The effect of patterns and distributions of physical activity on blood glucose control in individuals with Type 2 Diabetes Mellitus: an exploratory study

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Abstract

Physical activity (PA) is known to be beneficial for blood glucose control in individuals with Type 2 Diabetes Mellitus (T2DM). The American Diabetes Association (ADA) recommends 150 minutes or more of moderate to vigorous physical activity (MVPA) per week for individuals with T2DM, which may be perceived as an intimidating target. Recent evidence suggests that firstly, PA of all intensities, including lighter intensity activity, may be beneficial for blood glucose control and that secondly, the pattern in which PA is accumulated may be important, however this is poorly understood. The purpose of this thesis was to provide a detailed understanding of how the patterns (extent to which bouts of activity durations and intensities are accumulated within and between days) and distributions of all habitual PA (not just moderate and vigorous) influence daily glucose fluctations in individuals with T2DM. Free living PA was measured using an ActivPal accelerometer worn on the thigh and 24 hour glucose was measured using an iPro continuous glucose monitor in 33 participants (age, 72 ± 11 years). Stepping at a light-intensity and overall stepping time were associated with increased glucose time in target glucose range (TIR) and total daily area under the curve (AUC). Stepping at or above moderate intensity was associated with lower mean amplitude of glucose excursions (MAGE) (95% CI -0.016(-0.032, -0.001), p = 0.04). Individuals with high variation and high volumes of stepping time at or above moderate intensity and total daily steps were found to have significantly greater glucose TIR when compared to individuals with low variation and low volumes of activity. These findings suggest that daily light intensity activity is beneficial for daily glucose, and investigating activity on a daily basis rather than averaged over a week is crucial for improving the understanding of associations between glucose and activity in free-living.

Table of Contents

List of tables 5

List of figures 6

Symbols and abbreviations 8

1.0 General Introduction 9

1.1 Diabetes. Disease burden and pathology 9

1.2. The importance of physical activity for glycaemic control in T2DM 14

1.3. Mechanisms of glycaemic control 16

1.4. Physical activity. Recommendations and engagement 17

1.5. Current research, advances in research and opportunities for improving PA guidance for diabetes engagement 18

2.0 Literature review 20

2.1 Physical Activity in the management of T2DM 20

2.2 Acute effect of Physical Activity on glucose levels 20

2.3 Chronic effect of physical activity on glucose control 22

2.4 The importance of physical activity intensity on glycaemic control 25

2.5 Patterns of Physical Activity and glucose control 28

2.6 Methods of measuring glycaemic control 29

2.7 Methods of measuring Physical Activity 34

2.9 Summary 39

2.9.1 Aims and objectives 40

3.0 Methods 41

3.1 Overview 41

3.2 Participants 41

3.3 Study design and protocol 43

3.4 Continuous Blood Glucose and Accelerometer data 45

3.5 Data processing 47

3.6 Statistical analysis 48

3.6.1 Associations between PA and daily glucose **48**

3.6.2 Inter-daily variations in PA and daily glucose **49**

4.0 Results 51

4.1 Device compliance and acceptability of measurement procedure 53

4.2 Daily physical activity 56

4.3 Associations between glycaemic control and activity variables 56

4.4 Intradaily variation in activity. Activity around meal times and postprandial glucose 60

4.5 Intradaily variation in activity. Morning and afternoon activity 63

4.6 Interdaily variations in total daily activity 66

5.0 Discussion 70

5.1 Daily PA accumulation and daily glucose 70
5.2 Inter- and Intra-daily variability 77
5.3 Implications of findings 79
5.3.1 Implication for Researchers **79**
5.3.2 Implications for Clinicians **80**
5.3.3 Implications for Policy makers **82**
5.4 Possible application of these findings 82
5.4.1 Developing a points based PA monitoring system **82**
5.5 Strengths and limitations of this study 83
5.6 Future research 86
5.7 Conclusion 87
6.0 Appendix 88
6.1 Participant information sheet 88
6.2 Measurement Questionnaire 92
6.3 Study Diary 96
6.4 Calculation of sample size based on Kingsnorth et al (2018) 108
References 110

List of tables

Table 1. Participant characteristics

Table 2. Acceptability questionnaire responses for activPAL and iPro2 Continuous glucose monitor.

Table 3. Associations between physical activity variables and glycaemic control (time in target glucose range, 24h average glucose, mean amplitude of glucose excursions and area under the curve) in all participants, and in non-insulin dependent participants.

Table 4. Results from General Estimating Equation models for the effect of pre- and post-meal steps on average 2 hour Post prandial glucose and percentage time in target glucose range in all participants and non-insulin dependent participants.

Table 5. Generalised Estimating Equations on the effect of morning and afternoon activity on night and day average glucose (mmol.L).

Table 6. Number of participants and average of glucose variables for each group of inter-daily variability in physical activity in Total Daily Steps, Step Time (min) and moderate to vigorous intensity physical activity min).

Table 7. Regression analysis results for difference in glucose variables (average glucose, time in target glucose range mean amplitude of glucose excursions and area under the curve across the four groups in step time, total daily steps and moderate to vigorous intensity physical activity.

List of figures

Figure 1. Diagram to represent insulin mediated GLUT4 translocation and glucose uptake from plasma into peripheral tissue cells.

Figure 2. Schematic of the study protocol detailing events throughout Visits 1 and 2, and the 5 days free-living measurement period.

Figure 3. iPro continuous glucose monitor worn on the lower abdomen and ActivPAL activity monitor work on the mid-thigh.

Figure 4. Example of 5 days movement and glucose trace from a male participant estimated to undertake the recommended 150 minutes MVPA per week. Minutes of stepping at a cadence of >100 steps per minute (moderate intensity), <100 steps per minute (low intensity) and mean 24h glucose (mmol.L).

Figure 5. Acceptability questionnaire responses for the two devices a) activPAL, b) iPro2 CGM, reported as percentage of responses from 29 participants.

Figure 6. Graphical representation to show the distribution of activity volume and variation in the four groups: Low Variation Low Physical Activity (LVLP), High Variation Low Physical Activity (HVLP), High variation High Physical Activity (HVHP) and Low Variation Low Physical Activity (LVLP). a) Distribution of average step time and the mean SD of step time. b) Distribution of mean

total daily steps and mean SD of total daily steps. c) Distribution of mean MVPA and SD of MVPA time (mins).

Symbols and abbreviations

ADA	American Diabetes Association
AMP	Activated Protein Kinase
AUC	Area under the Curve
BMI	Body Mass Index
CGM	Continuous Glucose Monitor
CVD	Cardiovascular Disease
DPP	Diabetes Prevention Programme
EGO	Endogenous Glucose Output
FBG	Fasting blood glucose
FFA	Free Fatty Acids
HbA1c	Glycated Haemoglobin
iAUC	Incremental area under the curve
IGT	Impaired Glucose Tolerance
MAGE	Mean Amplitude of Glucose Excursions
MET	Metabolic Equivalent Task
MVPA	Moderate-Vigorous Physical Activity
NGT	Normal Glucose Tolerance
NHS	National Health Service
NIDDM	Non-insulin Dependent Diabetes Mellitus
OGTT	Oral glucose tolerance test

PA	Physical activity
PPG	Post Prandial Glucose
RCT	Randomised control trial
RR	Relative Risk
SD	Standard Deviation
T2DM	Type II Diabetes Mellitus
TIR	Time in target glucose range
WHO	World Health Organization

1.0 General Introduction

1.1 Diabetes. Disease burden and pathology

Diabetes is a chronic disease in which blood glucose (sugar) levels become elevated due to either pancreatic β-cells being unable to produce insulin (Type I Diabetes Mellitus), and/or reduced insulin sensitivity at target organs and tissues (Type II Diabetes Mellitus; T2DM) (WHO, 2018). Insulin's

function is to facilitate glucose uptake to muscles, adipose tissue and liver; a lack of insulin, or low insulin sensitivity can result in prolonged periods of elevated blood glucose, or hyperglycaemia (Saltiel and Kahn, 2001). In turn this can increase the risk of further health consequences (Karin and Tabas, 2011; Stratton et al, 2000; Sarwar et al, 2010).

Diabetes is the 8th leading cause of mortality worldwide (Tao, Shi and Zhao, 2015). In 2016 it was estimated that all diabetes directly resulted in 1.6 million deaths (WHO, 2018). Further health consequences are common in individuals with diabetes due to the impact of oscillating blood glucose levels causing stress on the body's systems. This stress increases the risk of vascular diseases, such as coronary heart disease, by two-to-three times (Sarwar et al, 2010). Large and persistent blood glucose oscillations can result in Diabetic retinopathy (vision impairment) as retinal blood vessels swell and leak (Fong et al, 2004), chronic kidney disease (difficulties in the filtration of blood in the kidneys) (Jha and Wang, 201)2, and diabetic neuropathy, (Juster-Switlyk and Smith, 2016) which describes peripheral nerve damage resulting from sustained periods of hyperglycaemia, and can result in pain and numbness in feet or legs, digestive system problems, bladder dysfunction and more (Tesfaye et al, 2010). These diabetic complications are associated with a poorer quality of life including lower physical and social functioning (Lloyd, Sawyer and Hopkinson, 2001).

The most common form of diabetes is T2DM, accounting for ~90% of all cases globally out of 382 million people in 2013 (Tao, Shi and Zhao, 2015). Furthermore, the number of individuals with T2DM is increasing. It is currently estimated that 4.7 million people in the United Kingdom have diabetes, of which 90% have T2DMwhich presents a significant challenge to the NHS (National Health Service; NHS Digital. National Diabetes Audit Report 1 Care Processes and Treatment targets 2017-2018). In 2018, the cost of blood glucose lowering drugs alone surpassed £1 billion (Stedman et al, 2019), and the treatment of diabetes and its complications is estimated to cost over £6 billion every year in the UK (National Health Service; NHS, Type 2 Diabetes and the importance of prevention, 2018).

It is understood that the underlying causes of T2DM involve a complex interaction of genetic and environmental factors (Lebovitz, 1999). The condition is defined by a deficiency in insulin release

from the pancreas and/or insulin resistance in target organs, the liver, and importantly tissues, such as muscle tissues (Chatterjee, 2017). Normal glucose homeostasis (a concentration in circulation of 4-7mmol.l^{-1}) is maintained by the balance of intestinal glucose absorption, liver glucose production and the uptake and subsequent metabolism of glucose by tissues such as muscles (Saltiel and Kahn, 2001). Insulin, a hormone produced and released by pancreatic β-cells, is involved in the maintenance of glucose homeostasis (Matthews et al, 1985). When glucose is ingested plasma glucose concentrations to rise, triggering the release of insulin which increases hepatic (liver) and peripheral (muscle) glucose uptake and decreases glucose production by the liver (Defronzo, 2004). Insulin acts on cells of peripheral tissue and stimulates the translocation of the glucose transporter protein type 4, or GLUT4, to cell plasma membranes (Saltiel and Kahn, 2001). GLUT4 allows glucose transport from plasma into peripheral cells to be used for metabolism; lowering plasma glucose concentration.

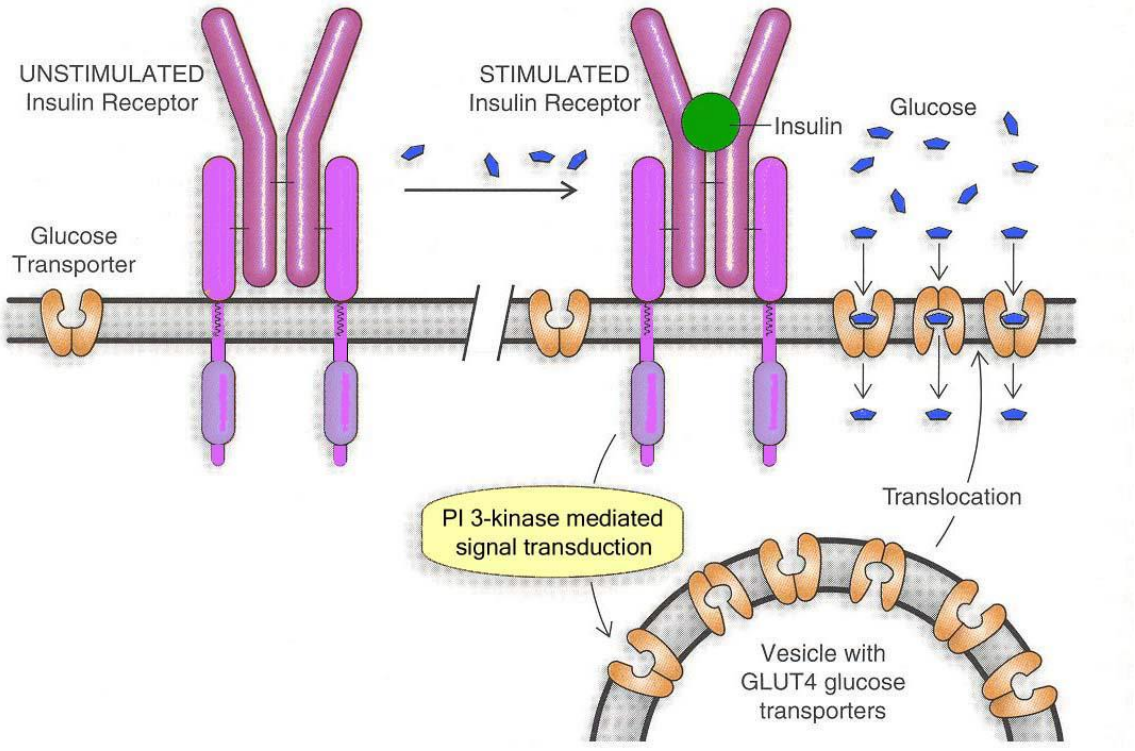


Figure 1. Diagram to represent insulin mediated GLUT4 translocation and glucose uptake from plasma into peripheral tissue cells.

The process of glucose uptake and translocation is impaired in individuals with T2DM; insulin release from the pancreas is impaired and/or insulin resistance is present at target tissues (Chatterjee, 2017). The consequence of this is hyperglycaemia as glucose is not removed from the blood stream sufficiently. Over time the process of Normal Glucose Tolerance (NGT) can deteriorate leading to a state of Impaired Glucose Tolerance (IGT), and without intervention this can develop into T2DM (Weyer et al, 2001). This can happen for a number of reasons which are described below.

Obesity is common in individuals with T2DM and is related to the development of insulin resistance, impaired insulin secretion and increased endogenous glucose output (EGO) (Weyer et al., 2001). Obesity is associated with an increase in plasma concentrations of free fatty acids (FFA) which can result in reduced insulin sensitivity and thus decreased glucose uptake (Scheen, 2000). Insulin secretion deficit can become present in obese individuals due to an inability of β-cells to sustain high volumes of insulin release to compensate for decreased insulin sensitivity, leading to the development of T2DM (Scheen, 2000; Robertson et al, 2004). The primary defect in reduced insulin sensitivity and later an inability of β-cells to produce sufficient levels of insulin results in T2DM. The progression from impaired glucose tolerance (pre-diabetes) to T2DM is determined by diagnostic criteria for circulating glycated haemoglobin (HbA1c) (ADA, 2019). Prediabetes is categorised as HbA1c of 42-47mmol·L^{-1} (6.0-6.4%) (Diabetes UK, 2019), and T2DM is diagnosed when HbA1c is ≥48 mmol·L^{-1} (≥6.5%) (Kilpatrick and Atkin, 2014).

Susceptibility to the development of IGT and subsequently T2DM may in part be attributable to predisposed genetic factors. The Framingham Offspring study estimated that the risk of developing T2DM is 3.5-fold higher when one parent has T2DM (compared to neither parents having T2DM), and is 6-fold higher when both parents have T2DM (Meigs, Cupples and Wilson, 2000). Although the authors agree that these differences are due to genetic factors, it is also possible that the dietary, PA, sleeping habits and norms within families where one or more parents has T2DM differ from families where neither parents do. Such environmental factors are strongly linked to the development of T2DM; a lifestyle including physical inactivity, excessive calorie consumption and

subsequent obesity can increase the risk of T2DM (Temelkavo-Kurktschiev and Stefanov, 2012), particularly in those with greater genetic susceptibility which interacts with environmental factors (Nolan, Damm and Prentki, 2011).

As well as playing a key role in minimising risk of T2DM, effective regulation of blood glucose levels in individuals living with T2DM is important, as prolonged hyperglycaemia resulting from poor blood glucose control, can cause neurological and/or vascular complications. Maintaining blood glucose within recommended values, 3.9-10mmol/L across the day including fed and fasted state (Battelino et al., 2019) reduces risk of potentially severe complications related to poorly controlled diabetes including atherosclerosis and cardiovascular disease, vision loss, diabetic neuropathy which can lead to amputation and disability (Schlienger.,1983).

