RMSSD** = Root mean square of successive RR interval differences, **SDNN** = Standard deviation of NN intervals.

4.2.3 Hyperglycaemia

In this study, hyperglycaemia was defined as BGL ≥ 12.0 mmol/L. Over the course of 450 hours of recording participants, n=33 instances of hyperglycaemia were captured, based on this criterion. As with hypoglycaemia, it was investigated whether there were any changes in HRV measures over the course of many hyperglycaemia events. HRV measures were calculated by the same methods, and the three HRV epochs were labelled similarly as Pre-hyper, Hyper, and Post-hyper, referring to HRV epochs that occurred 10 minutes before, during, and 10 minutes after the start of a hyperglycaemia event, respectively. For an initial analysis, line graphs were used on three instances of hyperglycaemia to inspect changes in HRV before and after the event starts (Figure 4.6).

Figure 4.6 Change in heart rate variability before and after hyperglycaemia in three events.

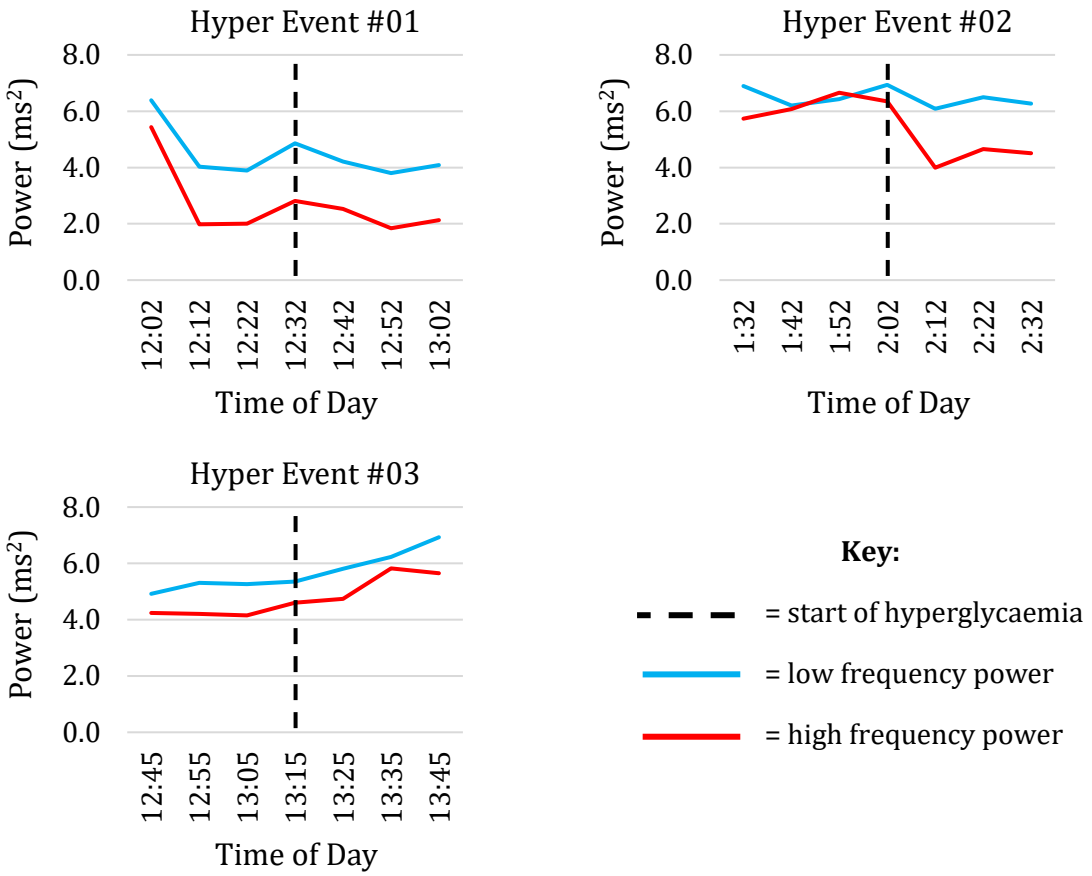

Figure 4.6 highlights the changes in low frequency power and high frequency power during individual hyperglycaemia events. Each of these events occurred in different participants, and for each individual graph, n=1. The black dotted line marks the start of the hyperglycaemia, when blood glucose rose above 12.0 mmol/L, and HRV data is provided for 30 minutes before and after this event start. **Hyper** = Hyperglycaemia, **ms²** = Milliseconds squared.

Following this, a statistical analysis was conducted on the means of the seven HRV measures investigated in this study. As with the hypoglycaemia events, a repeated measures ANOVA determined any significant changes in LF power between Pre-Hyper, Hyper, and Post-hyper means calculated from the n=33 hyperglycaemia events. This was then repeated for the other HRV measures, including HF power, total power, LF:HF, normalised LF power, SDNN, and RMSSD. The means and standard deviations of these HRV measures at each of the 10-minute epochs are provided in Table 4.3.

Table 4.3 Mean heart rate variability measures (n=33) at 10 minutes prior, during, and 10 minutes after hyperglycaemia. All the measures presented in this table are frequency-domain, except for SDNN and RMSSD, which are time-domain. The analysis of variance determined there was no significance difference between any of the repeated measures for all variables. **ANOVA** = Analysis of variance, **HF** = High frequency, **Hyper** = Hyperglycaemia, **LF** = Low frequency, **LF:HF** = low to high frequency ratio, **LFnu** = Normalised low frequency power, **ms** = Milliseconds, **ms²** = Milliseconds squared, **NS** = Non-significant.

Variable	Pre-hyper	Hyper	Post-hyper	ANOVA
n	33	33	33	-
LF power (ms^2)	6.1 ± 1.2	6.2 ± 0.9	6.0 ± 1.2	NS
HF power (ms^2)	4.9 ± 1.6	5.2 ± 1.3	5.0 ± 1.4	NS
Total power (ms^2)	6.6 ± 1.2	6.7 ± 0.9	6.5 ± 1.2	NS
LF:HF	4.2 ± 2.9	3.8 ± 2.7	3.6 ± 2.7	NS
LFnu (%)	74 ± 15	71 ± 17	71 ± 17	NS
SDNN (ms)	34 ± 19	33 ± 15	31 ± 17	NS
RMSSD (ms)	26 ± 21	27 ± 18	25 ± 21	NS

Figure 4.7 visualises the differences in LF power, HF power, and total power. According to Mauchly's test of sphericity, the HF power data set violated the assumption of sphericity, $\chi^2(2) = 8.066$, $p = 0.02$. As such, a Greenhouse-Geisser correction was used in conjunction with the repeated measures ANOVA. This determined no significant difference in mean HF power between the three time points ($F(1.627, 52.071) = 1.285$, $p = 0.28$). The assumption of sphericity was not violated by either the LF power data set, $\chi^2(2) = 2.134$, $p = 0.34$, or the total power data set, $\chi^2(2) = 2.781$, $p = 0.25$. As such, the repeated measures ANOVA was conducted without Greenhouse-Geisser correction. There was no significant difference in LF power ($F(2, 64) = 1.335$, $p = 0.27$) or total power ($F(2, 64) = 1.322$, $p = 0.27$) between the three time points.