Effective glucose management can be achieved through different approaches including pharmaceutical intervention, and lifestyle adaptations including increased Physical Activity (PA) and improved diet. Pharmaceutical interventions include the use of Metformin, injecting insulin to augment endogenous insulin action. Metformin is widely used as first line therapy for T2DM and acts by reducing glucose production through a series of complex events (Rena, Pearson and Sakamoto, 2013). Ingested metformin is transported into hepatocytes resulting in an inhibition of the mitochondrial respiratory chain (responsible for generating energy). This energy production deficit is balanced by reducing the energy consumption of cells, such as gluconeogenesis (making of glucose), in the liver (Rena, Pearson and Sakamoto, 2013). In doing so Metformin reduces circulating glucose by decreasing hepatic glucose production. Metformin is used extensively, primarily because of its ability to lower glucose levels with little/no impact on body weight, and also being low cost compared to other forms of medication. Nevertheless, medications such as metformin are not without their side effects; the most common of these being gastrointestinal issues such as diarrhoea, nausea and abdominal discomfort (Sanchez-Rangel and Inzucchi, 2017). Although pharmacological intervention for T2DM is common, it is not without its limitations, including variability in compliance to drug treatment regimes (WHO; Cramer et al, 2003), impact on lifestyle and cost for healthcare

providers. Lifestyle interventions including changes to diet and movement behaviours offer a cost effective alternative or complement to pharmacological interventions (Wing et al, 2010).

Dietary improvements can reduce body weight, which is associated with improved glucose control (Vitolins et al, 2017) and also directly impact carbohydrate, which may reduce circulating glucose levels. For improved glucose control and vascular health it is often recommended to consume mainly plant-based foods with high polyunsaturated and saturated fatty acid content, and limit intake of salt, trans-fats, high glycaemic index foods and those high in fructose and sucrose (Garber et al, 2018). These lifestyle interventions do not have side effects, unlike medications, however can be difficult to implement over long periods due to barriers such as participants negative perception of required changes, difficulty changing longstanding dietary habits (Booth et al, 2013). Interventions that target movement behaviours such as PA to improve glucose levels are also common.

1.2. The importance of physical activity for glycaemic control in T2DM

PA is defined as 'any bodily movement produced by skeletal muscles that results in energy expenditure' (Caspersen et al, 1985). PA includes exercise, a subset of PA which includes 'planned, structured and repetitive bouts of movement performed to improve or maintain one or more components of physical fitness' (Caspersen et al, 1985) and all other movement behaviours which occur during occupational and leisure time. PA is a key modifiable lifestyle factor which is known to improve blood glucose regulation, and can therefore play an important role in the management of T2DM (Wilmot et al., 2012; Bassuk, Shari and Manson, 2005). In addition, PA provides cardio-metabolic benefits which reduce the risk of cardiovascular and overall mortality (Wing et al, 2010).

Several studies demonstrate that achieving insufficient PA increases the relative risk (RR) of developing T2DM (Aune et al, 2015). A meta-analysis revealed that being sedentary (sitting or reclining activities with a low energy expenditure which displace PA) specifically for >14h per day, is associated with a 112% increased RR of developing T2DM (Wilmot et al, 2012). Furthermore, there is evidence from long term intervention studies to demonstrate PA aids the prevention of T2DM. The

Diabetes Prevention Program (DPP) Research Group, USA, investigated a lifestyle change programme compared to metformin or a placebo pill on T2DM development in individuals with pre-diabetes (DPP research group, 2009). The lifestyle change programme involved lowering calorie intake and increasing PA to ≥ 150 min per week, aiming to lose 7 percent body weight. In the 34 month follow up, diabetes incidence was reduced by 58% in the lifestyle intervention group compared to the placebo and 31% in the group taking metformin compared to the placebo. After a 10 year follow up, the lifestyle change programme delayed T2DM development by 34% compared to the placebo, on the other hand metformin delayed the development of diabetes by 18%. Although the DPP study focused on development of T2DM, there is evidence that in individuals with T2DM, increases in PA have demonstrated significant improvements in HbA1c (glycosylated haemoglobin; an indicator of glucose control over a period of approximately 3 months) and Body Mass Index (BMI; height to weight ratio) (Avery et al, 2012).

In addition to reducing diabetes risk, it is well established that PA can play an important role in improving short and long term blood glucose regulation both in individuals with diabetes and those without. Those who are regularly active (≥ 30 minutes per week) are less likely to have abnormal blood glucose control than those who take part in <30 minutes activity per week (Mainous 2017), and increased PA through behaviours such as walking can improve glucose profile in individuals at high risk of developing T2DM (Yate et al, 2011). In individuals already diagnosed with T2DM, increased sedentary time (low PA time) is associated with higher blood glucose (Paing et al, 2019 and PA has also been observed to benefit both acute (Metcalf 2018; Dempsey, 2016; Van Dijk et al, 2013) and longer term improvements in daily glucose (Boule et al, 2003; Wing et al, 2010), and also reduces the risk of further health complications (Tanasescu et al., 2003; Batty et al., 2002) and mortality (Batty et al., 2002; Tanasescu et al., 2003; Sadarangani et al., 2014; Loprinzi et al., 2015). A meta-analysis of exercise intervention studies (12 aerobic training studies and 2 resistance training) showed that regular PA was associated with reductions in HbA1c sufficient to reduce risk of diabetic complications (Boulé et al., 2003). A comprehensive discussion of evidence linking PA with blood

glucose, diabetes risk and management is provided in subsequent chapters (See chapter 2 Literature Review). The links between PA and exercise with induced improvements in daily glucose, HbA1c, and risk of diabetes and its complications can be explained by mechanisms by which muscular contraction which causes bodily movement impact glucose transport at a cellular level. These mechanisms are described briefly below, and in greater detail in Chapter 2.

1.3. Mechanisms of glycaemic control

At rest the main fuel source for the body is free fatty acids (FFA), any PA above rest changes the fuel source to a contribution of fat, glucose (from circulation and from endogenous stores) glycogen for energy. At an even higher intensity of PA (above 70% VO_{2max}) carbohydrate from muscle glycogen is the main source of energy (Jensen et al., 2011). During PA, blood glucose uptake into muscle is increased thus lowering blood glucose levels. This increased glucose uptake happens via the insulin dependent and insulin-independent pathways (Goodyear, Kahn 1998), each activate GLUT4 through different signals.

In insulin dependent pathway, GLUT4 is activated by insulin through a series of complex signals; first insulin binds to the insulin receptor on the target cell initiating a signalling cascade through the insulin signalling pathway. GLUT4-containing vesicles in the intracellular membrane translocate to the cell membrane. Insulin-dependent GLUT4 activation occurs when insulin is present, stimulating a cascade of complex signals (Zierth et al, 2000). Insulin-independent pathways such as muscle contractions active GLUT4 through the activation of a protein called 5'AMP (activated protein kinase) (Musi et al, 2001). Although insulin-mediated uptake is impaired in individuals with T2DM (Goodyear and Kahn, 1998), contraction-stimulated GLUT4 translocation is normal (Musi et al, 2001). Whereas the enzyme glycogen synthase involved in converting glucose to its stored form (glycogen) is impaired in individuals with T2DM (Christ-Roberts et al, 2004). With PA resulting from muscle contraction, improvements in glucose control have been reported to last for 24-72 hours after activity (Oberlin et al., 2014), implying that PA is beneficial for glucose control.

The chronic effect of PA is improved insulin sensitivity and glucose tolerance due to an increase in GLUT4 production and enzymes such as glycogen synthase, responsible for glucose phosphorylation, storage and oxidation (Ivy, 1997; O'Gorman, 2006; Christ Roberts, 2004). Long term engagement in PA, such as exercise training, improves insulin sensitivity as skeletal muscles become more responsive to insulin resulting in improved activation of GLUT4 transporter proteins (Jensen et al, 2011). Despite a clear understanding of the mechanisms underpinning a clear benefit regular PA for glucose control, participation both in the general population and amongst individuals with T2DM is low.

1.4. Physical activity. Recommendations and engagement

In the UK, individuals with T2DM are recommended to participate in ≥150 minutes MVPA per week. The UK Chief Medical Officer's guidelines recommend that adults (aged 16-60 years) should participate in ≥150 minutes moderate intensity PA or 75 minutes vigorous intensity PA, or a mixture of (MVPA), and include 2 days of strengthening activities (UK Chief Medical Officers' Physical Activity Guidelines, 2019). The clinical guidelines for individuals with T2DM set by the American Diabetes Association (ADA) are slightly more detailed including a recommendation not to allow more than two days separating PA sessions in order to enhance insulin action (Colberg, 2016).

Moderate intensity PA includes activities equivalent to 3.0-6.0 Metabolic Equivalent Task (MET) (Ainsworth et al, 2011). Ainsworth et al., (2011) describe one MET as the equivalent to sitting quietly, typically requiring 3.5ml of oxygen per kilogram body weight; other activities can then be classified using multiples of this resting value. For example an activity equal to 2.0 METs, such as walking (strolling) requires two times more energy than sitting quietly. Vigorous intensity PA is performed at METs greater 6.0, this includes activities such as jogging and cycling. In addition, moderate intensity activity is also described by Tudor Locke (2011) as a walking cadence of >100 step/min, and vigorous intensity as >120 step/min.

Despite the known benefits of PA for blood glucose management and weight loss, public participation in the recommended 150 minutes MVPA is low. PA engagement is particularly low in individuals with a high body mass index (BMI) and T2DM (Steeves et al, 2015; Jakici et al, 2010), which may increase risk of diabetic complications such as cardiovascular disease (Laakso, 2000).

Current public health and clinical guidelines provide little practical guidance on how best to accumulate PA for optimal benefit on blood glucose control. The volume of MVPA (>150 minutes) recommended in the ADA guidelines could be accumulated in myriad different ways over the course of a week, for example, it could be accumulated only on two days of the week with the other days being largely inactive, or being accumulated evenly in much smaller bouts across all 7 days. PA could also be accumulated in one bout per day or in multiple smaller bouts throughout the day. The ADA guidelines provide limited guidance regarding how best to accumulate PA. In addition, the current guidelines are limited to recommending MVPA, which is only undertaken by a minority of individuals with T2DM. Recent research has highlighted opportunities to improve practical guidance for PA to support effective glucose control in T2DM.

1.5. Current research, advances in research and opportunities for improving PA guidance for diabetes engagement

The majority of PA surveillance or interventions studies involving individuals with T2DM focus exclusively on achieving 150 minutes of MVPA. Although the ADA provides a slightly more detail explanation of how to achieve the recommended activity, it still only focuses on MVPA. Recent advances in behavioural measurement allows more precise measurement of PA which has improved our understanding of links between PA and health outcomes. The use of such precise measurements in recent research has highlighted two key opportunities which will help to understand the relationship between PA and glycaemic control in T2DM, and improve clinical guidance for patients to aid T2DM management.

Firstly both observational and experimental studies have demonstrated that PA of all intensities (light – vigorous) can benefit blood glucose regulation (Pulsford et al., 2017; Healy et al., 2007; Peddie et al., 2013; Dunstan et al., 2012). Secondly, the hourly, daily and weekly pattern in which PA is accumulated is critical for blood glucose regulation (Miyashita et al., 2008). The way in which PA is accumulated influences blood glucose regulation, for example long or short duration and in multiple or single bouts. Finally, the proximity of PA to food intake and time of the day (morning, afternoon, evening) influences the impact it has on blood glucose control (Haxhi et al., 2013).

Given the above, it is clear that improving practical guidance on PA for individuals with T2DM to aid blood glucose management is possible. However this requires a detailed understanding of how the patterns and distributions in which PA is accumulated over the course of a week are linked to glucose control, and this is currently lacking. Such understanding of how PA variables link to daily glucose has the potential to inform new methods of PA monitoring and goal setting, and for behavioural surveillance within this population.

This thesis will address this gap within the literature. The following sections of this thesis will describe the literature which provides the context and rationale for this work (Chapter 2.0), the methods used in this study (Chapter 3.0), the study findings (Chapter 4.0), and a discussion of these findings in the context of the wider field and their implications for research policy and healthcare practice (Chapter 5).

2.0 Literature review

2.1 Physical Activity in the management of T2DM

PA can contribute to the effective management of T2DM through the acute and chronic impact on blood glucose uptake and insulin sensitivity (Oberlin et al., 2014; Wing et al., 2010). By improving blood glucose control, PA can prevent or slow the progression of further complications such as cardiovascular disease or neurological complications. The subsequent sections of this chapter will review existing evidence for acute and chronic impact of PA on blood glucose, the influence of PA intensity, and issues surrounding the measurement of both PA and blood glucose which underpin our understanding of this relationship.

2.2 Acute effect of Physical Activity on glucose levels

PA has an effect of lowering blood glucose and improving insulin sensitivity which can be observed immediately during PA and afterwards, for at least 24 hours and up to 72 hours after activity (Oberlin et al., 2014 and Boulé et al., 2005). Repeated bouts or regular PA can therefore result in sustained benefits for day to day glucose control and to manage glucose excursions. As described above (Chapter 1,) the mechanisms behind the acute responses include changes to fuel utilisation and a stimulation of glucose uptake. PA is also effective in enhancing insulin sensitivity in a dose-response manner with a greater volume and intensity of PA leading too greater improvements in insulin sensitivity (Black et al., 2010). There is an increase in energy demands during PA compared to at rest which increases glucose uptake via the insulin dependent and independent pathways leading to lowered blood glucose concentration (Black et al., 2010)

Increased glucose uptake from the blood stream during PA occurs due to the increase in demand for glucose to resynthesise Adenosine Triphosphate (ATP), this happens through insulin dependent and independent pathways. Insulin-dependent glucose uptake is attributed to the increase in muscle insulin sensitivity, signalling GLUT4 translocation predominately in contracting muscles (Richter et

al., 1989). Independent of insulin, there is an increase in GLUT4 translocation to the membranes of skeletal muscles cells during PA due to muscle contraction. This is triggered by an increase in enzyme AMP-Activate Protein Kinase (AMPK), the extent to which AMPK is activated in individuals with T2DM is similar to individuals without T2DM during PA (Musi et al 2001). During PA involving muscle contraction, there are also changes in fuel utilization depending on the duration and intensity.

Fuel demands change from predominantly nonesterified fatty acids (NEFAs) at rest towards a mix of glucose, NEFAs and muscle glycogen during PA (Sigal et al, 2004). NEFAs and glucose are required for ATP resynthesis, the only energy source used by muscles to generate muscle contraction. Glycogen stored within muscles is the main source of energy at the start of PA. As duration increases, glucose within circulation and NEFAs become the main sources of energy as glycogen becomes depleted (Sigal et al, 2004). Fuel utilisation responses to PA are dependent on intensity and duration of activity. Kang et al (1999) compared changes in fuel demands at different PA intensities. Participants with T2DM completed two exercise protocols; 50% maximal oxygen uptake ($\dot{V}O_{2max}$) and 70% $\dot{V}O_{2max}$, duration at each intensity was adjusted so energy expenditure matched. Glucose utilisation rate was greater in the higher intensity condition, however total glucose utilisation was similar in both intensities. These results show PA increases plasma glucose use leading to decreased blood glucose concentrations, irrespective of intensity, in individuals with T2DM.

Historically research regarding the acute effects of PA on circulating glucose levels has largely focused on MVPA and exercise rather than lower intensity activities. For example a systematic review by Asano et al., (2014) concluded that acute physical exercise improves glucose tolerance, insulin sensitivity and reduces glycaemia during a period between 2-72h after exercise bout cessation. However there is now increasing evidence from experimental research pointing to acute benefits of light intensity activity, such as walking in single or intermittent bouts, for glycaemic control (Henson et al, 2016). Henson et al (2016) found that walking (5 min every 30 min over a 7.5 hr period) was significantly associated with improved glycaemic control in women at high risk of T2DM.

In summary, there is now good evidence suggesting that PA acutely effects circulating glucose and that these effects exist in a dose-response manner (Black et al, 2010) and improved insulin sensitivity is evident for 24-72 hours after the last bout of PA (Boulé et al., 2005). As a result it may be possible to obtain sustained benefit by undertaking physical activity regularly, in order that repeated acute single bout effects result in consistently lower glucose. In addition to the acute effect of PA on glucose control, regular physical activity may, over time, illicit more chronic adaptations which benefit glycaemic control. These are described below.

2.3 Chronic effect of physical activity on glucose control

This section will cover the effects of long term engagement in PA on blood glucose control. Benefits from regular or repeated PA participation, such an improved insulin sensitivity, can be see within one week (Winnick et al., 2008). By improving blood glucose control, hyperglycaemic excursions are reduced, in turn reducing vascular strain which is beneficial for preventing further health complications (Wing et al., 2010). Existing evidence points to three key mechanisms by which regular and long term engagement in PA improves glycaemic control; improved insulin sensitivity, increase in availability of GLUT4 in skeletal muscle cells and increased glycogen synthase content within skeletal muscle cells. Systematic reviews, longitudinal studies and intervention studies are discussed here.

Currently there are no systematic reviews into skeletal muscles adaptations to long term PA engagement in individuals with T2DM, however the below summarises the findings of a review of 18 studies by Wang et al, 2009 on skeletal muscle adaptations to exercise training in adults with T2DM or IGT.From the 18 studies reviewed, exercise training consisted of moderate to high intensity aerobic or resistance exercise over 4-52 weeks, with one study involving low intensity aerobic exercise. The main outcomes measured related to glucose control were glycogen synthase, glycogen, GLUT4 availability within the muscle and insulin signalling. Glycogen synthase (an enzyme involved in

converting glucose to glycogen to be stored) activity increased following training in three out of four studies in which it was measured. GLUT4 protein content in skeletal muscle was increased in 5 out of 7 training studies, contributing to overall improved glucose uptake into muscles. Improvements in insulin signalling were consistent, with the study involving a low intensity intervention showing a clinically significant increase in insulin sensitivity (Fritz et al, 2006). These changes from chronic exercise training in individuals with T2DM are related to improvements in overall blood glucose control.

A systematic review, conducted by Umpierre et al (2011), summarised the findings from studies involving structured exercise programmes or PA advice interventions of at least 12 weeks in duration. Overall, structured exercise in 23 studies was associated with a 0.67% decline in Glycated haemoglobin (HbA1c; marker of glycaemic control), PA intervention advice studies was associated with a 0.43% decline in HbA1c. Increased activity levels over a long duration, irrespective of type, are beneficial for improving overall glucose control (Umpierre et al, 2011) However, this review does not provide detail on the specific skeletal muscle adaptations.

In further support of the long term effects a longitudinal study, the Look AHEAD (Action for Health in Diabetes) project (Wing et al, 2010), investigated the impact of a 4 year lifestyle intervention on weight loss, fitness, blood pressure and HbA1c. The intervention included diet modification and increasing PA participation to 175 minutes or more of MVPA per week, compared to a control group receiving diabetes support and education. Over the 4 years, the lifestyle intervention group demonstrated greater weight loss, greater fitness improvement, lower HbA1c and lower blood pressure compared to controls. This demonstrates that improved diet and PA is beneficial for improving HbA1c and other health factors.

Associations between PA and habitual daily PA were investigated in a cross sectional study of healthy individuals (Balkau et al., 2008). PA was recorded using accelerometery over six days and insulin sensitivity was measured using a 2-h hyperinsulinemic-euglycemic clamp following the six days of activity measurement. Total PA was inversely related to insulin sensitivity, as were sedentary

time, light-intensity duration and activity intensity demonstrating the potential benefit of being habitually active, albeit in a health cohort. Houmard et al (2004) investigated a group of individuals who were overweight/obese and therefore at higher risk of T2DM, randomly assigned either low-volume moderate intensity (170 min/week), low-volume high intensity (115 min/week) or high-volume high intensity (170 min/week) training conditions over 6 months. The high-volume high intensity and low-volume moderate intensity groups had greater improvements in insulin sensitivity (~85%) than low volume high intensity group (~40%). These findings are again promising as they show improvements in insulin sensitivity is improved over 6 months of regular activity, but importantly demonstrate the importance of the volume of activity accumulated.

As GLUT4 is a key protein for glucose transportation from the vascular system into skeletal muscles, increases in the concentration of GLUT4 are beneficial for reducing blood glucose levels. In obese individuals with T2DM, exercise training on a cycle ergometer has shown to increase GLUT4 protein content by ~87% following 7 days training (O'Gorman et al., 2006). In addition to this, fasting blood glucose and whole-body insulin-stimulated glucose uptake were also improved. Although this provides evidence on skeletal muscle improvements from regularly activity as exercise training, it is not clear how long this adaptation lasts with training and crucially provides no evidence to whether lower intensity activities have a significant impact on GLUT4.

Glycogen synthase is involved in converting the glucose that enters muscle cells to its stored form (glycogen) by glycogen synthesis, a process which is impaired in individuals with T2DM (Christ-Roberts et al, 2004). Holten et al (2004) investigated the effects of one-leg strength training involving 30 minute sessions, three times a week for six weeks. Following training, glycogen synthase protein content and activity, and GLUT4 concentration GLUT4 were increased in the trained leg and improve glucose clearance. This finding provides evidence that exercise involving strength training improves glucose uptake which is attributed to contraction-mediated mechanisms (GLUT4 and glycogen synthase increases). As of yet, it appears that there is no literature on the associations between habitual PA and glycogen synthase content, only structured exercise.

There is good evidence that structured exercise benefits insulin signalling, GLUT4 and glycogen synthase activity in individuals who were obese and those diagnosed with T2DM (Christ-Roberts et al., 2004). Participants undertook an eight week training programme involving three sessions involving aerobic exercise on a cycle ergometer per week. Key findings were; training increased insulin-stimulated glucose disposal through the increase in GLUT4 expression, and glycogen synthase activity was increased resulting in glycogen synthesis. While the literature is not entirely consistent, Christ-Roberts et al, 2004) observed that insulin signalling did not improve following aerobic exercise. The results from this study highlight positive adaptations, including insulin sensitivity, that occur with training programmes involving an increase in PA. These findings showed little differences between the two groups of individuals (obese/overweight and diagnosed with T2DM), suggesting that metabolic effects of PA shown in previous studies can be applied to both obese/overweight individuals and in those with T2DM which could fill gaps within the literature into PA responses.

In summary the relationship between high intensity PA and structured exercise with blood glucose control in individuals with T2DM is well-established. There is a substantial body of evidence describing skeletal muscle adaptations to exercise training, yet evidence regarding chronic adaptations to higher levels of total free-living PA is far more limited. Bouts of daily PA varies significantly in intensity from lower intensity intermittent tasks of daily living, to more sustained light to moderate activity involved in active travel, to higher intensity structured exercise. The importance of PA intensity for glucose control will be discussed below.

2.4 The importance of physical activity intensity on glycaemic control

The intensity of PA and the influence it can potentially have on glycaemic control will be discussed here. The intensity of PA can vary substantially and may be important in determining both acute effects on glucose control and more chronic adaptations which occur. Current public health recommendations for the general public, and clinical recommendations for individuals living with

T2DM are that they should achieve 150 minutes MVPA per week (Bull et al, 2010; Colberg et al, 2010), implicitly suggesting that only activity of a moderate to vigorous intensity is beneficial. It has been suggested that PA of higher intensities are more effective than lower intensities for improved blood glucose control (Connolly et al, 2016; Little et al, 2011). However, recent evidence also suggests that PA of all intensities can be beneficial for lowering blood glucose levels after a bout of activity or over a period of time (Duviver et al, 2017; Pulsford et al, 2017; Dunstan et al, 2012; Van Dijk et al, 2011; Healy et al, 2007). This section will discuss the findings of how varied intensities of PA influence blood glucose control and insulin sensitivity.

A systematic review of 81 studies summarised the effects of specific types of PA and the risk of developing T2DM (Aune et al, 2015). Total PA, leisure-time and occupational activity, resistance exercise, cardiorespiratory fitness and low, moderate and vigorous intensity PA were measured. All were associated with a significant reduction in the risk of developing T2DM. A key finding was that vigorous intensity PA was more strongly associated with T2DM reduction than walking, however it is important to note that even with 2-3h walking per week there was a significant reduction in risk. The authors stated that RR of T2DM were reduced with leisure-time, vigorous or low intensity PA of 5-7h per week. This provides evidence that all PA is beneficial for improving blood glucose control and reducing the risk of T2DM.

These findings are supported by a number of experimental studies which have investigated possible benefits of light intensity PA on blood glucose control. Pulsford et al (2017) demonstrated that glucose and insulin AUC were lower when breaking up sitting with two minutes of light intensity walking every 20 minutes compared to two minutes of standing every 20 minutes or continuous sitting. The findings demonstrate accumulating regular light intensity activity in bouts as short as two minutes is beneficial. In addition, Dustan et al (2012) found glucose and insulin iAUC were reduced after light intensity and moderate intensity walking compared to the control (uninterrupted sitting), providing further evidence for benefits of not only moderate intensity activity, but light intensity too. These studies were undertaken in groups without a diagnosis of T2DM and as such we should

be cautious when generalising their findings however they provide good evidence that physical activity of any intensity, rather than only MVPA and exercise may be a useful tool for controlling blood glucose in T2DM.

In a randomised crossover trial in individuals with T2DM within free-living situations Duvivier et al (2017) examined 24 h glucose responses to an intervention which replaced 1.1h per day sitting with MVPA (cycling) or 4.7h per day light intensity walking in comparison to a control (14h per day sitting). Glucose iAUC calculated from continuous measurement was lower in both activity conditions compared to control; light intensity walking (1263 ± 189 min × mmol/l), MVPA cycling (1383 ± 194 min × mmol/l) and control (1974 ± 324 min × mmol/l). Insulin resistance was reduced in the light intensity walking condition to a greater extent than in cycling condition, possibly due to greater duration of the activity and the intermittent nature of the walking across the day.

In summary the findings presented here suggest that all intensities of PA, including light intensity activities such as walking, may benefit blood glucose control, and offer potential benefits for individuals living with T2DM (Duvivier et al, 2017) . As long-term participation is valuable for improved blood glucose control and reducing comorbidities, it would seem suitable to recommend PA of lower intensities for this clinical group as adherence may be better. However, the majority of existing research has focussed only on activities of a higher intensity, usually structured exercise, and this is reflected in existing public health and clinical guidelines. Understanding free-living activity and the prevalence of PA including low, moderate and vigorous intensities would be beneficial to provide realistic achieving recommendations for optimising PA and blood glucose control. However in addition to considering activity intensity, there is emerging evidence that the temporal pattern in which physical activity occurs may also be important in determining the benefit of physical activity for glucose control .

2.5 Patterns of Physical Activity and glucose control

In addition to the overall volume and intensity, temporal patterns of PA may also be important in determining the impact of PA on glycaemic control. Regular bouts of activity throughout the day are more effective in lowering glucose levels, in particular post prandial glucose (PPG) than single bouts of the same overall volume (Reynolds et al, 2016; Haxhi et al, 2015). As controlling postprandial hyperglycaemia is important to achieve recommended HbA1c values (Woerle., 2007), optimising the timing of PA around meals could control post-meal glucose surges. This section covers studies investigating how varying timings and distributions PA result in different glucose responses, the investigated timings include PA in the morning, afternoon, evening and before or after meals.

As discussed above, Duvivier et al., study demonstrated that PA in the form of low intensity walking was beneficial for reducing glucose iAUC in individuals with T2DM. The duration of low intensity walking reduced sitting time more than the cycling condition, but the bouts of walking were separated throughout the day rather than being one singular bout. In support of this, DiPietro et al (2013) investigated the effects of either 3 x 15-min bouts of post-meal walking at a moderate intensity or one 45 minute of sustained moderate walking away from meals at 10:30am or 3:30pm. Although both conditions significantly improved 24-h blood glucose control, it was also shown that 15 minutes post-meal walking was more effective for lowering 3-h post-dinner glucose than the 45 minute sustained walking. These studies provide evidence that PA in short bouts compared to a single volume bout matched in energy expenditure, is more beneficial for glycaemic control. The study by DiPietro et al (2013) also highlights that when physical activity occurs during a day or a week may be important, and that PA around meals may be crucial for controlling PPG.

Controlled trials demonstrate that PA of all intensities and forms; resistance exercises (Heden et al., 2014), walking (Colberg et al., 2009; Haxhi et al., 2015; Reynolds et al., 2016) and cycling (Larsen et al., 1997; Poirier et al., 2000) are more beneficial for managing hyperglycaemia when performed after meals compared to before meals. Chacko et al (2016) conducted a meta-analysis to summarise current findings. The timing of PA was separated into four different time periods; pre-meal, early

postprandial (15 - 29 min post meal), mid postprandial (30 – 120 min post meal) and late postprandial (>120 min post meal), and included different intensities (light, moderate and high). From analysing 30 studies, it was found that the optimal combination to reduce postprandial hyperglycaemic excursions was light – moderate intensity PA performed 30-45 min post-meal. In comparison, high intensity PA performed before meals resulted in an elevation of glucose levels suggesting a detrimental effect, however a delayed but modest improvement in insulin sensitivity was also present.

In summary, findings discussed here provide some insight into the importance of the temporal patterning of physical activity within and between days, an observation which may be important when considering physical activity advice to patient s with T2DM. However it should be acknowledged that, the majority of studies in this field involve controlled conditions that do not always represent normal free-living behaviour. Meals are controlled in composition and content (e.g. meal replacement shakes), unlikely to reflect a normal meal. In addition, PA conditions may be rigid and structured and not reflective of normal daily PA engagement. In addition these investigations generally examine 4 – 24 hour post-meal periods, so the full effect of PA on blood glucose control may not be represented. There is a clear need for further studies which can elucidate the precise temporal relationships between free-living activities of all intensities and glucose control in individuals with T2DM. Such understanding is now possible following advances in the real-time objective measurement of both glucose and physical activity. These methods are described below.

2.6 Methods of measuring glycaemic control

For clinical monitoring and population surveillance of glucose control in T2DM, HbA1c, Fasting blood glucose (FBG) and post prandial glucose (PPG) are the most common indicators used. Glycated haemoglobin (HbA1c) is the result of glucose combining with haemoglobin in red blood cells, and levels of HbA1C this can provide a reflection of average plasma glucose over the previous 2-3 months. FBG is blood glucose measured following 8-10h of no food or water consumption, the aim for individuals with T2DM is to keep FBG below <7mmol.L^{-1} (NICE, 2015). PPG refers to glucose levels

in the period following a meal. In individuals with T2DM this is generally at 2-h post-prandial as this is when glucose peaks, in some cases glucose is measured continually over this 2 hr period. And an area under the concentration vs time curve is calculated. The effectiveness of these methods for measuring glycaemic control and more recently the use of CGM are summarised below.

The use of CGMs is becoming increasingly more accessible for research and improving the ability of individuals with Type 1 and Type 2 diabetes mellitus to improve their blood glucose control. Various monitors are available which are worn on the body (often on the back of the upper arm or abdomen) and obtain frequent interstitial glucose readings automatically, for example every 5 minutes (Funtanilla et al, 2019). Frequently used monitors consist of the FreeStyle Libre System, Dexcom, Medtronic iPro2. The most recent FreeStyle Libre System is worn on the back of the upper arm, measures interstitial glucose every 15 minutes and can be worn for up to 14 days with no daily calibration required, however can be expensive (Blum, 2018). The Dexcom has three CGMs, worn either on the lower abdomen or on the back of the upper arm, these last up to seven days and require calibrations every 12 hours (Funtanilla et al, 2019). Both the FreeStyle Libre system and Dexcom provide immediate results to the individuals, and more recently can be viewed on mobile applications (Funtanilla et al, 2019). Medtronic has produced a range of devices, the iPro2 CGM used in the current study is worn on the lower abdomen above the iliac crest measuring interstitial glucose every five minutes for up to five days and required calibration every 12 hours. The device stores the glucose data which is then manually uploaded to provide a report of 24 hour glucose, this means patients are blinded to their glucose and is more frequently used in research (Leinungg et al, 2013). The iPro 2 was the preferred choice of monitor in the current study due to the frequency of measures (every 5 minutes), providing good detail on glucose control.

HbA1c is a preferred method of testing glycaemic control as it can be performed at any time and does not require fasting beforehand. Assessing HbA1c levels has been recommended by the ADA as a method of diagnosing T2DM, with high values of HbA1c in individuals with T2DM also associated with a greater risk of diabetic complications. Typical HbA1c goals are <7% (53mmol/mol) (ADA,

2018), however this can vary between patients depending on medication and duration of T2DM. Therapies to reduce HbA1c values have resulted in a significant decline in the risk of developing vascular complication in T2DM (Holam et al, 2008). However this chronic measure does not reflect the importance of daily glucose fluctuations such as the varied responses in glucose around meals, after PA and overnight. Although HbA1c is used as a tool for diagnosing T2DM, it is not accurate in determining the risk of further complications as fluctuations in blood glucose are more detrimental for cardiovascular function than constant high glucose (Ceriello et al, 2008).

FBG can also be indicative of glycaemic control with aims of treatment for individuals with T2DM to keep FBG below <7mmol.L^{-1} (NICE, 2015). It can be argued that measurements of FBG simply provide a value of basal blood glucose levels and do not provide an indication of the overall glycaemic control of an individual (Bouma et al, 1999). Relative to both HbA1c and PPG, FBG has shown to be less effective in predicting glycaemic control. Avignon et al, (1997), reported that correlations between PPG and HbA1c are better than FBG and HbA1c, when comparing pre-breakfast, post-lunch and extended post-lunch glucose to HbA1c values within a group of individuals with diagnosed T2DM. However, as noted above HbA1c (used as the reference measure in this study) may also not be reflective of further complications risks. This finding suggests that FBG is not a strong reflection of glycaemic control and insulin concentrations, other measures such PPG and HbA1c may be more reliable measure (Bonora et al, 2011).