Figure 4.7 Comparison of mean low frequency, high frequency, and total power at three time points during hyperglycaemia (n=33).

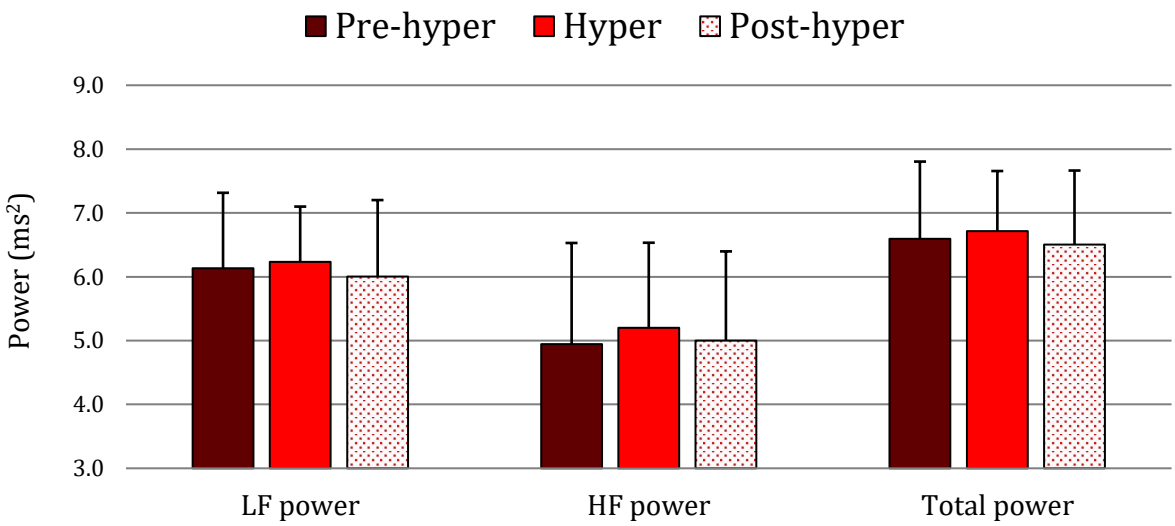

Figure 4.7 shows the difference in frequency-domain measures of heart rate variability before, during, and after hyperglycaemia occurred. These measures are presented as means with error bars as standard deviations. Though these measures are presented on the same axis, it is inappropriate to compare values between different measures of heart rate variability. **HF** = High frequency, **LF** = Low frequency, **ms²** = Milliseconds squared.

The change in two other frequency-domain measures of HRV, LF:HF and normalised LF power, is visualised in Figure 4.8. Mauchly's test indicated that the assumption of sphericity was violated in both the LF:HF ($\chi^2(2) = 12.008$, $p < 0.01$) and normalised LF power ($\chi^2(2) = 8.457$, $p = 0.02$) data sets. Repeated measures ANOVA with Greenhouse-Geisser correction showed that there was no significant difference in mean LF:HF ($F(1.483, 43.003) = 1.488$, $p = 0.24$) or normalised LF power ($F(1.615, 51.664) = 1.091$, $p = 0.33$) between the three time points.

Figure 4.8 Comparison of mean low to high frequency ratio and normalised low frequency power at three time points during hyperglycaemia (n=33).

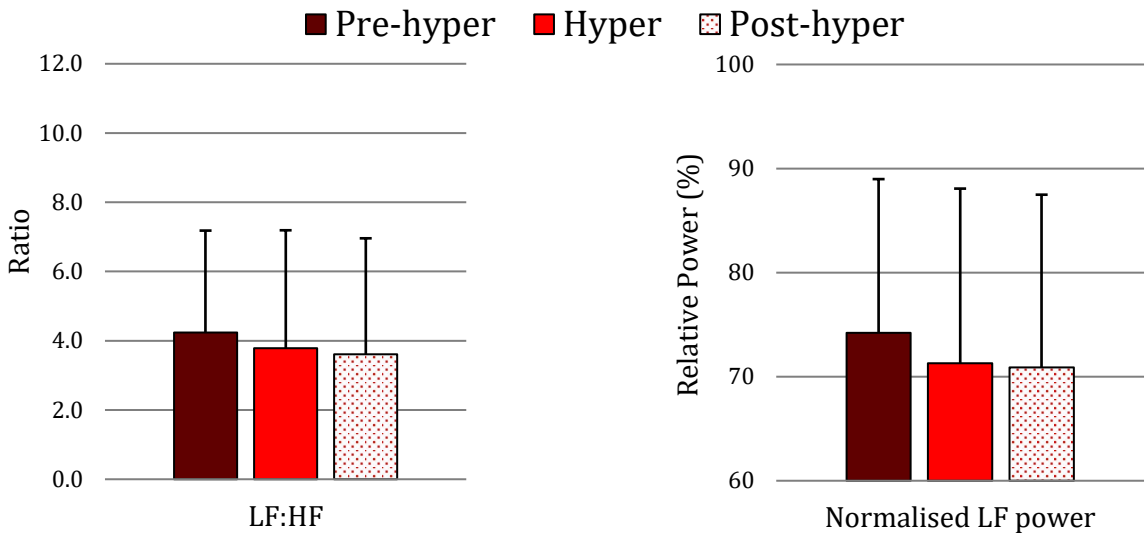

Figure 4.8 demonstrates differences in certain frequency-domain measures of heart rate variability during a hypoglycaemic event. Data is presented as means with error bars as standard deviations. LF:HF is a ratio, and has no units, and normalised LF power was calculated from the relative value of LF power compared to HF power. **LF** = Low frequency, **LF:HF** = low to high frequency ratio.

Analysis was also performed on time-domain measures of HRV during hyperglycaemia, and a graphical representation of the means is shown in Figure 4.9. Mauchly's test of sphericity showed the assumption of sphericity was violated for SDNN, $\chi^2(2) = 12.492$, $p < 0.01$, and so a one-way repeated measures ANOVA was conducted with Greenhouse-Geisser correction. The ANOVA determined that mean SDNN was not significantly different between the three time points ($F(1.502, 48.060) = 0.524$, $p = 0.55$). There was also a violation of the assumption of sphericity for RMSSD, $\chi^2(2) = 6.307$, $p < 0.05$, and so a repeated measures ANOVA was conducted with Greenhouse-Geisser correction. Mean RMSSD was not found to be significantly different ($F(1.681, 52.118) = 1.961$, $p = 0.16$)

Figure 4.9 Comparison of time-domain measures at three time points during hyperglycaemia (n=33).

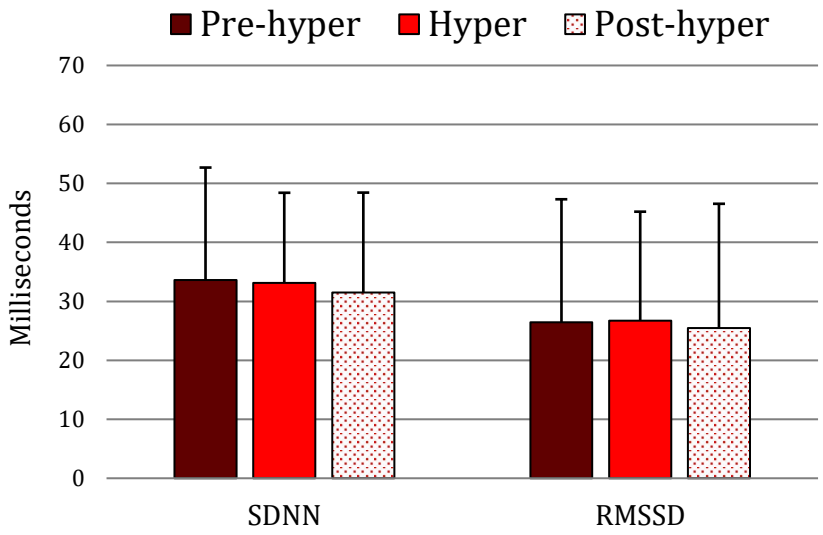

Figure 4.9 represents the mean activity of time-domain measures at three time points during hyperglycaemia. Errors bars represent standard deviations, and each bar indicates a mean value. Time-domain measures of HRV such as these are measured in milliseconds. Differences between mean SDDN and mean RMSSD were not investigated. **RMSSD** = Root mean square of successive RR interval differences, **SDNN** = Standard deviation of NN intervals.

4.2.4 Summary of Results

Preliminary correlation analysis of the first participant in the study revealed that daily fluctuations in BGL and HRV measures were largely unrelated, and scatter plots of the variables of interest were equally distributed. Line graphs showing HRV activity 30 minutes before and after three instances of hypoglycaemia, each occurring in a different participant, were inspected and no obvious trends were identified. This was followed-up with a statistical analysis. The repeated measures ANOVA determined there was no significant difference in any HRV measure in the 10-minutes before, during, or after a hypoglycaemic (n=22) or hyperglycaemic (n=33) event based on means of the 15 participants.

4.3 Discussion

Glycaemic excursions are prevalent in people living with T1D and represent a significant threat to their continued health and wellbeing. Current standards in glucose monitoring are inadequate given the severity of T1D and its complications, and recent interest in improving these standards is justified. A non-invasive and convenient measure of glycaemic excursions may significantly improve effectiveness of stringent glucose monitoring, which will lead to better glycaemic outcomes and quality of life for those with T1D. In the RNSH T1D Study, people with T1D participated in a 24-hour study continuously monitoring their HRV and glucose levels. The aim was to determine whether glycaemic excursions, including hypoglycaemia and hyperglycaemia, were marked by significant changes in measures of HRV. Preliminary analysis of these results focused on whether HRV measures were associated with glucose fluctuations across the day, as well as whether individual glycaemic excursions were associated with identifiable changes in HRV. These early analyses showed no meaningful findings, and machine learning techniques may be required to identify trends in HRV during glycaemic excursions, which would require a larger sample size. Additionally, the follow-up statistical analysis by ANOVA indicated there were no significant changes in any HRV measure during hypoglycaemia or hyperglycaemia. This section will explore these findings in the context of the wider literature and discuss the limitations of this research.

4.3.1 Preliminary Analysis of the Sample

Due to the small number of subjects, it is difficult to judge whether the sample was reflective of the wider Australian T1D population. The sample was of similar age and BMI compared with samples observed in other studies in this specific area of research.[491, 506] In terms of physiology, the values for systolic and diastolic blood pressure were generally within optimal ranges.[507] Glucose levels were significantly above the optimal range,[406] though this was expected as this is a clinical characteristic of T1D. The mean HbA_{1c} for the sample was 6.9 ± 0.9%, and as with the UTS T2D Study, roughly half of the sample presented with an HbA_{1c} level below 7.0%, which is the target glycaemia level. This is similar to the proportion of Australians with diabetes who achieve target glycaemia, based on large-scale epidemiological data.[104] The preliminary correlation analysis revealed no significant relationship between HRV measures and BGL values measured at the same time points. A significant finding here would have indicated that fluctuations in HRV are closely related to fluctuations in BGL, and that HRV may be used to estimate glucose levels at any time. This would have been a novel finding, as this has not been demonstrated anywhere in the literature to date. Furthermore, analysis of individual glycaemic

events revealed no consistent relationship between autonomic activity and the onset of hypoglycaemia and hyperglycaemia. This was more likely to have yielded a significant result, as other studies have shown significant changes in HRV measures at the onset of hypoglycaemia. These will be discussed in the next section.

4.3.2 Changes in Autonomic Activity during Hypoglycaemia

In a study conducted by Klimontov, Myakina & Tyan (2016) in 11 older women with T1D, LF power was lower during hypoglycaemia (n=12), as defined by CGM glucose reading ≤ 3.9 mmol/L, compared to fasting LF power.[396] HF power was similar during hypoglycaemia compared to fasting HF power, and LF:HF was lower during hypoglycaemia only in subjects with cardiac autonomic neuropathy. Additionally, LF power, HF power, and LF:HF during hypoglycaemia were all similar to fasting levels when the hypoglycaemia event occurred at night.[396] As such, the key finding was that daytime LF power was significantly diminished during hypoglycaemia, a finding which was not shared in the RNSH T1D Study. This is likely related to differences in the sample, as the present study recruited an equal number of men and women and with a much younger mean age compared to the study by Klimontov, Myakina & Tyan. In another study, Myakina, Klimontov & Safarov (2015) recruited n=73 subjects with T2D (aged 48-78 years old) who underwent simultaneous CGM and ECG monitoring. They demonstrated that LF power, HF power, and LF:HF all increased significantly ($p<0.01$) during hypoglycaemia (BGL < 3.9 mmol/L), for both daytime and nocturnal events.[475] In subjects with cardiac autonomic neuropathy, LF power decreased during hypoglycaemia. Further, Deshmukh and colleagues (2021) observed a significant change in RMSSD by Holter monitoring of T1D subjects with hypoglycaemia unawareness. This study also demonstrated that this change in RMSSD persists following islet cell transplantation and the restoration of euglycaemia in T1D subjects.[380]

These are just two examples of studies which demonstrated there are marked changes in certain HRV measures during hypoglycaemic events. Due to publication bias, it is difficult to identify studies in the literature which found no significant changes in HRV measures during hypoglycaemia, as was the case with the RNSH T1D Study. This may be the first study to demonstrate that autonomic activity remains relatively stable during hypoglycaemia in T1D, as determined by 10-minute HRV epochs in 15 subjects. It is possible changes in HRV in this sample were significant based on five-minute HRV epochs, however shorter epochs are less accurate and thus were not investigated in the statistical analysis. One recent study published by Lundqvist and colleagues (2021) reported no significant changes in HRV during

hypoglycaemia in a sample of T2D participants,[508] though to date these findings have not been reported in T1D.