PPG can be measured using the laboratory based technique OGTT, or within free-living conditions after meals. These tests measure blood glucose responses after consumption of glucose or a meal, providing information into glycaemic control and fluctuations in glucose caused by a food ingestion. An OGTT involves consumption of a bolus of liquid typically containing 75g of glucose for adults, followed by a blood glucose measurement at 2 hours after consumption or a prolonged OGTT (Stern et al, 2002). Additionally, research often uses frequent blood samples over the 2 hours, such as every 15 minutes, to determine glucose iAUC and assess glycaemic control (Sakaguchi et al, 2016). This has shown to be a preferred method for monitoring glucose control compared to fasting

glucose (Monnier and Colette, 2006). However this method is often not representative of a 'normal' meal consisting of carbohydrate, fat, protein and fibre. Consumption of a pure load of glucose is not experienced on a normal daily basis and does not represent glucose challenges in free-living conditions.

Using a meal challenge that better represents food more typically consumed in free-living, a 'mixed-meal tolerance test' can provide a more realistic challenge on the body to control glucose levels Comparisons between OGTT and mixed-meal tolerance tests show a correlation in plasma glucose responses over two hour post-consumption (Marena et al, 1992; Meier, Baller and Menge, 2009; Traub et al, 2012), but the correlation does not represent the extent of the effect. Using a mixed-meal tolerance test is more representative of human meal, and further causes marked islet B-cell secretion (which is dysfunctional in those with T2DM) more than glucose alone as other macronutrients such as protein are present (Marena et al, 1992). A limitation of using mixed meal tolerance test is that there is no accepted standardised meal composition or size which makes comparability between studies difficult. This method is also typically limited to a laboratory based environment preventing insight into glucose control in free-living situations.

The use of continuous glucose monitors (CGM) is now common practice in clinical and research settings. A small sensor is inserted into the lower abdomen or upper arm and a CGM is attached to take interstitial glucose reading every 3-15 minutes 24 hours a day. The use of these enables glucose data collection outside a laboratory setting, providing information about an individual's glucose control during free-living and improving understanding of responses to meals and activity. These first became commercially available in 2000 for individuals living with diabetes to use (Rodbard, 2016). Some monitors provide immediate glucose readings, which can be used by an individual to help control their glucose through immediate diet or activity changes. In a research setting CGMs are used to understand daily blood glucose control and how activity, meals and medication influence it (Duvivier et al, 2017; Van Dijk et al, 2011).

Van Dijk et al (2011) investigated hyperglycaemic excursions and HbA1c values in individuals with T2DM, assessing daily blood glucose using CGMs. A CGM was inserted for a 3-day experimental period to assess daily blood glucose concentrations every three minutes, during which habitual PA patterns were maintained and a standardised diet was provided. The results of the study demonstrated that the prevalence of hyperglycaemia is high in individuals with T2DM even in those who are considered to have good glycaemic control according to their HbA1c values (>7.0%). This suggests that HbA1c values do not provide evidence of the frequency and duration of hyperglycaemic excursions, particularly after meal. This highlights the importance of understating the daily variations in glucose levels and suggests that a shift towards monitoring and managing this variation (rather than focussing only on longer term markers like HbA1C may be important for future clinical care and for future research into diabetic health. .

An RCT involving three activity regiments compared the effect on glucose incremental area under the curve (iAUC), mean 24h glucose and hyperglycaemia over 4 days using a CGM in individuals with T2DM (Duvivier et alF, 2017). The three activity conditions were: 14 h sitting/day; 1.1 h/day of sitting replaced by moderate- to vigorous-intensity cycling and 4.7 h/day of sitting replaced by standing and light-intensity walking. Average glucose over 24h was lower in the condition with less sitting time vs. condition with high sitting time (7.35 ± 0.19 vs. 7.69 ± 0.23 mmol.l), but was not significantly different to the condition involving exercise. The CGM data enabled 24h iAUC to be investigated, finding that it was also significantly reduced after Sit-less compared to sitting (1974 ± 324 vs. 1263 ± 189 min x mmol/l). However, although 24h iAUC was significantly lower with exercise (1383 ± 194 min x mmol/l) compared to sitting, this finding was not significantly significant. This study highlights the benefits of using CGM to collect data on daily changes in glucose by providing detailed data, and again demonstrates that reducing sitting time with standing and light-intensity activity is beneficial for lowering daily blood glucose and hyperglycaemic excursions.

In support of CGMs use, a recent study investigated the impact that sedentary behaviour in free living has on glucose regulation, specifically relating to pre and post-meal glucose, and the 'Dawn

Phenomenon' (Paing et al, 2019). Participants wore CGMs and activity monitors over a period of 10 days which enabled associations between habitual sedentary behaviour and glucose levels to be investigated. Increased sedentary time was shown to be associated with higher glucose levels before breakfast and dinner and after lunch and dinner. The Dawn Phenomenon is a rise in glucose which is due to a release in glucose from the liver in response to certain hormones with no dietary carbohydrate intake (Schmidt et a, 1984), this is commonly experience in individuals with T2DM (Monnier et al, 2013). This was increased with more sedentary time, and decreased with a reduction in sedentary time (Paing et al, 2019). These findings highlight the benefit of CGM use to investigate glucose responses to behaviour that otherwise would not be easily studied. As of yet, this is the only observational study found using CGM to measure free-living activity and glycaemic control in individuals with T2DM.

The use of these devices has enabled the collection of precise and detailed data on daily glucose variations including, during waking hours, overnight and around meals. Establishing this is important as oscillating blood glucose levels have a more negative effect on endothelial function and oxidative stress, increasing the risk of vascular complications to a greater extent than constant high glucose levels (Ceriello et al., 2008). The availability of concurrent precise information on PA would allow detailed understanding of how movement patterns and glucose are linked and fluctuate together. Discussion of the assessment of PA is provided below.

2.7 Methods of measuring Physical Activity

PA has been assessed in population health studies since the 1950s (Paffernbarger, 2011). PA assessment was initially limited to the used of self-report questionnaires and activity diaries which relied on individuals to accurately remember and report activities of interest. Subsequently the use of wearable activity monitors, such as accelerometers, has become common. Such devices can

provide valid and reliable data on an individual's PA through direct movement measurement. In this section, the methods of PA measurement will be describe and evaluated.

Self-report questionnaires rely on accurate recall of PA through; written or online recall questionnaires or diaries, in-person or telephone interviews or similar. Questionnaires vary in detail depending on measurement aims, period and duration of assessment (Shephard et al., 2003). Importantly, there are variations in what they aim to measure such as frequency, intensity and duration of PA, and have differences in what activities are included such as assessment of leisure time, household, occupational or transport PA. The data collected from questionnaires is reported in different ways depending on research aims; activity scores, time spent in PA categories of different intensities, sedentary time or calorific expenditure (Sylvia et al., 2014). There are extensive volumes of questionnaires to assess PA, but no questionnaire is superior for measuring PA, the validity and reliability of a questionnaire is dependent on the aims of research and PA outcomes (Poppel et al, 2010). Questionnaires are still used extensively to understand PA behaviour, largely due to their relative ease of use, low participant burden, cost effectiveness and flexibility.

While there are a large number of studies that have examined associations between self-reports of PA and risk for T2DM (Cleven et al, 2020; Patterson et al, 2019; Aune et al, 2015), there are few studies using objective methods to investigate PA and glycaemic control. Kriska et al, (1963) investigated how fasting and 2h-PPG were associated to current and historical PA measured through a self-report interview in individuals with and without T2DM. Higher rates of T2DM were reported in individuals with low levels of historical PA, and a negative correlation between leisure time PA and fasting glucose and 2h PPG. In support of this, total weekly hours of MVPA were found to be associated with 2h-PPG after an OGTT; greater PA, lower 2h-PPG (Montero et al, 2016). In addition, Sadarangani et al (2014) found PA measured in individuals with T2DM through self-report interview to be associated with all cause and CVD mortality in a dose-response relationship when adjusted for covariates including BMI and hypertension (high blood pressure). These studies provide examples of

how the use of PA measurement through self-report techniques could be used to explain the relationship between PA and blood glucose control in individuals with T2DM and healthy individuals.

However it must be acknowledged that the use of self-report measures to assess habitual PA has some limitations, including the potential impact of errors in recall error and social desirability bias. Recalling exercise behaviours or activities which occur regularly or are more structured may be relatively simple. However, recalling all PA including often short and sporadic occupational activity, activities of daily living, and transport regardless of duration is very difficult. Although it is possible that recall error is greater in some population subgroups compared to others, it is also likely that there will be a degree of measurement error across any given measured population. This non-differential exposure misclassification can lead to a weakening or underestimation of links between PA exposures and health outcomes. Social desirability bias refers to the provison of self-report data which conforms better with societal or moral expectations. Activities such as physical activity which are perceived to have a (moral or social) worth are often, conciously or unconciously, over reported. Over reporting of PA due to social desirability bias can lead to an underestimation of the effect PA has on glucose outcomes. Hence, where possible accelerometers, which are not subject to such error or bias, are often used to assess PA in free-living and experimental settings.

Accelerometers are wearable devices which detect movement acceleration, usually on 3 axes, and have been widely used to measure PA in population research due to their ease of use for participants and ability to generate large amounts of reliable and accurate data. Accelerometers are typically worn on the waist, thigh, wrist, back or ankle and measure accelerations in motion of objects along an axes of reference (Yang and Hsu, 2010). Acceleration is defined as change in velocity over time, expressed as a multiple of gravitational force ($g=9.8m.s^2$) (Welk, 2002). Within these devices acceleration is detected by piezoresistive elements (Welk, 2002) and collected to determine the intensity, duration and frequency of movement over time (Yang and Hsu et al, 2010) and can be used within laboratory or free-living environments to reflect habitual PA.

Accelerometers of different models are regularly used within PA research include but are not limited to: ActivPAL, GENEactiv and ActiGraph. The ActivPAL device, used in the current study, is a thigh worn monitor which detects changes in movement and posture (Lynden, 2017). The GENEactiv is worn on the non-dominant wrist and measures accelerations in 3 axes, acceleration cut points are used to determine intensity of activity (REFERNCE). Lastly, ActiGraph accelerometers are worn at the hip, also measuring triaxial accelerations. Each have been validated for use of measuring sedentary behaviour and PA in adults, however the ActiGraph and GENEActiv monitors rely on thresholds and could misclassify activity intensities, specifically for clinical populations whereas the ActivPAL allows for postural allocation to identify sitting, standing and stepping time. Lyden (2017) found the accuracy of the ActivPAL activity monitor (a thigh worn accelerometer used to detect changes in movement and posture) to be 96.2% for measuring time spent in varying intensities of PA and sedentary time in free-living. It is suggested that the minimum accelerometer wear time required to accurately measure weekly activity behaviour is 5 days, and to include a combination of weekday and weekend days (Aguilar-Farias et al 2019). The activPAL has demonstrated to be reliable and valid for measuring ambulatory PA and sedentary behaviour; the devices are small and often attached to the thigh with medical tape such as tegaderm, compliance is high and can provide good detail on waking wear time (Edwardson et al, 2017).

In addition to measuring variations in activity intensity, devices such as the ActivPal provide high quality information on specific behaviours such as walking. For many, walking makes up the majority of their daily or weekly PA (Ham, Kruger and Tudor-Locke, 2009), and as such walking behaviour itself is of interest for researchers interested in how daily free-living PA impacts glucose control. Steps can be objectively measured to calculate cadence (steps/min) (Tudor-Locke et al, 2011) and walking cadences can be used to determine intensity. Cadence has been defined as slow (60-70 steps/min), medium (80-99 steps/min) or brisk (100-119step/min), faster forms of locomotion (jogging and running) are 120 + steps/min (Tudor-Locke et al, 2011). Moderate intensity is estimated to be ~100 steps/min, and >130 steps/min is estimated to be vigorous intensity (Tudor-Locke et al,

2018). Walking cadence and intensity can be used to understand the habitual activity patterns and glucose control relationship, this can be used to improve public health guidelines for PA. There are a number of studies using these devices to assess associations of sedentary behaviour and PA participation with glycaemic control (Buckley et al, 2014; Hansen, 2013; Healy et al, 2007). Healy et al (2007) investigated sedentary time and PA measured over 7 days in relation to markers of glucose control; FBG and HbA1c in individuals with T2DM. The majority of waking hours were spent sedentary and most PA performed was of a light intensity, which was significantly associated with lower fasting plasma glucose. However, due to the use of summary rather than continuous glucose measures examination of how PA influenced daily variation in glucose was not possible. Hansen et al., (2013) observed that with greater PA energy expenditure, (assessed over 7 days using combined accelerometers and heart rate monitors) insulin sensitivity was higher suggesting better glycaemic control. Individuals with higher PA energy expenditure also spent less time at the highest level of plasma glucose following OGTTs. This demonstrates this is a promising area to investigate in more detail to understand patterns of PA and glycaemic control.

The use of accelerometers is not without limitation, they are increasingly used to determine free-living PA in both population and clinical research. However while existing studies of PA and glucose have included the use of accelerometers the use of accelerometer data limits insight into daily variations of PA and glucose. Almost all existing studies of PA and glucose in T2DM (and in the wider PA literature) have tended to summarise activity data into average values for a particular measurement period (often a week) which prevents examination of how between and within day variation in PA might impact glucose.

2.8 Recent evidence for associations between PA and glucose assessed using contemporaneous objective measures

Two recent studies (Kingsnorth et al, 2018; Paing et al 2019) have examined associations between movement behaviours and free-living glucose using both accelerometers and CGMs. Kingsnorth et al

(2018), observed significant associations between average daily MVPA with average daily glucose. While very relevant the study was conducted in healthy individuals and therefore offers limited insight into how daily movement patterns may impact free-living glucose in individuals with T2DM. Paing et al (2019) conducted similar research in individuals with T2DM but only investigated the effect of sedentary behaviour on glucose, therefore offering no insight into impact into the effect of physical activity of different intensities. In addition neither studies have investigated how between and within day variations in PA and the impact this may have on glucose control, particularly in individuals with T2DM.

2.9 Summary

There is a significant body of evidence that PA can be a valuable tool in the management of T2DM. However detailed understanding of how precisely day to day changes in activity and glucose are related is lacking, and this is reflected in clinical guidance for individuals with T2DM which is still very general and focusses largely on activity of higher intensities. Firstly, methods of measuring glucose control previously do not adequately quantify important oscillations in daily blood glucose which may determine subsequent disease outcomes. With the use of CGM this is now possible in observational research. In addition assessment of PA in studies examining links between activity and glucose are limited to aggregate values which summarise activity over a given measurement period. This ignores potentially important information on how daily patterns of PA vary. By collecting data contemporaneously using CGMs and accelerometers, the influence of daily variations in PA of all intensities on daily variations in glucose can be studied in detail. This information could improve guidance for individuals living with T2DM. While two studies to date have taken some of these steps; PA and glucose control in individuals with T2DM including within day variations has yet to be investigated. Therefore, a precise and comprehensive understanding of how the distributions of PA influence glucose control in T2DM is needed.

2.9.1 Aims and objectives

The study aim was to inform improved practical guidance for PA for individuals with T2DM by providing the first detailed understanding of how the daily patterns and distributions, including intra- and inter-daily variations, of habitual PA of all intensities (not just moderate and vigorous) influence blood glucose regulation in free-living. Using contemporaneous assessment of movement and glucose.

Specifically this study has addressed existing gaps in the literature by:

1) Providing a detailed understanding of the associations between daily PA and glucose and

2) Provide the first exploration of the impact of between and within-day variability in PA.

3.0 Methods

3.1 Overview

This cross-sectional study was approved by the University of Exeter Sport and Health Sciences Research Ethics Committee (Approval reference: 190311/A/02) prior to commencing participant recruitment.

The study involved individuals with T2DM wearing a CGM and an activity monitor over five days to measure interstitial glucose and PA over 24h/day, while continuing usual free-living daily activities. Timing of food intake and sleep were recorded in a bespoke study diary. Subsequently PA metrics (including stepping time volume and cadence), and between and within day variations in activity were examined in relations to a number of clinically meaningful metrics of daily glucose. Risk assessments for the procedures were completed, and University and Sport and Health Science health and safety guidelines were closely followed to minimise any possible risks.

3.2 Participants

The target sample for this study was 33 participants with diagnosed T2DM. This target sample size was calculated based on the magnitude of the effect of daily accelerometer-defined moderate to vigorous PA on continuously measured glucose observed by Kingsnorth et al (2018) in a similar study within low-fit but otherwise healthy individuals. For detailed information on the calculation of the target sample size please see supplementary material (appendix 6.4, page 106). As Kingsnorth et al (2018) observed a significant correlation at the lower end of what would be considered a moderate effect size, a within individual correlation for minutes of daily MVPA and glucose was assumed to be r=0.25 (R^2 = 6%, f^2= 0.065). Based on these parameters our sample-size calculation indicated that we would require 123 days of complete paired accelerometer and CGM data to observe a significant association with 80% power. The CGMs record glucose data for 5 days. For 123 days of complete data, 31 participants would need to be recruited but to account for some data loss due to device non-wear or failure or participant drop out (conservatively 20% data loss, equivalent to achieving 4 out of 5 days per participant), 33 participants was the target.