Recently, Bekkink and colleagues (2019) published an original research article with findings that are particularly relevant to this PhD thesis. They investigated changes in LF:HF, SDNN, and RMSSD during hypoglycaemia (n=66 events) in 23 participants (mean age 42 ± 11 years). In their study, mean LF:HF increased significantly ($p<0.05$) by 0.2 ± 0.4, mean RMSSD decreased significantly by 4.4 ± 18.1, and there was no change in SDNN.[491] These findings are promising as they indicate a potential pattern in HRV at the initiation of hypoglycaemia. The presence of specific increases in LF:HF corresponding with specific decreases in RMSSD may be detected non-invasively by a heart rate monitor, as demonstrated in this study, and used to detect the onset of hypoglycaemia. Such research is similar to that of the present study and aims to develop HRV as a non-invasive predictor of hypoglycaemia. However, as Bekkink and colleagues did not investigate LF power, HF power, or total power, it is difficult to compare the results with those of the RNSH T1D Study. Of the three HRV measures which were investigated in both studies, LF:HF and RMSSD changed significantly during hypoglycaemia according Bekkink and colleagues (2019) but did not change significantly according to the results of the RNSH T1D Study. This discrepancy will be explored further.

In their paper, Bekkink and colleagues (2019) reported the mean glucose level of the n=66 hypoglycaemic events to be 3.1 mmol/L, and ranging between 1.6 - 3.9 mmol/L.[491] In their methodology, the authors defined their hypoglycaemic events as any glucose level equal to or below 3.9 mmol/L. Considering this information, it is clear a more generous glucose cut-off was implemented in this study for defining hypoglycaemia, as in the RNSH T1D Study the glucose cut-off value for hypoglycaemia was < 3.0 mmol/L. The criterion for the RNSH T1D Study was more stringent as it incorporated findings from previous literature and based this cut-off value on the findings of numerous clinical studies. However, this more stringent cut-off value meant that the glycaemic events recorded in the present study were more severe than those of the study conducted by Bekkink and colleagues (2019). Despite the added clinical significance, changes in mean HRV measures were not statistically significant in the present study but were significant in the study from Bekkink and colleagues who assessed a larger, but less severe sample of hypoglycaemic events. Differences in the approach of the statistical analysis may have affected these different findings, for example in the present study a one-way ANOVA with repeated measures was used, but Bekkink and colleagues (2019) implemented paired sample t-tests to analyse mean changes of HRV measures before and during hypoglycaemia.

One other key observation was made in the paper published by Bekkink and colleagues. They reported that 39% of their sample had impaired awareness of hypoglycaemia, reflecting a

reduced autonomic response to hypoglycaemia. Despite this, the authors were able to identify significant changes in LF:HF and RMSSD during hypoglycaemia. They reported that changes in HRV were not different between subjects with impaired awareness of hypoglycaemia compared with those with intact awareness, though initially they expected there would be because hypoglycaemia unawareness reflects decreased sympathetic nervous system activity during hypoglycaemia. The authors did not explain this finding, though limitations in the scope and design of their study may have limited their ability to comment on why impaired awareness of hypoglycaemia had little clinical significance in their sample. Hypoglycaemia unawareness is of particular importance to this area of research as it may limit the potential for heart rate monitors to detect autonomic responses to hypoglycaemia in theory, though findings from some studies, such as the one from Bekkink and colleagues, indicate this may not be the case in real-world settings.

4.3.2.1 Clinically Significant Hypoglycaemia

To further explore the limitations of the present research, as well as other research in this area, it is relevant to discuss the impact of clinically significant hypoglycaemia and impaired awareness of hypoglycaemia. The term clinically significant hypoglycaemia strictly refers to hypoglycaemic events which are associated with symptoms and impaired functioning. For individuals without diabetes, clinically significant hypoglycaemia generally occurs when BGL ≤ 3.9 mmol/L, as BGL does not fall below this level under optimal physiological conditions, according to American Diabetes Association consensus.[495] At this level, symptoms may arise such as sweating, confusion, and blurred vision. For people who experience hypoglycaemia more often, such as people with T1D who take insulin to counter their chronic hyperglycaemia, the onset of symptoms due to low BGL may not occur around the threshold of 3.9 mmol/L. Due to impaired awareness of hypoglycaemia, characterized by a reduced autonomic response to low BGL in as many as 30% of all T1D subjects, people with T1D may not experience symptoms until their BGL falls as low as 2.3 mmol/L, and some may not experience symptoms at all. Problematically, because of the pathophysiology of this unawareness and how it develops, it is likely that most people with T1D are affected by it to a certain degree. This unawareness may be graded, and as such some people are affected more strongly whilst others are affected only minimally by it. It is clear clinically significant hypoglycaemia may not occur in many people with T1D at the threshold of ≤ 3.9 mmol/L, and there are different views in the literature on how hypoglycaemia should be defined.

A 12-week study on n=3,912 subjects demonstrated that less than 40% of the hypoglycaemia episodes experienced by people with diabetes are symptomatic when hypoglycaemia is defined as BGL ≤ 3.9 mmol/L (Figure 4.10). However, as discussed, this threshold does not equate with symptoms of hypoglycaemia in people with T1D. Amiel and colleagues (2008) argue that defining hypoglycaemia as simply any glucose value equal to or below 3.9 mmol/L leads to overestimation of clinically significant hypoglycaemia.[509] The members of the International Hypoglycaemia Study Group agree that BGL < 3.0 mmol/L is sufficient to indicate clinically relevant or important hypoglycaemia.[510] According to Swinnen and colleagues (2009), when hypoglycaemia is defined by a more clinically relevant threshold, such as < 3.0 mmol/L, roughly 70% of subjects experience autonomic symptoms, and only 30% do not.[494] This is also demonstrated in Figure 4.10. This 30% is similar to the rate of hypoglycaemia unawareness observed in large samples of people with T1D and is the proportion of people with T1D who experience no autonomic symptoms at almost any level of low BGL. In the present study, it was therefore accepted that lowering the threshold further when defining hypoglycaemia would not significantly affect the observed proportion of subjects who experience symptoms, and thus < 3.0 mmol/L was an appropriate threshold for defining clinically significant hypoglycaemia. It was accepted that there will always be a proportion of people with diabetes who do not experience symptoms of hypoglycaemia due to hypoglycaemia unawareness.[494] This is the main reason subjects were not screened for impaired awareness of hypoglycaemia when participating. In a real-world scenario, this unawareness is likely to be present in about 30% of subjects, and changes in HRV that precede hypoglycaemia may be identified even in the absence of symptoms, i.e., HRV may predict hypoglycaemia even when it is not clinically significant. The research of Bekkink and colleagues (2019) suggests as much, as they implemented a glucose cut-off of ≤ 3.9 mmol/L when defining hypoglycaemia in their study. However, the results of the present study do not indicate that HRV measures may predict hypoglycaemia.

Figure 4.10 Proportion of asymptomatic hypoglycaemia at different definitions.