Male and female adults (aged 18 years old and above) with a diagnosis of T2DM were invited to participate. Exclusion criteria included: any pre-exiting medical conditions or injuries that prevent usual habitual PA; smoking; and pregnancy. Injury or illness preventing habitual movement would inhibit the collection of useful data on the associations between PA and glucose control. As this study is concerned with associations between daily movement behaviours and glucose, injury or illness that prevents glucose behaviour would prevent the collection of meaningful data. Smoking impacts vascular function and glucose control (Chang., 2012; Frati, Iniestra and Ariza., 1996) which may have a confounding effect on observed associations between PA and glucose control. Diabetes during pregnancy (gestational diabetes) involves additional clinical factors and determinants which are beyond the scope of the current enquiry. Additionally pregnancy may alter daily movement patterns which may confound findings.

Participants were volunteers recruited via two methods; 1) advertisements, posters and presentations to an Exeter Diabetes Support group (Diabetes UK), and 2) invitation letter to eligible participants from an existing research volunteer database 'Exeter 10 thousand' (https://exetercrfnihr.org/about/exeter-10000/). Individuals from the Diabetes Support group based in Exeter, were able to express interest following a short talk during meetings and informal coffee mornings. Individuals with T2DM on the Exeter 10 Thousand register were contacted via post in batches of 30 and responded if they were interested in taking part.

Before taking part, all prospective participants were given an information sheet (appendix 6.1, page 84) detailing experimental procedures, potential benefits and risks involved to consider for at least 24 hours. Participants were given the opportunity to discuss any further questions or concerns regarding the study with investigators before providing written, informed consent. All were informed of their right to withdraw at any given time if they did not wish to complete the study, without reason or disadvantage to themselves.

3.3 Study design and protocol

The present study was a cross-sectional observational study which investigated how habitual PA patterns and volumes influence daily glucose in individuals with T2DM. Over a 5-day measurement period, continuous interstitial glucose and PA were measured.

A suitable date and time was arranged for the first visit with each participant. Arrival at the laboratory was by transportation of choice, parking permits were provided to participants who drove to St Luke's campus, University of Exeter. At the start of the first visit, the nature of the study and methods involved were explained, giving an opportunity for participants to ask any questions to be answered before giving informed consent. After providing written informed consent, baseline anthropometric measures were taken including: height (cm; Seca statiometer SEC-225, Seca, Hamburg, Germany), weight (kg; Seca digital column scale SEC-170, Seca, Hamburg, Germany), waist circumference (cm; Harpenden anthropometric tape, Holtain LTD, Hoechstmass, West Germany), body fat percentage (Omron Body Fat Monitor BF306, Omron Healthcare Europe B.V., The Netherlands) and blood pressure (mmHg; Dinamap Pro 100V2, GE Medical Systems Information Technologies 2002, Tampa, Florida, USA). Before measuring blood pressure, participants sat still for 5 minutes, the measurement was taken on the left arm which was raised to heart level. Three measures were taken, and an average of the three was recorded. Participants were request not to speak during measurements.

Following baseline measures, a CGM was fitted on the non-dominant sleeping side in the lower abdominal region slightly above the level of the iliac crest. The CGM consists of a small sensor which is inserted below the skin and glucose monitor attached to the sensor to measure and record interstitial glucose. The accelerometer was attached to the thigh (Activpal). Both devices were secured using adhesive 3M tegaderm. Further details on the use and validity of these measures is provided below.

Participants received instruction regarding the finger-tip blood sampling required to calibrate the CGM 3 times per day (morning, afternoon and before bed). A bespoke study diary (appendix 6.3, page 92) was provided to record timings of food intake, finger-tip glucose readings and sleep timing. This bespoke study diary also contained the information already provided, as well as answers to commonly asked questions about the use of accelerometers and CGMs. Thereafter, normal daily activities were resumed for 5 full days (including 2 weekend days) while glucose and activity were measured, before returning to the laboratory at a pre-arranged suitable time. The devices were removed and the study diary collected, followed by completion of an acceptability questionnaire (appendix 6.2, page 88) to determine ease of use, comfort and wear time compliance from participants. A schematic detailing the study protocol is presented in Figure 1.

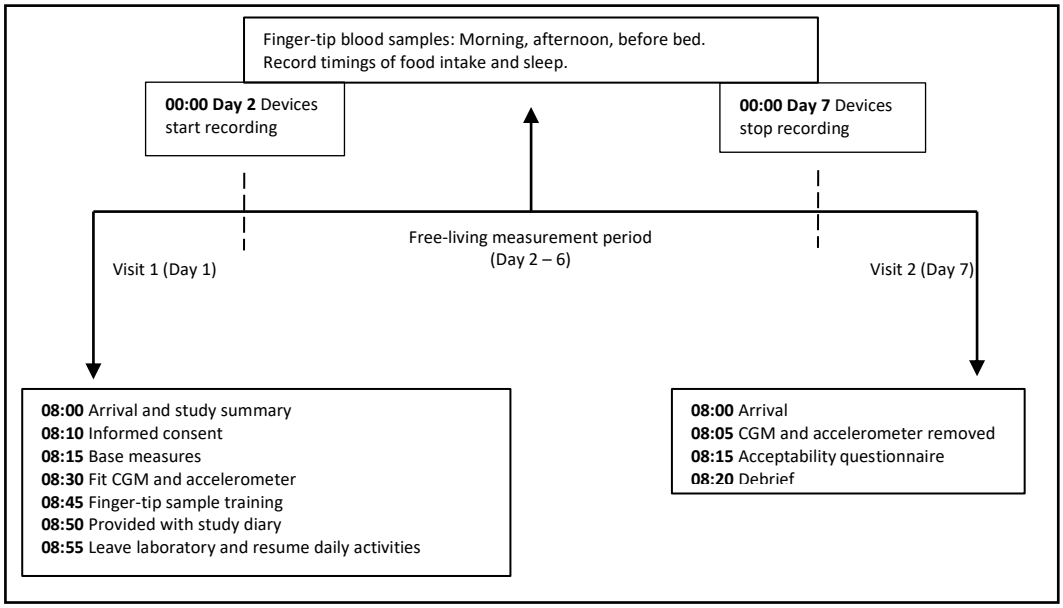


Figure 2. Schematic of the study protocol detailing events throughout Visits 1 and 2, and the 5 days free-living measurement period.

3.4 Continuous Blood Glucose and Accelerometer data

PA measures

The accelerometer used in this study was the activPAL (activPAL4, PAL Technologies, Glasgow, UK) accelerometer which was attached to the anterior aspect of the mid- thigh. The activPAL device has found to be valid for accurately measure time in varying PA intensities and sedentary time in adults (Lyden, 2013). Through its positioning on the thigh and its internal inclinometer the activPal measures sitting standing and stepping across a predetermined measurement period. A combination of movement acceleration and step cadence is also used to predict activity intensity and energy expenditure in METs (Metabolic equivalents).

Data from the devices was used to determine the following exposure variables:

- Total volume of activity by day and across the measurement period
- Volume of activity at light intensity (<100 step/min) and moderate to vigorous intensity (>100 step/min)
- Stepping that occurs within 2 hours before and after food intake
- Proportion of daily activity (min) that occurs in the morning vs the afternoon
- Between day variation in activity (further details below)

Continuous blood glucose measures

The CGM (iPro2 continuous glucose monitor, Medtronic, UK) fitted in the lower abdominal region slightly above the level of the iliac crest, records an interstitial glucose reading every 5 minutes for 24 hours per day allowing observation of detailed information on glucose throughout the 24 hour period. The iPro2 continuous glucose monitor used does not provide live feedback so participants were blinded to knowing their glucose reading apart from when completing the calibrations, a portable blood glucose meter (CONTOUR® NEXT LINK 2.4 glucose meter, Ascensia Diabetes care),

was provided to take 3 finger-tip glucose readings per day (at least 1 required every 12h) to calibrate the iPro2 CGM.

Figure 3. iPro continuous glucose monitor worn on the lower abdomen and ActivPAL activity monitor work on the mid-thigh.

This data was used to determine the following outcomes variables:

- Total time spent in target glucose ranges (TIR). These are 3.9-8.5mmol.L (the recommended range following meals) and 4.0-7.0mmol.L (the recommended fasting range) respectively (Diabetes UK, 2015). TIR provides a dynamic measure of daily glucose which can inform treatment to reduce the exposure to hyperglycaemia.
- Mean Amplitude of Glucose Excursions (MAGE). MAGE is considered as the Gold Standard measure of glucose variability (Monnier et al, 2008) and hence was selected for analysis. It is the mean of upward or downward glucose excursions that exceed the threshold of typically 1 standard deviation from the average within a 24h period (Akasaka et al, 2014).
- Average 24-hour glucose. This provides a measure of the amount of high and low glucose values. 2-hour postprandial glucose was used to measure the postprandial glucose responses following a meal to provide details into glucose tolerance (Sakaguchi et al, 2016).

- 24 hr Glucose Area Under the Curve (AUC). Provides an indication of glucose excursions (Sakaguchi et al, 2016) and total daily exposure to glucose
- Average waking and nocturnal glucose (mmol.L)
- 2-hour postprandial glucose (mmol.L)

3.5 Data processing

Data was downloaded from the activPAL device using PALconnect (PAL Technologies) and converted to .csv files using the PALanalysis software (PAL Technologies). CGM data was downloaded using Medtronic software as a .csv file. MATLAB (R2019a) was used to further reduce the data and extract the variables: TIR, average 24-hour average glucose, average waking and nocturnal glucose, and 2-hour postprandial glucose. Using the open source R package 'cgmanalysis' (Vigers et al, 2019) key variables MAGE and glucose AUC were extracted. The process of these is detailed below.

Using the programme MATLAB (R2019a), variables required for analyses were extracted from the activPAL accelerometer and CGM csv. files. The timestamped activity and glucose data were aligned (in MATLAB) to allow paired days of data to be examined contemporaneously. Sleep and waking times were used to determine day and night glucose. Reported meal times were used to determine post prandial periods for examination of postprandial glucose (PPG), and the number of steps and time spent stepping 2 hours before and after meals. This included the activity variables: total daily steps, stepping time (min), sitting time (min), standing time (min), stepping time at >100 step/min, stepping time <100 step/min, pre-meal steps, post-meal steps, morning (am) steps and afternoon (pm) steps.

To investigate the impact of inter-daily variations in PA on glucose, the standard deviation (SD) of activity between days (for example; Monday and Tuesday, Tuesday and Wednesday, Wednesday and Thursday) was calculated. The average between day SD for each participant was used to determine the variability in activity; those with an average SD above the group median were classed as 'high variability' and those with an SD lower than the median as 'low variability'. For analysis

participants were also classified as having a high or low volume of activity (defined as above or below the group median for each of the activity variables). Based on activity variability and volume participants were then grouped into one of four categories: Low Variability, Low PA (LVLP); High Variability Low PA (HVLP); High Variability, High PA (HVHP); Low variability, High PA (LVHP).

Glucose variables included: number of TIR readings per day (n), glucose TIR (%), 24h average glucose (mmol.L), average nocturnal glucose (mmol.L), average waking glucose (mmol.l) and 2h PPG average (mmol.L).The package 'cgmanalysis' in R created by Vigers et al (2019) was used to extract and calculate MAGE and AUC from the CGM files, the process consisted of 3 functions: cleandata(), cgmvariables() and cgmreport(). In the cleaning function, 20minutes gaps or less in glucose data were filled in, but 24h periods with gaps larger than 24h were removed. The CGM variables were then calculated in cgmvariables(); MAGE was calculated using Baghurt's algorithm (Baghurst, 2011) with blood glucose excursions greater than 1SD from the mean. In cgmreport() the results of the analysis were presented in an excel file.

3.6 Statistical analysis

Up to 5 days of paired glucose and PA data was available from each participant. A measurement day was not included in the analysis if accelerometer wear did not meet the valid wear time criteria of 13h/day (Herrmann et al, 2013), or if glucose finger prick calibration readings were not taken at 12 hour intervals.

3.6.1 Associations between PA and daily glucose

Generalised Estimating Equations (GEE) models were used to assess the relationship between daily PA and glucose variables, this allows for the analysis for correlated observation. Using the quasi-likelihood under independence model criterion (QIC) value, the correlation structure was evaluated to determine which would be a better fit. The two correlation structures assessed were: unstructured and autoregressive order 1 (AR 1). In AR1 structure, repeated measures have a relationship in the first-order, for this analyses it would assume there is a stronger relationship

between day 1 and day 2 than day 1 and day 5. Unstructured is a general correlation matrix. AR1 demonstrated a better fit as the QIC values were lower in comparison to unstructured. Univariate (model 1) and multivariate (model 2) and multivariate (model 3) GEE analyses were conducted. Model 1 was unadjusted, Model 2 was adjusted for age and BMI, and model 3 was used for PPG adjusted for age, BMI and pre/post-meal steps. A sensitivity analysis was conducted as some participants reported being insulin dependent, a sensitivity analysis was conducted in which these participants were removed from the analysis to see if any associations changed. GEE were conducted on data from all participants (then separately on data only from those who were not insulin dependent) to examine the following:

- associations between daily PA metrics (total steps, stepping time (min), stepping at cadences above and below 100 steps per minute) with daily glucose outcomes (24 average glucose, TIR, MAGE, and AUC)
- associations between both pre and post prandial stepping and PPG and postprandial TIR
- associations between activity accumulated in the morning and afternoon with average waking time and nocturnal glucose

3.6.2 Inter-daily variations in PA and daily glucose

Simple linear regression analyses (for categorical exposure variables) was conducted to assess the relationship between interdaily activity variability and 5-day glucose variables. Each of the four categories (LVLP, HVLP, HVHP, LVLP) were assigned a numerical value in an arbitrary order, these were then recoded to create dummy variables. Regression analyses were done for average TIR% (4.0-7.0mmol.L), average glucose (mmol.L), MAGE and AUC with the LVLP group as the reference category.

Statistical Package for the Social Sciences (SPSS), version 23, was used to conduct all statistical analysis. Unless otherwise stated, all data is presented as unstandardized B coefficient (95%

confidence intervals), mean ± standard deviation (SD) and was considered significant at the level $p >$ 0.0.

4.0 Results

Complete data for interstitial glucose and daily movement activity data was collected for 31 participants. Data from two participants was excluded as minimum standard for the instructed calibration of the CGM (at least one calibration reading every 12 hours) was not met on all days, therefore data from 29 participants was analysed. In total, 145 days of paired glucose and movement data was included in analyses of all participants and 110 days of paired glucose and movement data was analysed in sensitivity analysis for those participants not taking insulin. Participant characteristics of those included in the final analytical sample are summarised in table 1. An example of paired glucose and PA data for 1 participant is illustrated in Figure 4.

Table. 1 Participant characteristics

Participant characteristics	
Number of participants (male/female) (*n*)	29 (m = 14, f = 15)
Age (years)	72 ± 11
BMI (kg/m^2)	34.6 ± 11.0
Waist circumference (cm)	109 ± 13
Diabetes management (*n*)	
No medication	4
Metformin	8
Metformin + Gliclazide	4
Metformin + Sitagliptin	1
Gliclazide	1
Metformin + Insulin	4
Insulin	3
Other	2
Activity Variables	
Total daily steps (n)	6760 ± 3958
Sitting time (min)	1102 ± 131
Standing time (min)	240 ± 101
Stepping time (min)	99 ± 78
Stepping time >100spm (min)	14 ± 22
Stepping time <100spm (min)	85 ± 75
Pre-meal Steps (n)	3495 ± 2542
Post-meal steps (n)	2947 ± 2402
Glucose Variables	
Daily average glucose (mmol.L)	9 ± 2.5
Time in Target Range 4.0-7.0mmol.L (n)	77.3 ± 77.3
Time in Target Range (% of day)	26.8 ± 26.8
Time in Target Range 3.9-8.5mmol.L (n)	154 ± 87
Time in Target Range (% of day)	53.5 ± 30.1
Post-meal glucose (mmol.L)	9.3 ± 2.8
Mean Amplitude of Glucose Excursions (MAGE)	4.4 ± 2.7
Total daily Area Under the Curve (AUC)	212.9 ± 58.9

Data are mean ± SD unless otherwise stated

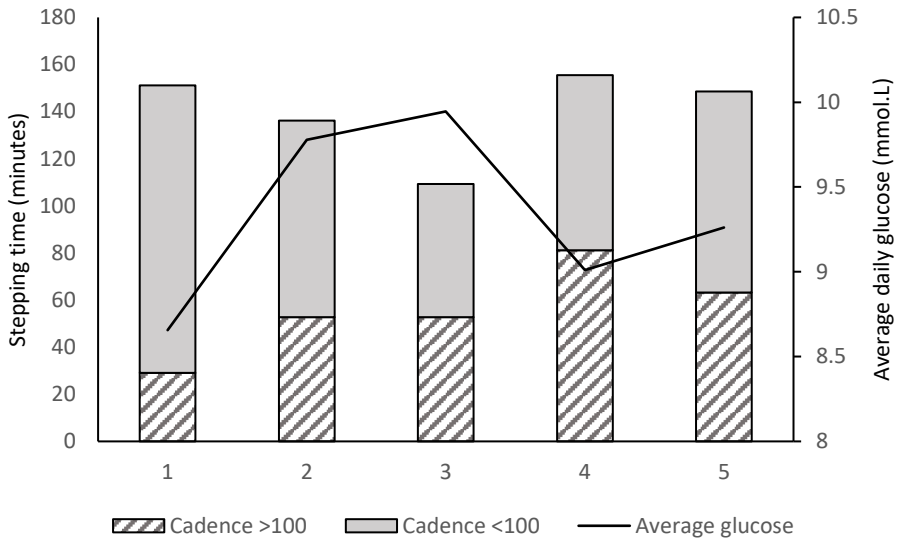


Figure 4. Example of 5 days movement and glucose trace from a male participant estimated to undertake the recommended 150 minutes MVPA per week. Minutes of stepping at a cadence of >100 steps per minute (moderate intensity), <100 steps per minute (low intensity) and average 24h glucose (mmol.L).