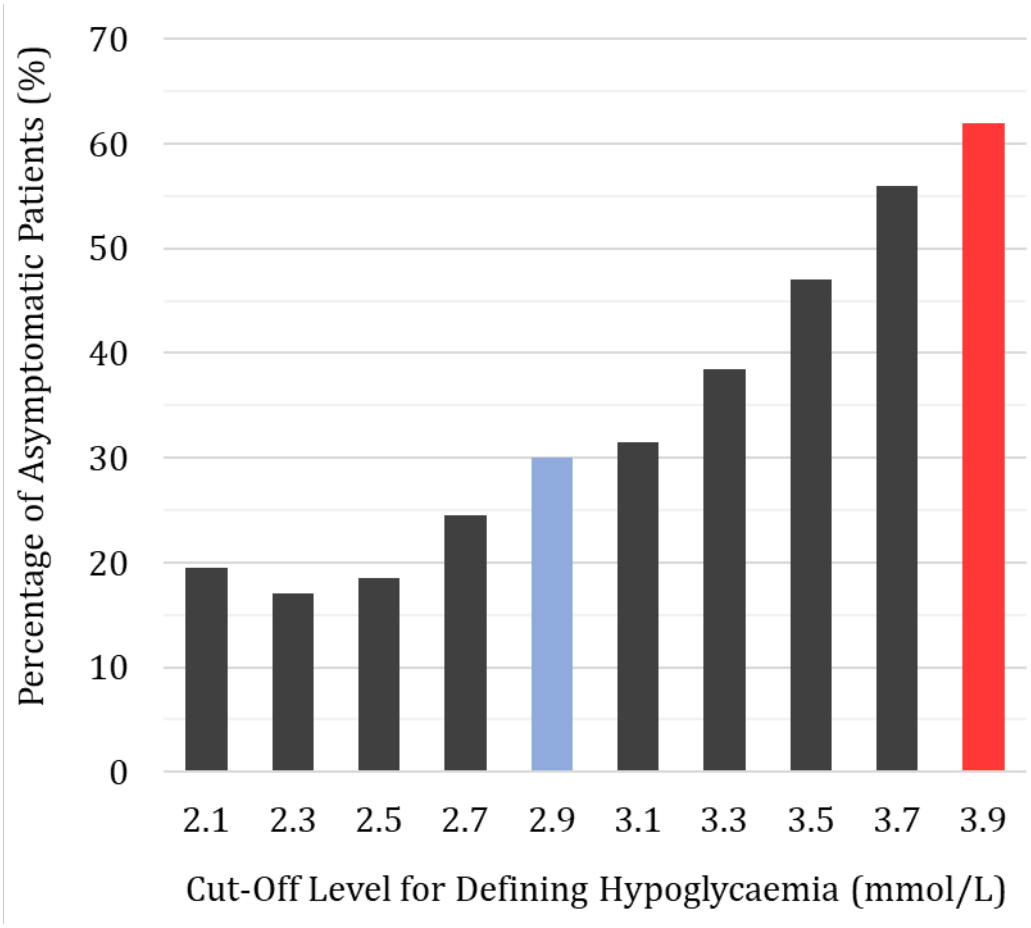

Figure 4.10 presents the different thresholds for hypoglycaemia used in the literature and the percentage of subjects who reported no symptoms at each of the thresholds. BGL ≤ 3.9 mmol/L (in red) is distinguishable as the pathological state of hypoglycaemia, as BGL does not exist below this level in non-pathological states. As indicated by the red column, over 60% of subjects have no symptoms of hypoglycaemia when it is defined as BGL ≤ 3.9 mmol/L. Defining hypoglycaemia by a symptomatic standard is more relevant in clinical practice, for example BGL ≤ 2.9 mmol/L (in blue). This is the cut-off used in the present study, though for consistency it was described as '< 3.0 mmol/L' in this thesis. Adapted from Swinnen and colleagues (2009).[494]

4.3.2.2 Neurophysiology of Hypoglycaemia

The autonomic nervous system is responsible for maintaining glucose homeostasis, which includes responding to hypoglycaemia. The detection of low BGL leads to efferent activation of the branches of the splanchnic nerve which connect to the pancreas, liver, and adrenal medulla. This results in secretion of glucagon from the pancreas, glucose from the liver, and catecholamines from the adrenal medulla.[511] As described previously, the neurophysiological response to hypoglycaemia involves autonomic symptoms, including sweating, heart palpitations, and trembling. If BGL continues to decline, such as when insulin medication continues to drive the uptake of glucose into cells from the blood, then neuroglycopenic

symptoms may predominate, including confusion, dizziness, and difficulty concentrating.[89] A clinical study on n=10 young adults demonstrated that awareness of hypoglycaemia was significantly higher in subjects during clamped hypoglycaemia with adrenergic blockade (phentolamine and propranolol) compared to subjects with pan-autonomic blockade (phentolamine, propranolol and atropine).[512] The authors, Towler and colleagues (1993), concluded that awareness of hypoglycaemia was largely the result of autonomic symptoms (shakiness, heart pounding, nervous) rather than neuroglycopenic symptoms (confusion, drowsiness), reinforcing the idea that the autonomic response to hypoglycaemia acts as an early warning system. In individuals with reduced awareness of hypoglycaemia, this is driven by reduced autonomic response to low BGL. The absence of autonomic symptoms lowers the threshold for clinically significant hypoglycaemia, and individuals may not realise they are experiencing a hypoglycaemic event until BGL becomes low enough that it causes problems with concentration and decision making. This raises an important concern for the RNSH T1D Study and similar research. HRV measures may be unable to reliable predict the onset of hypoglycaemia because the physiological variable they are assessing – autonomic activity – may remain unchanged in a large proportion of those who experience hypoglycaemia, as documented in this sample. Based on the literature, it is likely that autonomic changes were present in this sample, so the lack of significant findings may be due to the small sample size or the definition of hypoglycaemia. Hypoglycaemia was defined by a short duration of 10 minutes instead of 15 minutes, and subjects were not required to validate hypoglycaemia by confirmation finger stick BGL. As such, some of the events defined as hypoglycaemia in these analyses may have not met the standardized criteria for hypoglycaemia.

4.3.3 Changes in Autonomic Activity during Hyperglycaemia

Hyperglycaemia is an independent risk factor for cardiovascular disease as well as numerous microvascular complications of diabetes. The ability to non-invasively predict the onset of hyperglycaemia may facilitate early intervention and prevention of hyperglycaemia, and thus reduce long term complications of diabetes. There is substantially less research in the area of non-invasive HRV measures predicting the onset of hyperglycaemia compared to hypoglycaemia. The RNSH T1D Study investigated changes in frequency-domain HRV measures during clinically relevant hyperglycaemia, as defined by BGL ≥ 12.0 mmol/L, and presented novel non-significant findings. There were no significant changes in autonomic activity, as assessed by HRV, at the onset of hyperglycaemia, as assessed by CGM. This section aims to provide greater context and meaning to these findings.

In a clinical study of n=30 young females without chronic illness, Majeed and Yar (2020) demonstrated that LFnu and LF:HF were significantly higher during hyperglycaemia compared to the fasting state.[513] However, in the present study, HRV measures were compared before, during, and after hyperglycaemia onset, rather than compared with fasting levels. Additionally, there are marked differences in the neurophysiological response to suboptimal BGL in people without diabetes compared to people with T1D, and impaired awareness of hypoglycaemia is just one factor involved in these differences. Lundqvist and colleagues (2021) investigated autonomic and hormonal changes during hypoglycaemia and hyperglycaemia in n=15 overweight subjects with T2D and n=15 lean weight subjects with T2D.[508] Though there are marked physiological and pathological differences between T1D and T2D, it may be relevant to compare the results of the present study with the results of the lean weight group with T2D. In this group, the authors observed no change in HRV frequency-domain HRV measures during hyperglycaemia (BGL ≥ 13.0 mmol/L), including LF power, HF power, and total power, and LF:HF. This is consistent with the findings of the RNSH T1D Study.

For clinical studies on people with T1D, there are few significant findings. According to a 96-hour study on n=37 individuals with T1D, hyperglycaemia (BGL ≥ 15.0 mmol/L) is not associated with clinically relevant cardiac arrhythmias,[514] and the authors suggest that cardiac autonomic control may not be impaired during hyperglycaemia. Based on the work of Berkelaar and colleagues (2013), hyperglycaemia (BGL ≥ 10.0 mmol/L) is not correlated with HRV measures, including RMSSD and HF power, or cardiac vagal tone,[515] and the authors did not assess any other HRV measure relevant to the present study. As discussed, there are few clinical studies in this area, and more research is required to develop a better understanding of autonomic changes during hyperglycaemia. Future studies should consider clinical relevance when defining hyperglycaemia, as inconsistencies in how hyperglycaemia is defined by different researchers may explain differences in the statistical results. Specifically for frequency-domain HRV measures, more studies with larger sample sizes are required to form a consensus.

4.3.3.1 Neurophysiology of Hyperglycaemia

The response of the autonomic nervous system to increasing BGL has been discussed in a previous chapter. To summarise, the autonomic nervous system effectively decreases BGL by increasing insulin release from the pancreas, as well as by directly innervating the liver. The elevation of BGL above the optimal limit is most commonly caused by a lack of insulin response to food or caloric intake. In T1D, the near or total deficiency of circulating insulin leads to large glycaemic excursions in the postprandial state, as somatic cells in the body are unable to take up

glucose from the blood without insulin signalling. This results in chronically high levels of blood glucose, even hours after the ingestion of a meal. In a clinical study on n=20 newly diagnosed people with T2D, Marfella and colleagues (2000) observed acute increases in systolic blood pressure, diastolic blood pressure, heart rate, and plasma catecholamines during hyperglycaemia.[516] They concluded that, in the absence of diabetes complications including autonomic neuropathy, hyperglycaemia causes an autonomic-mediated hemodynamic response. This research is part of a growing body of evidence showing that hyperglycaemia is a risk factor for cardiovascular events, and this applies to people without diabetes as well.[517]

Early literature from Legler and colleagues (1982) established that cortisol levels increase after a meal,[518] and this has been ratified by randomized clinical research from Reynolds and colleagues (2001).[519] However, hyperglycaemia in people with T1D is not always the result of a postprandial response, as it may result when the effects of glucose-lowering medications wear off. Lundqvist and colleagues (2020) contend that the hormonal response to hyperglycaemia, including cortisol release, varies between individuals, but in general the autonomic response to rising BGL is characterised by a stress response.[508] In people with autonomic neuropathy or diminished autonomic tone, this stress response, as measured partly by LF power, is smaller.[508] This is similar to hypoglycaemia, in which decreasing BGL leads to a 'fight or flight' stress response mediated by the sympathetic branch of the autonomic nervous system. It may be inferred that the sympathetic nervous system responds to both high or low BGL to maintain glucose homeostasis, and inability to maintain BGL in the target range may predispose an individual to further glycaemic excursions, such as repeated hypoglycaemia risk in people with impaired awareness of hypoglycaemia. An important conclusion to be made from this is that even if HRV measures cannot accurately predict BGL in an individual with diabetes, as suggested by the results of the RNSH T1D Study, they may still be useful in identifying individuals with diminished autonomic response to glycaemic excursions. This information may empower individuals to more stringently monitor their BGL using CGM or more stringently manage their condition with lifestyle interventions.

4.3.4 Strengths and Limitations

The potential to non-invasively predict glycaemic events represents a significant landmark in improving the quality of life and health outcomes for people with diabetes. A major strength of the RNSH T1D Study is that it provided further insight into a novel area of research. In terms of study design, the 24-hour monitoring period was an effective means of capturing multiple glycaemic events for each participant. Shorter monitoring periods would have reduced the

amount of data points captured and reduced the statistical power of the analysis. There were two key elements of the methodology which were important in strengthening the scope of this research. During the monitoring period, participants continued their routine work and life schedules, including medications and meals, with as little interruption as possible. Additionally, the criteria used to define glycaemic events were based on values that were clinically relevant. Due to the two key design elements, the findings presented in this research are applicable to real-world clinical settings. Based on these results, it can be concluded that HRV measures do not change significantly immediately before, during, or after the onset of glycaemic events in people with T1D during their regular work and life routines. Though clinical studies have observed significant differences in hypoglycaemia induced by clamp techniques, the RNSH T1D Study may be the first study to demonstrate no statistical significance in a real-world setting.

Some limitations of the RNSH T1D Study have been provided already and will be consolidated in this section. In this study, participants were recruited based on their likelihood of experiencing a glycaemic event within 24-hours of attending the clinic at RNSH. This was to increase the number of glycaemic events captured during the monitoring period which would improve statistical power, as the average person with T1D does not experience many glycaemic events in a 24-hour period. This in turn likely led to the recruitment of more subjects with impaired awareness of hypoglycaemia, as this condition is more prevalent in people with T1D who experience recurring episodes of hypoglycaemia (see Section 1.5.1.3). Due to the pathophysiology of impaired awareness of hypoglycaemia, subjects with this condition were likely to have a reduced autonomic response to hypoglycaemia, which could explain why mean HRV measures did not change significantly in this cohort during hypoglycaemic events. As subjects were not screened for impaired awareness of hypoglycaemia, it is unknown how many in this study had reduced autonomic response during hypoglycaemia, and this limits the conclusions which can be made here. Future studies should consider screening for this condition, as well as restrict the proportion of recruited subjects with this condition to a level reflective of the general T1D population, roughly 30%. In people with T2D who depend on insulin, this proportion is roughly 10%. Future studies which attempt to outright exclude participants based on the presence of impaired awareness of hypoglycaemia may not be able to apply their findings to the broader population of people with diabetes. Duration of diabetes for each subject was not recorded by the researchers in error. This would have been useful since a longer mean duration of diabetes in subjects may have indicated an increased risk or presence of autonomic neuropathy.