4.1 Device compliance and acceptability of measurement procedure

Compliance was determined based on wear time from the activPAL reports and CGM readings. Five complete days of activPAL and CGM data was collected for all 29 participants. Acceptability of measurement procedures reported in the questionnaire are reported in figure 3.

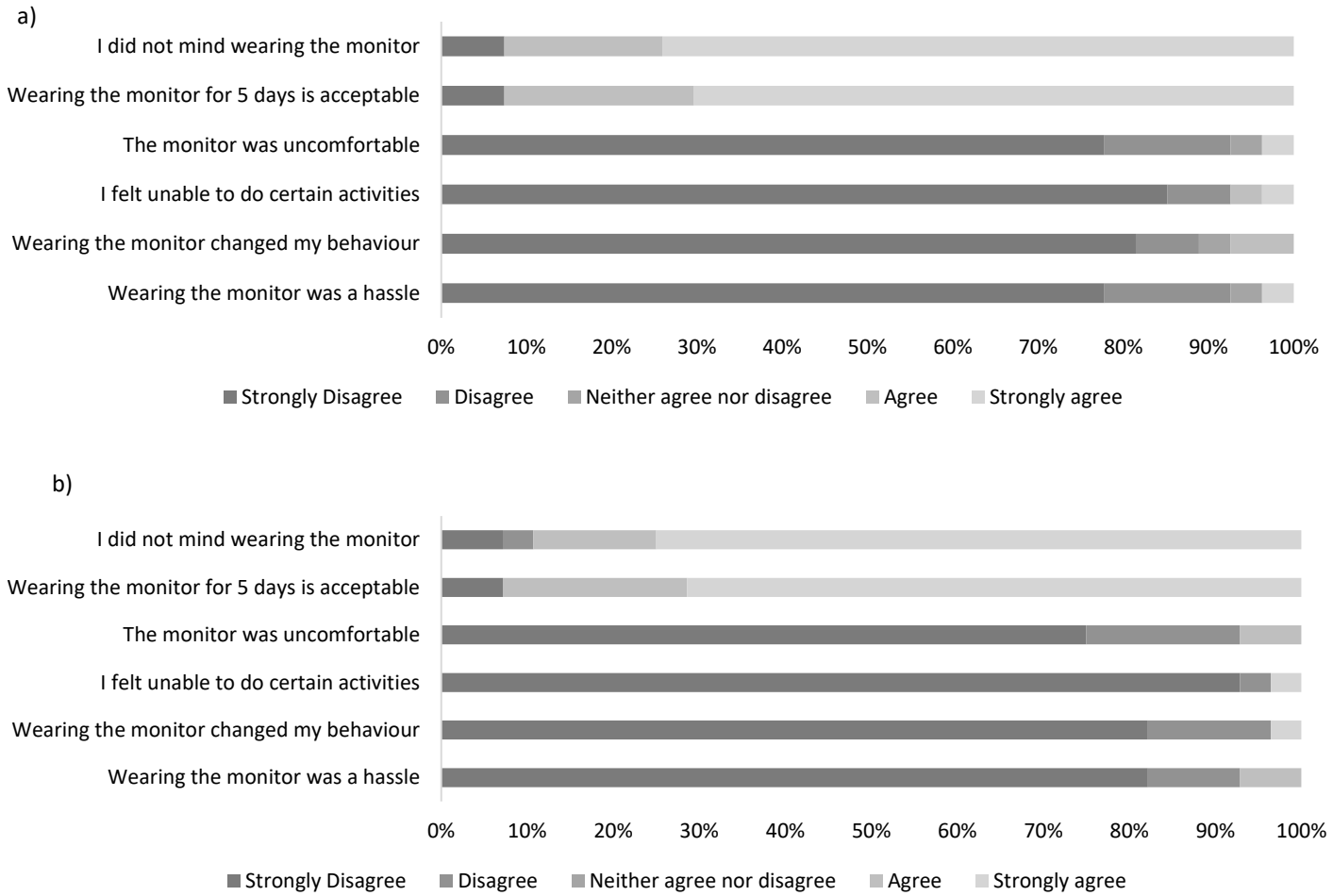


Figure 5. Acceptability questionnaire responses for the two devices a) activPAL, b) iPro2 CGM, reported as percentage of responses from 29 participants.

	ActivPAL		CGM	
	Agree	Disagree	Agree	Disagree
Wearing the monitor was a hassle	4%	93%	7%	93%
Wearing the monitor changed my behaviour	7%	89%	4%	96%
I felt unable to do certain activities	7%	93%	4%	96%
The monitor was uncomfortable	4%	93%	7%	93%
Wearing the monitor for 5 days is acceptable	93%	93%	93%	7%
I did not mind wearing the monitor	93%	7%	89%	11%

Table 2. Acceptability questionnaire responses for the two devices: activPAL and iPro2 Continuous glucose monitor.

4.2 Daily physical activity

The ActivPAL data showed most activity was of a light intensity rather than moderate, as presented in table 1. ActivPAL intensity was determined through walking cadence. Moderate intensity (14± 22 minutes) and light intensity (85 ± 75 minutes) were determined using duration at selected stepping cadences; >100 steps per minute and <100 steps per minute respectively. This data was used to estimate participation in MVPA; an average of at least 21.43 minutes moderate intensity PA was required to achieve 150 minutes MVPA per week. Seven participants achieved this based on ActivPAL data out of a total of 29 participants.

4.3 Associations between glycaemic control and activity variables

4.3.1 Total daily activity and glycaemic control

Comparisons between activity variables (total steps, stepping duration, stepping duration at a cadence of >100 and <100 step/min) and glycaemic control (TIR% at 4.0-7.0mmol.L and 3.9-8.5mmol.L, 24h average glucose mmol.L, MAGE and AUC) are presented in Table 3.

All participants

GEE analyses on all participants revealed no significant associations between activity variables and percentage TIR (3.9-8.5mmol.L) in either model. When GEE analyses were conducted with TIR as 4.0-7.0mmol.L, total daily steps ($\beta = 0.001$, $p = 0.021$), stepping time ($\beta = 0.125$, $p = 0.01$) and stepping at a cadence of <100 step/min ($\beta = 0.151$, $p = 0.012$) were significantly associated with TIR percentage in model 1. In model 2 total stepping time ($\beta = 0.156$, $p = 0.038$) and stepping at a cadence of <100 step/min ($\beta = 0.182$, $p = 0.033$) were significantly associated with TIR percentage. For context; when accounting for age and BMI a one minute increase in stepping time is associated with a 0.16% increase in TIR, meaning a 10 minute increase in stepping time could increase daily TIR by ~22minutes.

GEE analyses examining the association between activity variables and MAGE found stepping time at >100 step/min was significantly associated with lower MAGE values (β = -0.017, p = 0.022) in model 2. No other significant associations were found between MAGE and activity variables in analysis of all participants. Stepping time at <100 step/min and AUC were significantly associated in model 2 (β = -0.386 p = 0.04). These findings demonstrates that a higher stepping time >100 step/min decreased MAGE, and stepping time <100 step/min decreased AUC. No significant associations between activity variables and 24h average glucose were found in analyses on all participants.

Non-insulin dependent participants

GEE analyses comparing TIR (3.9-8.5mmol.L) and activity variables in the 21 non-insulin dependent participants revealed that total daily steps (β = 0.001, p = 0.03) and stepping time (β = 0.102, p = 0.026) were significantly associated with TIR% in model 1. There were no significant associations between TIR (3.9-8.5mmol.L) percentage and activity variables in model 2. GEE analyses comparing TIR (3.9 – 7.0 mmol.L) percentage found total daily steps (β = 0.001, p = 0.013), stepping time (β = 0.137, p = 0.008) and stepping time at a cadence of <100spm (β = 0.147, p = 0.027) were significantly associated with TIR percentage in model 1. In model 2, total daily steps (β = 0.002, p = 0.025) stepping time at a cadence <100spm (β = 0.207, p = 0.024) and moderate intensity PA (β = 0.256, p = 0.033) were significantly associated with TIR percentage. Stepping time at >100 step/min was significantly associated with MAGE in model 2 (β = -0.017, p < 0.001). Stepping time at <100 step/min was significantly associated with AUC (β = -0.386, p = 0.04). No significant associations between activity variables and 24h average glucose were found in analyses on participants not dependent on insulin.

Table 3. Associations between PA variables and glycaemic control (TIR, 24h average glucose, MAGE and AUC) in all participants and in non-insulin dependent diabetes mellitus (NIDDM) participants.

		TIR[a] (%; 3.9 - 8.5mmol.L)		TIR (%; 4.0 - 7.0 mmol.L)		24h Average Glucose	
		B (95% CI)	***p***	**B (95% CI)**	***p***	**B (95% CI)**	***p***
NIDDM	**Total Steps**						
	Model 1	**0.001(0.00,0.002)**	***0.03***	**0.001(0.00,0.003)**	***0.01***	-0.5x10^4(0.00,0.12x10^4)	*0.11*
	Model 2	0.001(-0.001,0.003)	*0.35*	**0.002(0.00,0.005)**	***0.03***	0.00(0.00,0.39x10^4)	*0.13*
	Stepping (min)						
	Model 1	**0.102(0.012,0.192)**	***0.03***	**0.137(0.037,0.238)**	***0.01***	-0.004(-0.010,0.002)	*0.20*
	Model 2	0.113(-0.095,0.321)	*0.29*	**0.207(0.027,0.387)**	***0.02***	-0.013(-0.028,0.002)	*0.09*
	>100 step/min (min)						
	Model 1	0.138(0.014,0.291)	*0.08*	0.154(-0.026,0.334)	*0.09*	**-0.008(-0.017,0.26x10^4)**	***0.05***
	Model 2	0.044(-0.295,0.383)	*0.80*	0.305(-0.122,0.732)	*0.16*	-0.008(-0.026,0.013)	*0.44*
	<100 step/min (min)						
	Model 1	0.095(-0.018,0.208)	*0.10*	**0.147(0.017,0.277)**	***0.03***	-0.002(-0.013,0.009)	*0.76*
	Model 2	0.163(-0.095,0.421)	*0.22*	0.206(-0.004,0.416)	*0.06*	-0.017(-0.035,0.001)	*0.06*
All	**Total Steps**						
	Model 1	0.001(0.000,0.002)	*0.11*	**0.001(0.00,0.002)**	***0.02***	-0.46x10^4(0.00,0.25x10^4)	*0.20*
	Model 2	0.001(-0.001,0.003)	*0.31*	0.002(-0.0001,0.004)	*0.06*	0.00(0.00,0.249x10^4)	*0.11*
	Stepping (min)						
	Model 1	0.081(-0.015,0.178)	*0.10*	**0.125(0.031,0.220)**	***0.01***	-0.004(-0.008,0.000)	*0.07*
	Model 2	0.100(-0.073,0.272)	*0.26*	**0.156(0.008,0.303)**	***0.04***	**-0.006(-0.012,-0.001)**	***0.02***
	>100 step/min (min)						
	Model 1	0.121(-0.018,0.260)	*0.09*	0.108(-0.045,0.261)	*0.17*	-0.007(-0.016, 0.001)	*0.07*
	Model 2	0.089(-0.223,0.401)	*0.58*	0.160(-0.212,0.532)	*0.40*	-0.010(0.029,0.009)	*0.30*
	<100 step/min (min)						
	Model 1	0.069(-0.054,0.192)	*0.27*	**0.151(0.033,0.270)**	***0.01***	-0.003(-0.008,0.002)	*0.19*
	Model 2	0.122(-0.088,0.332)	*0.25*	**0.182(0.015,0.349)**	***0.03***	**0.006(-0.011,-0.001)**	***0.02***

		MAGE[b]		AUC[c]	
		B (95% CI)	***p***	**B (95% CI)**	***p***
NIDDM	**Total Steps**				
	Model 1	$0.14x10^4$(0.00,0.00)	0.82	-0.001(-0.002,0.001)	*0.428*
	Model 2	$-0.54x10^4$(0.00,$0.91x10^4$)	0.47	-0.003(-0.008,0.001)	*0.136*
	Stepping (min)				
	Model 1	0.002(-0.009,0.013)	*0.73*	-0.046(-0.214,0.122)	*0.59*
	Model 2	-0.004(-0.018,0.009)	*0.54*	-0.360(-0.764,0.045)	*0.082*
	>100 step/min (min)				
	Model 1	-0.009(-0.023,0.005)	*0.23*	-0.13(-0.346,0.086)	*0.238*
	Model 2	**-0.016(-0.032,-0.001)**	***0.04***	-0.171(-0.696,0.353)	*0.522*
	<100 step/min (min)				
	Model 1	0.008(-0.006,0.023)	*0.27*	-0.007(-0.324,0.311)	*0.967*
	Model 2	$0.44x10^4$(-0.018,0.018)	*1.00*	-0.505(-0.983,-0.027)	***0.038***
All	**Total Steps**				
	Model 1	$-0.717x10^6$(0.000,0.000)	0.91	-0.001(-0.002,0.001)	*0.5*
	Model 2	$-0.6813x10^4$(0.00,$-0.69x10^4$)	*0.33*	-0.003(-0.006,0.001)	*0.1*
	Stepping (min)				
	Model 1	-0.001(-0.011,0.010)	*0.90*	-0.059(-0.235, 0.117)	*0.51*
	Model 2	-0.006(-0.018,0.006)	*0.32*	-0.298(-0.612,0.015)	*0.06*
	>100 step/min (min)				
	Model 1	-0.009(-0.023,0.004)	*0.19*	-0.108(-0.318,0.101)	*0.31*
	Model 2	**-0.017(-0.032, -0.002)**	***<0.001***	-0.212(-0.669,0.245)	*0.36*
	<100 step/min (min)				
	Model 1	0.003(-0.011,0.017)	*0.68*	-0.047(-0.329,0.234)	*0.74*
	Model 2	-0.003(-0.019, 0.012)	*0.67*	**-0.386(-0.755,0.017)**	***0.04***

Values are presented as B coefficient and 95% confidence interval. Model 1 represents univariable association, model 2 is adjusted for age and BMI. [a]Time in range percentage (TIR%) as 4.0-7.0mmol.L [b]Mean Amplitude of Glucose Excursions (MAGE). [c]Total glucose area under the curve (AUC).

4.4 Intradaily variation in activity. Activity around meal times and postprandial glucose

GEE analyses on all participants comparing number of pre- and post-prandial steps with 2h-PPG and TIR (%) are presented in Table 4. Analyses revealed that number of post meal steps was significantly associated with 2h-PPG in model 1 (β = 0.000, p = 0.012), model 2 (β = 0.000, p = 0.046) and model 3 (β = 0.000, p = 0.046), presented in table 4. GEE analyses on only non-insulin dependent participants show post-meal steps was significantly associated with PPG in model 1 (β = 0.000, p = 0.02). No other associations between pre- and post-prandial step were found to be significant predictors of 2h-PPG or TIR percentage at the level p > 0.05 in all models. In both models for all participants and non-insulin dependent participants, the significant associations had unstandardized beta values of <0.000, this would suggest that the changes in glucose per step are so minute at this level.

Table 4. Generalised estimating equation on the effect of pre- and post-meal steps on average 2 hour Post prandial glucose and percentage TIR in all participants and non-insulin dependent diabetes mellitus participants (NIDDM).