Though there have been significant improvements with recent models, there are still known lag-times in the detection of glycaemic excursions by CGM systems. Participants in this study

utilised several different CGM system models to record their glucose levels continuously. The difference between when participants were undergoing a glycaemic excursion compared to when the excursion was detected by the glucose monitor may have been as much as seven minutes on average, though based on current data this varies between CGM system models. For a rough example, a participants BGL may have decreased to 2.9 mmol/L at 10:00am during the study. As CGM systems estimate BGL based on glucose levels in the interstitial fluid, instead of directly measuring glucose levels in the blood, it is unlikely the CGM system would record this value of 2.9 mmol/L until approximately 10:07am (see Section 1.7.2). When analysing the data, the hypoglycaemic event would be registered as 10:07am, and changes in autonomic activity would be calculated from heart rate epochs 10-minutes before, during, and after this exact time. However, there was no corresponding lag-time with the heart rate monitors when recording autonomic activity. As such, there was likely a mismatch between the time when the glycaemic events were recorded compared to when they actually occurred in participants. This is a difficult problem to address, as a predictive model using HRV measures to predict glycaemic events will require large amounts of data, which currently can only be provided by a CGM system. This may change with technological advances, however.

Improvements should be made on this study design in future studies. Using a cut-off for hyperglycaemia as BGL ≥ 10.0 mmol/L may provide more data points to analyse, though clinical relevance of these events should be considered. A participant questionnaire could be provided to collect subjective experiences of glycaemic events, indicating clinical relevance if there are signs or symptoms concurrent with each event. Hypoglycaemic events should be validated by a finger stick confirmation assessment. Improvements could have been made to the autonomic monitoring aspects of this study. Firstly, future research may calculate HRV measures from the full 24 hours of monitoring and compare against other groups. Secondly, the FirstBeat heart rate monitor may have lacked sensitivity required to detect changes in autonomic activity during glycaemic events, so a Holter monitor may be considered as an alternative as it is the hold standard for HRV measurement. Overall, this study did not provide evidence for a change in HRV measures during glycaemic events and did not indicate that HRV measures may act as an 'early warning' system for hypoglycaemic or hyperglycaemia in T1D. The evidence does not support the hypothesis stated in Chapter 1. CGM remains the gold standard for identifying these events in the absence of symptoms.

Chapter 5. Conclusion

The scope of diabetes is significant. The cost to individuals and to global healthcare systems is substantial, and due to its nature as a 'Disease of Civilization', the threat of T2D, the main type of diabetes, is not likely to ease without meaningful social and cultural changes. The initial chapter of this thesis introduced numerous concerns about the current standards in treating and managing diabetes from the perspective of the medical science community. According to scientific consensus, currently available glucose monitoring tools are inadequate given the scope of diabetes. The improvement of these tools, or replacement with superior ones, has been a major priority for decades, as has improvement of pharmacological treatments. Currently available tools have improved markedly in recent years, but core problems, such as their invasive nature, have yet to be addressed. There is growing interest in non-invasive approaches to glucose monitoring, such as tools that estimate BGL based on saliva or sweat samples, as there is evidence that non-invasive alternatives to current invasive measures are more efficient. To summarize, better efficiency is associated with higher quality glucose monitoring and management of diabetes, and this in turn is associated with better quality of life for the many people living with diabetes. However, to date there is no non-invasive measure of blood glucose that is commercially successful. All clinically relevant glucose monitoring tools are invasive, and this has been identified as a core problem in the scientific literature.

HRV is an emerging technology in this area with novel applications. There is strong evidence that HRV measures can predict diabetes complications, and the literature advocates for the use of routine HRV assessments in clinical settings for monitoring of complications such as autonomic neuropathy. HRV is also an excellent marker of autonomic control of the heart. The autonomic nervous system is responsible for regulating various functions throughout the body, including the regulation of blood glucose. It achieves this through innervation of various organs, such as the liver and pancreas, to release specific hormones in response to fluctuating glucose levels. Though the action of the autonomic nervous system on these endocrine organs cannot be measured easily, its action on the heart can be, and it can be measured non-invasively. The level of autonomic innervation that an individual exerts on their heart is inversely related to their glycaemia. This is due to various reasons, such as the fact that total autonomic activity is lower in people with poor physical fitness, and these people tend to have high levels of blood glucose. However, it is also related to the acute response of the autonomic nervous system to food intake. The autonomic nervous system prepares the body for food intake through innervation of various organs and nerve pathways. Though the mechanism is not well understood, changes in autonomic modulation of the heart are associated with changes in blood glucose. It was this physiological principle which first led to the development of the novel hypothesis that

autonomic innervation of the heart, as measured by HRV, may estimate glycaemia. Therefore, HRV measures may be a non-invasive alternative to current glucose monitoring.

This novel hypothesis was tested by Rothberg and colleagues (2016) in people with and people without diabetes. The authors set out to determine whether HRV measures were related to BGL in a way that could lead to the development of them as non-invasive markers of BGL. Their work was continued by Jarman and colleagues (2021) in subjects without chronic illness, and in the UTS Pilot Study over a shorter time period. These studies aimed to accumulate sufficient clinical data to justify further exploration of HRV measures as non-invasive markers of BGL. The UTS T2D Study was the main study conducted as part of this PhD candidature and aimed to consolidate years of exploratory research into a study on people without diabetes and people with T2D, the most common type of diabetes. While several HRV measures accounted for the variability in BGL, no one measure was significant in a multivariate model, and therefore HRV is limited in its capacity to diagnose diabetes or to predict BGL from HRV measures alone.

The ability to predict glycaemia using HRV measures represents a landmark in diabetes management, as it would improve the quality of life and health outcomes for those living with diabetes and reduce the overall burden of the condition on individuals and society. The ability to predict overall glycaemia is also important in the screening and diagnosis of diabetes, and undiagnosed diabetes is cause for significant disability and morbidity worldwide. Early detection of diabetes would be facilitated with the introduction of a convenient and reliable non-invasive measure of blood glucose, and early detection and intervention is associated with reduced risk of diabetes complications and increased quality of life. HRV may not just be relevant in the prediction of fasting and postprandial glycaemia. Another study conducted as part of this PhD candidature, the RNSH T1D Study, aimed to justify the use of HRV measures in the prediction of glycaemic events, which are prevalent in T1D. Though T1D affects only a minor percentage of people with diabetes compared to T2D, people living with T1D are dependent on insulin to survive, and this reliance on exogenous insulin predisposes individuals to hypoglycaemia. Due to the limitations of CGM systems, the ability to reliably predict glycaemic events using HRV measures would significantly improve glucose monitoring for those living with T1D, and this would in turn improve their quality of life as well as potentially reduce the costs associated with managing their condition. The results of the RNSH T1D Study are relevant in a real-world clinical setting, however they do not indicate that HRV measures can predict glycaemic events, such as hypoglycaemia. The effect of impaired awareness of hypoglycaemia on recruited subjects may cause problems for researchers attempting to develop a real-world application of this technology. Nevertheless, some future directions for this novel area of research have been provided in this thesis. The next section will address some of the broader

future directions for researchers in this area, as well as discuss the research which has been growing in recognition in recent years.