		2 hour PPG[a] average		TIR %[b]	
		B (95% CI)	***p***	**B (95% CI)**	***p***
NIDDM	**Pre-meal steps**				
	Model 1	-0.00005(0.000,0.000)	*0.566*	-0.001(-0.004,0.001)	*0.342*
	Model 2	0.000(0.000,0.00008)	*0.155*	-0.001(-0.005,0.003)	*0.656*
	Model 3	0.000(0.000,0.00008)	*0.155*	-0.001(-0.005,0.003)	*0.656*
	Post-meal steps				
	Model 1	**0.0001(0.000,-0.00002)**	***0.02***	0.001 (-0.001,0.002)	*0.337*
	Model 2	0.0001(-0.001,0.00002)	*0.065*	0.001(-0.003,0.005)	*0.486*
	Model 3	0.0001(-0.001,0.00002)	*0.065*	0.001(-0.003,0.005)	*0.486*
	Pre and post-meal steps				
	Model 1	0.000(0.000,0.001)	*0.388*	0.001(0.000,0.002)	*0.131*
	Model 2	0.000(0.000,0.00001)	*0.061*	0.001(-0.002,0.004)	*0.424*
All	**Pre-meal steps**				
	Model 1	-0.00007(0.000,0.00008)	*0.383*	-0.002(-0.004,0.001)	*0.152*
	Model 2	0.000(0.000,0.00003789)	*0.101*	-0.001(-0.004,0.002)	*0.609*
	Model 3	0.000(0.000,0.00003789)	*0.101*	-0.001(-0.004,0.002)	*0.609*
	Post-meal steps				
	Model 1	**0.0001(0.000,-0.00003)**	***0.012***	0.000(-0.001,0.002)	*0.717*
	Model 2	**0.0001(0.000,-0.000005)**	***0.046***	0.001(-0.002,0.005)	*0.5*
	Model 3	**0.0001(0.000,-0.000005)**	***0.046***	0.001(-0.002,0.005)	*0.5*
	Pre and post- meal steps				
	Model 1	-0.00006(0.00,0.000005)	*0.069*	0.000(-0.001,0.002)	*0.409*
	Model 2	0.000(0.000,0.000006)	*0.061*	0.001(-0.001,0.003)	*0.429*

Unstandardised β coefficient and 95% confidence intervals for Generalised Estimating Equations on the effect of pre- post- or total meal steps on Post Prandial glucose and Time in target glucose range % in all participants and in only participants not taking insulin. [a]Post Prandial Glucose (PPG), [b]Time in

target glucose range percentage (TIR%). Model 1 is univariable analysis, model 2 is adjusted for age and BMI, and model 3 is adjusted for age, BMI and post-meal steps for pre-meal steps, and vice-versa.

4.5 Intradaily variation in activity. Morning and afternoon activity

GEE analyses revealed no significant association between steps done in the morning or afternoon on day or night average glucose. Stepping time was found to be significantly associated with daytime glucose in model 2 for all participants ($\beta = -0.008$, $p = 0.039$) and non-insulin dependent participants ($\beta = -0.008$, $p = 0.021$), suggesting that increased stepping time is associated with a decrease in average day glucose values. Stepping time at a cadence of <100 step/min was also associated with day glucose but only in participants not dependent on insulin ($\beta = 0.021$, $p = 0.043$). No other significant associations were found. These analyses are presented in table 5.

Table 5. Generalised Estimating Equations on the effect of morning and afternoon activity on night and day average glucose (mmol.L) in non-insulin dependent diabetes mellitus participants and all participants.

		Night glucose (mmol.L)		Day glucose (mmol.L)	
		B (95% CI)	***p***	**B (95% CI)**	***p***
NIDDM	**Total Steps**				
	Model 1	$-0.108x10^4$(0.000,0.000)	*0.862*	$-0.30x10^4$($-0.98x10^4$,$0.37x10^4$)	*0.376*
	Model 2	$-0.321x10^4$(0.000,0.000)	*0.97*	0.000(0.000,$0.9214\ x10^5$)	*0.067*
	am steps				
	Model 1	$-0.84x10^5$(0.000,0.000)	*0.972*	$0.739x10^4$(0.000,0.000)	*0.635*
	Model 2	$0.744x10^4$(0.000,0.001)	*0.734*	0.000(-0.001,0.000)	*0.347*
	pm steps				
	Model 1	$-0.122x10^4$(0.000,0.000)	*0.871*	$-0.245x10^4$(0.0,$0.6466\ x10^4$)	*0.59*
	Model 2	$-0.922x10^5$(0.000,0.000)	*0.932*	0.000(0.000,$0.1184\ x10^4$)	*0.068*
	Total stepping time				
	Model 1	-0.003(-0.007,0.001)	*0.201*	-0.001(-0.006, 0.002)	*0.395*
	Model 2	-0.003(-0.008,0.001)	*0.145*	**-0.008(-0.014,-0.001)**	***0.021***
	>100 step/min(min)				
	Model 1	0.053(-0.066,0.172)	*0.383*	-0.004(-0.012,0.003)	*0.271*
	Model 2	0.033(-0.054,0.119)	*0.458*	0.009(-0.032,0.013)	*0.416*
	<100 step/min(min)				
	Model 1	0.026(-0.041,0.092)	*0.449*	0.004(-0.007,0.014)	*0.461*
	Model 2	0.028(-0.050,0.105)	*0.485*	**-0.021(-0.041,0.001)**	***0.043***

Table 5. Continued

		Night glucose (mmol.L) B (95% CI)	*p*	Day glucose (mmol.L) B (95% CI)	*p*
All	**Total Steps**				
	Model 1	-0.509x10^4(0.000,0.298 x10^4)	*0.217*	-0.310x10^4(-0.940x10^4,0.321x10^4)	*0.336*
	Model 2	-0.564x10^4(0.000,0.000)	*0.489*	0.000(0.000,0.118 x10^4)	*0.68*
	am steps				
	Model 1	0.000(-0.001,0.000)	*0.551*	0.375x10^4(0.000,0.000)	*0.828*
	Model 2	0.000(-0.001,0.000)	*0.689*	0.000(-0.001,0.000)	*0.389*
	pm steps				
	Model 1	-0.575x10^4(0,000,0.414x10^4)	*0.254*	-0.147x10^4(-0.926x10^4,0.632x10^4)	*0.711*
	Model 2	-0.664x10^4(0.000,0.000)	*0.545*	0.000(0.000,0.108x10^4)	*0.063*
	Total stepping time				
	Model 1	-0.004(-0.008,0.000)	*0.064*	-0.001(-0.005, 0.003)	*0.591*
	Model 2	-0.005(-0.009,0.000)	*0.06*	**-0.008(-0.016,0.000)**	***0.039***
	>100 step/min(min)				
	Model 1	0.011(-0.025,0.003)	*0.136*	-0.007(-0.016,0.002)	*0.12*
	Model 2	-0.007(-0.027,0.013)	*0.472*	-0.013(-0.032,0.007)	*0.218*
	<100 step/min(min)				
	Model 1	0.004(-0.016,0.007)	*0.455*	0.003(0.007,0.013)	*0.573*
	Model 2	-0,005(-0.020,0.010)	*0.527*	-0.015(-0.031,0.001)	*0.073*

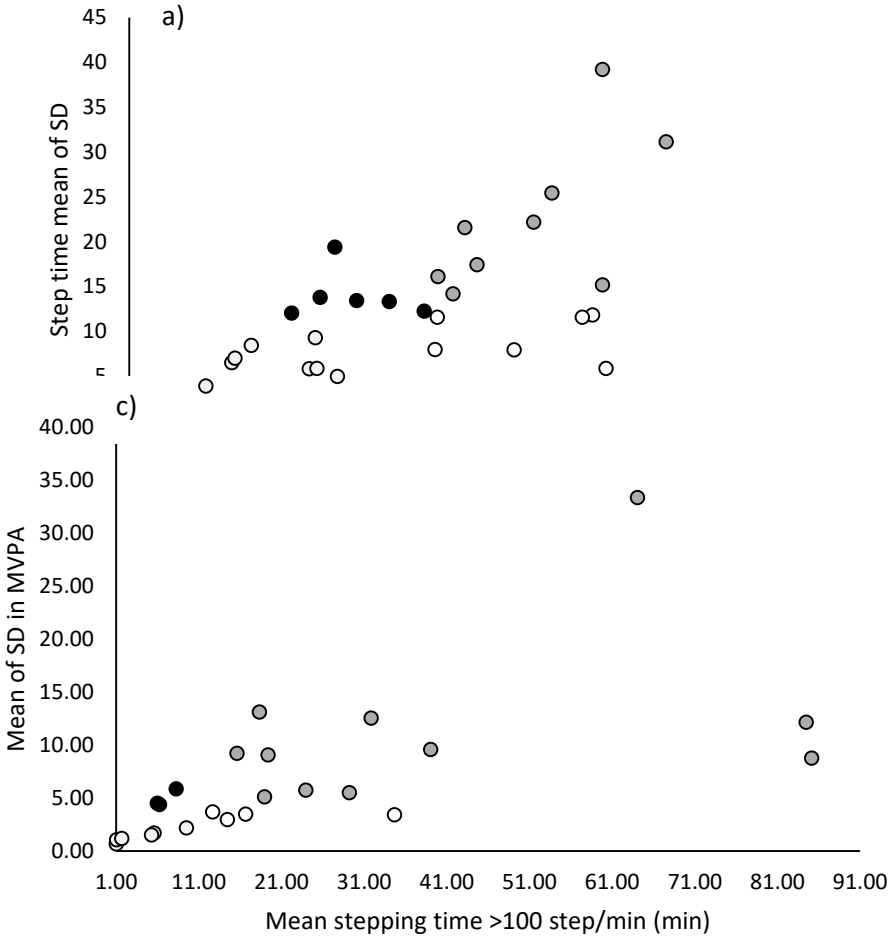
Unstandardised β coefficient and 95% confidence intervals for Generalised Estimating Equations on the effect of total daily steps, am steps, pm steps, total stepping time (min), stepping time at >100 step/min and stepping time at <100step/min. Model 1 is unadjusted and model 2 is adjusted for age and BMI.

4.6 Interdaily variations in total daily activity

Within participants

Group averages for glucose variables and number of participants in each group are presented in table 6. HVHP grouping had the highest TIR % and lowest 24h average glucose in total daily steps (46.6 ± 25.1%; 7.6 ± 1.3mmol.L), stepping time (39.2 ± 29.7%; 7.7 ± 1.3mmol.L) and MVPA (42.5 ± 25.1%; 7.7 ± 1.2mmol.L). LVHP grouping had the lowest TIR % and highest average glucose in total daily steps (14.6 ± 12%; 10.1 ± 1.5mmol.L), stepping time (16.7 ± 11.9%; 9.9 ± 1.4) and MVPA minutes (17.5 ± 13.2%; 9.7 ± 2.1). Graphs to show the distribution of individuals within the four groups are presented in figure 4.

Regression analyses revealed that glucose TIR% was significantly higher in the HVHP group compare to LVLP for total steps (46.6 ± 25.1% vs 23.7 ± 14.9%, p = 0.023) and MVPA (42.5 ± 25.1% vs. 20.3 ± 13.5%, p = 0.021). No other significant differences were found for TIR%, 24h average glucose, MAGE and AUC across all groups when LVLP was used as the reference group in all activity variables (total daily steps, average stepping time and minutes of MVPA). Results of the regression analyses are presented in table 7.



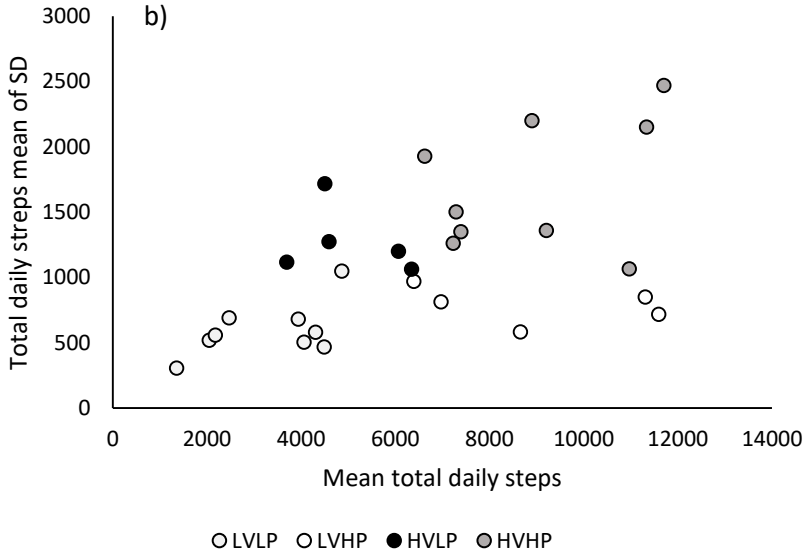


Figure 6. Graphs to show the distribution of activity volume and variation in the four groups: Low Variation Low Physical Activity (LVLP), High Variation Low Physical Activity (HVLP), High variation High Physical Activity (HVHP) and Low Variation Low Physical Activity (LVLP). a) Distribution of average step time and the mean SD of step time. b) Distribution of mean total daily steps and mean SD of total daily steps. c) Distribution of mean stepping time >100 step/min (min) and SD.

Table 6. Number of participants per grouping, and average of glucose variables for each group of inter-daily variability in PA in Total Daily Steps, Step Time (min) and >100step/min (min).

		Low Variability Low PA	High variability Low PA	High Variability High PA	Low Variability High PA
Total steps	**n**	9	5	10	5
	TIR%[a]	23.7 ± 14.9	21.9 ± 17.3	46.6 ± 25.1	14.6 ± 12
	Glucose average (mmol.L)	8.9 ± 1.4	9.7 ± 3.0	7.6 ± 1.3	10.1 ± 1.5
	MAGE[b]	4.6 ± 1.8	3.5 ± 2.3	3.9 ± 2.6	5.4 ± 2.0
	AUC[c]	1130.9 ± 217.9	1239.7 ± 513.5	958.1 ± 169.7	1340.4 ± 166.0
Step time (min)	**n**	8	6	9	6
	TIR%	26.7 ± 13.0	31.3 ± 20.6	39.2 ± 29.7	16.7 ± 11.9
	Glucose average (mmol.L)	8.8 ± 1.4	9.3 ± 3.2	7.7 ± 1.3	9.9 ± 1.4
	MAGE	4.3 ± 1.9	4.3 ± 2.5	4.1 ± 2.7	4.9 ± 1.8
	AUC	1148.7 ± 210.7	1308.8 ± 538.1	972.0 ± 217.4	1309.9 ± 165.8
>100 step/min[4]	**n**	11	3	12	3
	TIR%	20.3 ± 13.5	25.3 ± 20.3	42.5 ± 25.1	17.5 ± 13.2
	Glucose average (mmol.L)	9.2 ± 1.3	10.4 ± 3.7	7.7 ± 1.2	9.7 ± 2.1
	MAGE	4.6 ± 1.7	4.7 ± 3.2	3.9 ± 2.5	4.7 ± 2.8
	AUC	1138.5 ± 177.5	1383.9 ± 743.9	1009.4 ± 214.5	1289.6 ± 330.7

Values are mean ± SD [a]Time in range percentage (TIR%) as 4.0-7.0mmol.L [b]Mean Amplitude of Glucose Excursions (MAGE). [c]Total glucose area under the curve (AUC). [4]Stepping time at a cadence of >100 steps per minute.

Table 7. Regression analysis results for difference in glucose variables (average glucose, TIR%, MAGE and AUC) across the four groups defined by interdaily variability in step time, total daily steps and time stepping at > 100 step/min.

		Glucose average (mmol.L)		TIR %[a]		MAGE[b]		AUC[c]	
		β Coefficient	*p*	β Coefficient	*p*	β Coefficient	*p*	β Coefficient	*p*
Step Time	**[e]LVLP (reference)**	8.843		26.709		4.312		1130.914	
	[f]HVLP	0.523	*0.616*	-0.667	*0.956*	-0.806	*0.482*	108.815	*0.485*
	[g]HVHP	-1.330	*0.164*	16.808	*0.13*	-0.395	*0.697*	-172.782	*0.222*
	[h]LVHP	1.096	*0.298*	-10.031	*0.409*	1.111	*0.334*	209.297	*0.184*
Total steps	**LVLP (reference)**	8.93		23.741		4.097		1148.675	
	HVLP	0.724	*0.494*	-1.880	*0.871*	0.196	*0.874*	160.11	*0.326*
	HVHP	-1.407	*0.115*	**22.812**	***0.023***	-0.01	*0.992*	-176.706	*0.191*
	LVHP	1.13	*0.29*	-0.9116	*0.433*	0.81	*0.514*	161.254	*0.322*
>100 step/min[d]	**LVLP (reference)**	9.218		20.321		4.424		1183.481	
	HVLP	1.19	*0.343*	4.957	*0.728*	0.235	*0.873*	200.437	*0.304*
	HVHP	-1.51	*0.067*	**22.148**	***0.021***	-0.568	*0.536*	-174.103	*0.167*
	LVHP	0.472	*0.705*	-2.775	*0.45*	0.306	*0.834*	106.096	*0.583*

B coefficient and p value presented as a difference from the reference group (LVLP). [a]Time in range percentage (TIR%) as 4.0-7.0mmol.L [b]Mean Amplitude of Glucose Excursions (MAGE). [c]Total glucose area under the curve (AUC). [d]Stepping time at a cadence of > 100 steps per minute. [e]Low variation low physical activity, [f]high variation low physical activity, [g]high variation high physical activity, [h]low variation high physical activity.