5.1 Future Directions

Limitations in the design of these studies conducted as part of this PhD candidature may have contributed to the lack of significant findings and the contradictions compared with current literature. If repeated, several improvements should be made to these studies. For the UTS T2D Study which investigated correlations between HRV measures and BGL in people with T2D and people without, subjects should be seated during the full assessment period and meal size should be standardized. Participants with T2D should be excluded if they have HbA_{1c} above 7.5% or autonomic neuropathy, and this should be assessed during the screening process and prior to study participation. Alcohol consumption should also be limited in the exclusion criteria. For the RNSH T1D Study, hypoglycaemia should be defined in accordance with the standardized reporting guidelines, which means a minimum of 15 minutes in duration and validated by a finger stick BGL. Subjects should be carefully screened or assessed for autonomic neuropathy, and a questionnaire should be provided to subjects to collect information on clinical significance, or the presence of symptoms, during glycaemic events. Using a lower threshold for hypglycaemic cut-off, such as 10.0 mmol/L, may help to capture more events, and calculating HRV measures from the full 24-hours of recording may provide additional insights. Studies should be designed around these improvements.

This thesis investigated the potential of HRV as a non-invasive alternative to invasive glucose monitoring, and thus priority was given to lightweight, portable devices when selecting a device capable of determining HRV. There are several heart rate monitors available in the form of wearable smartwatches. Whilst these are convenient and lightweight to use, concerns have been raised regarding their ability to accurately estimate HRV. Smartwatches do not directly measure the electrical activity of the heart, but rather detect an individual's radial pulse under the skin, usually using a technique known as photoplethysmography. Regardless of the method used to detect a person's pulse, the use of pulse rate variability as an estimate of HRV remains a contentious topic. Pulse rate variability is only sufficiently accurate as an estimate of HRV in participants that are young, at rest, and without chronic illness.[520] Physical or mental activity have been shown to exaggerate differences in pulse rate variability and HRV, and short-term variability is somewhat overestimated in pulse rate variability. A promising exception to these is the Polar S810, which has demonstrated excellent agreement with an ECG in providing time-domain HRV parameters for three-minute[521] and five-minute recordings.[522] Given that there is

no evidence to support the viability of a smartwatch to estimate frequency-domain HRV parameters and for long-term recordings, smartwatches were not considered for use within this study. In future, however, the accuracy of these wearable devices may be improved to a point where they can provide reliable estimates of glycaemia. This would be a significant improvement to current management of BGL and diabetes. Modern smartwatches are already capable of tracking stress, sleep hygiene, hear rate, and more, and one day may also be capable of tracking blood glucose.

There is growing interest in the applications of machine learning in predicting glycaemia. In their 2018 paper, Zhu and colleagues presented the results of their neural network which had been trained on 115,200 glucose data points from CGM systems attached to n=6 subjects across 40 days.[523] Their model outperformed many existing algorithms, despite the fact machine learning models usually require training data with a much larger size to be effective. However, they noted that glucose levels predicted by their model lagged behind the true glucose levels observed in their subjects , and the error in prediction was high when subjects consumed a meal or took their insulin medication. A neural network developed by Zecchin and colleagues (2011) provided competitive predictive value for its time by learning carbohydrate (meal) intake alongside learning glucose information.[483] An algorithm developed by Li (2019) achieved minimal time lag in a real subject dataset by also training their model using carbohydrate data, suggesting that it is an industry standard to manage or account for the effect of meals on glycaemia.[524] Meals are an inevitable source of glucose spikes, and thus glycaemic variability, in people living with diabetes. Goldner and colleagues (2018), who trained their machine learning model on nearly two million BGL measurements from 14,706 people with T2D, have indicated their model could be improved by incorporating meal behaviours into their training set, such as carbohydrate amount.[525] Pustozerov and colleagues (2020) advocate the importance of predicting postprandial BGL in diabetes, as machine learning algorithms may predict the magnitude of a glucose spike after consuming a meal.[526]

In terms of predicting low BGL, Wang and colleagues (2020) created a machine learning model by Bayesian inference capable of predicting hypoglycaemia in T1D with 96% accuracy and 92% precision, which has potential clinical utility.[527] As mentioned, there are few studies on the applications of machine learning in SMBG. However, a probabilistic model developed by Sudharsan, Peeples, and Shomali (2014) had a sensitivity of 92% and a specificity of 70% in predicting a hypoglycaemic event within the next 24 hours.[486] They achieved this using only 10 SMBG values per week, which is a reasonable number of blood glucose assessments for someone living with diabetes, based on current standards. A novel algorithm presented by Cichosz and colleagues has shown promising results by combining information from a Holter

monitor with concurrent values from a continuous glucose monitoring system. The algorithm detected 16/16 hypoglycaemic events in subjects with T1D with a sensitivity of 79% and a specificity of 99%.[452] This study represents an appealing line of research, as it aims to overcome some of the current problems people with diabetes face. Current SMBG is costly,[305] though a portable ECG, such as a Holter monitor, may present a reduced financial burden and, at the very least, would provide a non-invasive option. The R-waves from an ECG, used in the determination of HRV parameters, have distinct profiles that make them suitable for detection by computer algorithms.[520] A real-world application of this technology would incorporate the predictive model within the monitoring device, allowing for real-time analysis of autonomic activity. Consequently, an inexpensive portable heart rate monitor capable of estimating blood glucose may be commercialised.

It is clear from emerging research that a non-invasive, accurate measure of BGL will almost certainly involve advanced machine learning.[528] The findings of the multiple studies conducted as part of this thesis add to the growing body of evidence that HRV measures may be an ideal marker of glycaemia. However, further research is needed. Continuous glucose monitoring (CGM) remains the gold-standard for measurement of BGL in diabetes, however HRV represents a promising area which can add to detection of diabetes and glycaemic events in tandem with CGM. Though the scope of this PhD limits the applications of these findings, it can be concluded that the future of HRV is promising in this area of research. The UTS Pilot Study provided a framework and preliminary data which was used to design a larger study. Following this, the UTS T2D Study demonstrated that HRV measures obtained whilst individuals are eating may be used to predict their postprandial BGL, a novel finding. The RNSH T1D Study investigated changes in HRV measures during glycaemic events, and though no statistically significant findings were observed, HRV measures may still be relevant in the management of T1D. There are certain confounders which future researchers should consider the impact of when investigating the clinical applications of HRV measures in predicting glycaemic events.

Overall, these findings presented in this thesis indicate that HRV possesses clinical relevance and real-world utility in the management of diabetes, and further investigation is warranted. Future studies should focus on gathering data in real-world situations, such as allowing subjects to continue their regular routines, including medications and meals. Studies may also improve sample power, which is a common limitation in this area of study, by pooling subjects from their individual groups and assessing correlations between HRV measures and BGL across a wider, unified sample. A useful follow-up study to this PhD research might investigate correlations between the change in HRV and change in BGL in postprandial states. Further, large amounts of data points may be used to train more sophisticated machine learning tools, such as deep

learning artificial intelligence, and clinically relevant thresholds of glycaemic events should be considered when developing therapeutic technologies. It is important that researchers in the scientific community empathise with those living with the condition they are attempting to improve, and to consider their specific needs when developing potential solutions. This has been a core focus of this thesis, as empathy will invariably facilitate the real-world applications of clinical research.

Chapter 6. References

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