5.0 Discussion

The aim was to inform improved practical guidance for PA for individuals with T2DM by providing the first detailed understanding of how the daily patterns and distributions, including intra- and inter-daily variations, of habitual PA of all intensities (light, moderate and vigorous) influence blood glucose regulation in free-living. To our knowledge this is the first study to adopt this approach to analysing important patterns of free-living PA including both intra and interdaily variability in individuals with T2DM. We observed that daily stepping time, daily stepping volume and steps accumulated below a moderate intensity were associated with increase daily time in TIR and decreased glucose AUC. Post-meal steps were inversely associated with 2-hour PPG. Steps accumulated at or above moderate intensity were associated with a decrease in MAGE. No significant associations were observed between inter-daily variability in PA and glucose variables (MAGE, glucose AUC, TIR and 24-hour average glucose).

The following sections of this thesis will discuss the principle findings of this investigation, including; a description of PA accumulation across days of the measurement period and associations between PA variables with glucose outcomes, the possible applications of these findings, and wider implications for research policy and practice.

5.1 Daily PA accumulation and daily glucose

Previous research using contemporaneous measurement has investigated links between PA and glucose in healthy individuals (Kingsnorth et al, 2018) and habitual sedentary behaviour in individuals with T2DM (Paing et al, 2019). The current study followed a similar methodology involving measurement of habitual PA and glucose over five days to provide a detailed observation of interstitial glucose responses to activity. It was observed that that higher volumes of stepping time, duration of walking cadence <100 steps per minute (light intensity) and total daily steps were significant predictors of daily glucose TIR (when the target range was 3.9-7.0mmol.L).

Our finding relating to the association between light intensity PA and glucose TIR is consistent with those from Paing et al (2019), who examined links between sedentary behaviour (the inverse of light intensity PA) using similar methods (CGMs and accelerometers) to measure movement and glucose contemporaneously. Paing et al observed that increased sedentary time (and hence lower light intensity PA) was associated with decreased glucose TIR (3.9-10mmol.L), demonstrating, like the present study, the importance of reducing sedentary time and increasing light intensity PA. However, it is important to note that the target range used in the study by Paing et al was 3.9-10mmol.L. In the present study we employed the target range 4.0-7.0 mmol.l and 3.9-8.5mmol.L which are the NICE and Diabetes UK recommendations for target glucose range before meals and post-prandial respectively.

Similarly, Kingsnorth et al (2018) used paired glucose and accelerometer data to investigate associations between glucose variability and both PA and sedentary behaviour in individuals with a lower cardio-respiratory fitness and healthy individuals. Glucose variability was determined based on mean glucose, SD of glucose and MAGE. It was observed that increased light intensity walking and sedentary time were associated with the most consistent changes in glucose variability, and MVPA was not associated. Findings from the current study are consistent with those of Kingsnorth et al in that time spent in light intensity PA was negatively associated with mean 24h glucose when age and BMI were accounted for. The magnitude of the effect in Kingsnorth et al was smaller than in the current study (b = -0.0004 (-0.00078, -0.0006), compared to -0.006 (-0.011,-0.001). The differences in effect size could be attributed to the fact that individuals with T2DM tend to experience greater reductions in PPG and insulin following PA than metabolically health individuals which could be because individuals with insulin resistance have a poorer glucose metabolism, transport and uptake compared to those who are comparatively more insulin sensitive (Loh et al., 2020).

The current findings are consistent with experimental studies that have demonstrated that even short bouts of PA are associated with improvements in measures of glucose control, including glucose uptake and insulin sensitivity, in individuals who are healthy (Pulsford et al, 2017,

Brocklebank et al, 2017; Peddie et al, 2013; Healy et al, 2007), obese/overweight (Henson et al, 2016; Dustan et al, 2012), and diagnosed with T2DM (Dempsey et al., 2016). Unlike the present study these studies involved regimented PA protocols, for example Peddie et al (2013) investigated walking for 1min40s every 30 minutes, and observed that glucose and insulin concentrations were lower compared to sustained inactivity. However this activity protocol may not reflect habitual activity patterns (Peddie et al, 2013). Despite the measures of glucose control varying from the present study (e.g. insulin sensitivity and glucose uptake, only compared to TIR, MAGE), the main findings from these studies are consistent with the present findings in that they suggest that even small amounts of PA at lower intensities may be beneficial for glucose control.

In the current study, no associations between glucose and MVPA were observed. Previous research has shown acute moderate intensity PA to be beneficial for lowering glucose and insulin iAUCs when investigated within a laboratory setting (Duviver et al., 2017, Dunstan et al., 2012) and in observational studies (Healy et al., 2007). The absence of an observed association in the current study is likely due to the recorded volumes of MVPA within the study sample being very low (14 ± 22min average per day), rather the majority of activity was of a light intensity. Out of 29 participants only seven accumulated daily MVPA equivalent to 150 minutes MVPA per week. Amongst the other 22 participants the average MVPA was 7 ± 11 minutes, including three who recorded 0 minutes of MVPA. It may therefore not have been possible to detect the true impact of MVPA within this sample of individuals with T2DM. Despite this, it is clear that greater time spent being physical active, rather than being sedentary can be beneficial for increasing glucose TIR in individuals with T2DM highlighting the importance of targeting improvements in all PA rather than focusing on MVPA.

Findings from the current study although focused only on more acute changes on day to day glucose are broadly consistent with the message from observational evidence which suggests a benefit of PA for individuals with T2DM, and more generally for glucose control. Evidence from observational studies shows that PA is beneficial for reducing risk of further health complications associated with

T2DM, such as cardiovascular disease (Tanasescu et al, 2003; Batty et al, 2002) and mortality (Batty et al, 2002; Tanasescu et al, 2003; Sadarangani et al, 2014; Loprinzi, 2015). Longitudinal analysis by Tanasescu et al (2003) found the RR of developing CVD over 14 years, with follow ups every two years, was 39% lower (OR 0.61) in those reporting that they accumulate ≥ 37.2 METhours/week of PA compared to the reference group who reported accumulating up to 5.1 MET-hours/week.

Sadarangani et al (2014) found that in individuals with T2DM, there was a dose-response relationship between self-reported PA and all cause and CVD mortality after adjusting for covariates including BMI and hypertension. When compared to individuals who were inactive (no participation in non-occupational MVPA), those who reported some activity (less than the recommended 150minutes MVPA per week) and those who met the PA recommendations (≥150minutes MVPA per week) had 26% and 35% lower risk of all-cause mortality, respectively. Nevertheless, self-reported PA is subject to reporting error and bias, this increases the possibility of PA volume misclassification (Sadarangani et al, 2014). Loprinzi et al (2015) investigated morality rates among 712 individuals with T2DM from the 2003-2006 National Health and Examination Survey (NHANES). PA was objectively measured using ActiGraph (accelerometer) technology and mortality up until December 31, 2011 was recorded. Out of the 712 individuals, 134 died; it was found that an increase in 60 minutes of daily ambulatory movement could decrease the risk of premature mortality by 29% (adjusted for covariates including age, gender and medication) (Loprinzi et al., 2015). Given the importance of managing glucose levels for the prognosis of T2DM, it is clear that individuals who are more physically active have better glycaemic control and reduced risk of complications such as cardiovascular disease and mortality.

Further evidence of the link between PA and glucose control exists in the general population. Huang et al (2019) examined the relationship between device (ActivPal) measured sedentary time and PA with cardiometabolic health markers including blood pressure and total cholesterol and a measure of glucose control, HbA1c, in 4,634 participants from the 1970 British Birth Cohort study without diagnosed T2DM. It was observed that daily prolonged sedentary time of >60 minutes was

associated with higher HbA1c values, whereas breaks in daily sedentary time was inversely associated, showing that the reallocation of sedentary time to light intensity PA is beneficial for glucose profile. The main findings demonstrates the potential utility of increasing PA of any intensity to benefit glucose control, not only in individuals with T2DM but across the population. This could prevent poor glucose control, the development of T2DM and other negative cardiometabolic health markers.

As the apparent association between PA and glucose is now supported by a substantial evidence base, a number of lifestyle-based intervention studies which aim to improve glucose profiles and reduce risk and severity of T2DM through PA have been piloted. A number of such interventions have shown that increasing activity, such as walking, can improve an individual's glucose profile in those at high risk of developing T2DM (Yates et al., 2011). These links are consistent across healthy individuals and those who have T2DM, individuals who are more physically active tend to have a better glucose profile than those who are inactive and are less likely to develop T2DM (Hamer et al., 2019). Based on the evidence from observational studies, PA has been investigated and used as an intervention to prevent T2DM (Lindström et al, 2006) and slow the progression of the disease in those already diagnosed with T2DM (Wing et al, 2010).

The Finnish Diabetes Prevention Study (Lindström et al, 2006) was key for demonstrating the benefit of identifying early deterioration in glucose tolerance and using an intervention programme to prevent progression to T2DM in. 522 overweight individuals were randomly assigned to an intensive lifestyle programme or control group. The intervention involved detailed and individualised counselling in which the goals were weight reduction from reducing energy intake and increased PA to 30mins moderate intensity per day or more, all individuals were monitored for up to 7 years. After 4 years of the intervention, those who did not develop T2DM continued for a further 3 years. When compared to a control group (received written and verbal health behaviour information) the incidence of T2DM was lower showing that the lifestyle intervention reduced the RR by 43%. As

increasing PA was one of the key goals in the intervention, this demonstrates the importance of it for the prevention of T2DM.

Similarly, the Look AHEAD (Wind et al, 2010) study investigated an intensive lifestyle intervention in individuals already diagnosed with T2DM. This involved individualised sessions to implement behaviour change, the goals were to decrease energy intake and increase participation in PA starting at 50min per week increasing to at least 175min per week, with the main goal also being weight loss. The intervention group was compared to a control group which maintained their normal care and received general recommendations of health eating and PA. A follow up after one year found that the intensive lifestyle intervention decreased energy intake and increased PA resulting in decreased weight loss; these had a direct impact on health-related outcomes and disease complications.

The findings of the current study, along with those from existing studies described above show that PA has an acute and chronic impact on glucose control. As explained in the literature review, this is due to the mechanisms of insulin secretion and action, and subsequent glucose uptake and utilisation (Richter et al., 1989. At the onset of activity involving muscular contraction there is an increase in GLUT4 translocation resulting in increased glucose uptake from the blood into muscles or target organs. In the long term GLUT4 transporters is increased, improving the sensitivity of muscles and target organs to glucose; increasing glucose uptake (Zierath, Krook and Wallberg-Henriksson, 2002). These mechanisms support the current findings showing that any increases in activity involving muscular contraction (such as walking) improve glucose uptake resulting in a greater time in target glucose range.

In summary, there is a growing body of literature including observational studies of free living behaviour, controlled experimental studies and large scale lifestyle interventions that demonstrate that PA of any intensity is beneficial for glucose control, often in a dose-response manner. The present findings are consistent with this existing work. However, insight into precisely how PA influences glucose is limited due to the methods that have been predominantly used in the existing literature. Firstly studies examining links between PA and glucose have almost exclusively summary

measures of activity, often average values for MVPA only aggregated over an entire measurement period (Sadarangani et al., 2014; Loprinzi et al, 2015). Secondly the majority of studies have focussed on summary glucose measures such as HbA1c which is indicative of glucose control over the previous months (Huang et al, 2019). Measuring activity and glucose in these ways obscures the day-to-day interplay between movement patterns and glucose. Given the acute impact of small amounts of PA, investigation of how variations in the patterns in which activity is accumulated might impact clinically meaningful glucose measures is required. The current study makes a novel contribution to the evidence base by assessing the importance of not just average activity and glucose values, but the inter- and intra-daily variations in PA and how these impact on glucose.

5.2 Inter- and Intra-daily variability

The novelty of the present study is not limited to the use of contemporaneous measures of movement behaviour and glucose in individuals with T2DM. To our knowledge this is also the first study to consider the importance of inter and intra-daily variability in PA and its impact on glucose from contemporaneous measures of PA and glucose. Intra-daily variations refers to the extent to which the pattern of accumulation of a given volume of PA can vary within a day. The present analysis focusses on: PA accumulated around meal times and its impact on post-prandial glucose responses, and how diurnal variation in PA (accumulated in the morning compared to afternoon) may impact 24h average glucose, TIR %, waking glucose and nocturnal glucose.

PA accumulated around meals, particularly activity in the post-meal period, has been shown to beneficial for reducing PPG and insulin responses (Reynolds, 2016; Chacko, 2016 and Haxhi et al, 2013).The current study found that PA in the 2 hours following meals was inversely associated with 2h-PPG; increased post-meal steps was associated with a lower 2h-PPG whereas pre-meal steps and total steps within 2 hours of meals were not associated. Unlike in previous research, activity was not limited to either pre- or post-meal. Colberg et al (2009) investigated walking performed for 20 minutes before or after an evening meal compared to a control (no activity), it was found that walking after a meal had a significant effect on lowering the PPG response. As participants kept to their normal daily routines, it is likely that the post-meal steps of one meal could be the pre-meal steps of another, therefore distinguishing between pre- and post-meal steps is difficult. Also as the precise composition of each meal was not recorded the findings may be confounded by the influence of carbohydrate intake. It is known that the responses to food intake and PA around meals can vary depending on the meal time (Colberg et al, 2009), whereas in the current study meals, such as breakfast, lunch and dinner, were not analysed separately. PPG for all meals was analysed together, thus the only conclusion is that activity in the 2h following a meal can be beneficial for lowering post prandial glucose.

5.2.1 Activity in the morning vs. afternoon

Research into glucose responses to morning or afternoon activity, excluding meal timings, is limited. There is evidence that PA undertaken in the afternoon may be more beneficial for blunting hyperglycaemic excursions than activity undertaken in the morning (Savikj et al 2019). However this study focussed on high intensity interval training rather than the accumulation of habitual PA. While this allows some insight into possible effect and likely mechanisms it tells us little about the impact of accumulating daily PA in different ways. In the present study total daily stepping time and steps accumulated at a light intensity were associated with lower daytime average glucose and there was some evidence of an effect of daily stepping time and night glucose with increase daily activity being weakly associated with night glucose. There was no evidence to suggest that morning or afternoon activity alone were differently associated with any of the glucose outcomes.

5.2.2 Inter-daily variations

To our knowledge this is the first study to examine the possible effect of inter-daily variations in activity on glucose profile. The current study has investigated how variations in activity between days and the volume of activity across all measured days relates to daily glucose. When investigating inter-daily variation in total daily steps, stepping time and participation in MVPA, it was found that the high variation high PA group had the highest glucose TIR %, lowest average glucose, smallest AUC and lowest MAGE (for all exposures apart from in total daily steps) suggesting a better glucose profile. The low variation high PA group consistently had the lowest glucose TIR %, highest average glucose, smallest AUC (apart from in MVPA) and largest MAGE suggesting a poorer glucose profile. However, these differences did not reach statistical significance. The high variation and high PA group was found to have significantly greater TIR% than the reference group (low variation and low PA) when variability was defined by total daily steps and MVPA. There were no further statistically significant differences between the reference category (low activity and low variability) with any of other three groups.

As the groups were not equally distributed in size, this may have influenced the findings, for example the low variability and high PA group was generally the smaller of the four (step time n= 6, total daily steps = n5 and MVPA = n3), so variations in glucose variables would have a larger impact on the group average than if the group size was larger as in the high variation, high PA group (step time n= 10, total daily steps = n9 and MVPA = n12). As well as being unequal the number of participants in all groups was fairly small which may have contributed to the null findings (see section 5.5 for further explanation).

The findings of the present study are of relevance to researchers investigating associations between human behaviour and T2DM severity/prognosis, or T2DM risk, clinicians working with individuals with T2DM, and policy makers responsible for public health policy regarding PA. The implications of these findings for these different fields are discussed below.

5.3 Implications of findings

5.3.1 Implication for Researchers

Historically most observational and clinical research into PA and health markers in individuals with T2DM has focussed on the impact of differences in the average of only MVPA through the use of self-report methods or accelerometers. The current study demonstrates that PA below a moderate intensity is beneficial, and the way in which activity is accumulated can vary significantly between days in the same individual which might have implications on daily glucose. In addition to this, oscillating glucose can be more detrimental to diabetic health markers than a high average glucose (Ceriello et al., 2008). Previous research has also shown that activity around meals can also have a significant impact on daily glucose (Haxhi et al., 2013; Chacko et al., 2016). Based on the current findings, inter- and intra-daily variations in PA require further investigation and may be important when determining glucose profiles in individuals with T2DM. Collectively, these findings and others highlights the importance of moving beyond using average values for MVPA and glucose in surveillance and observational studies of individuals with T2DM, as important behavioural and clinical information may be missed.

The need to capture inter- and intra-daily variations in all PA and glucose in individuals with T2DM has implications for the measurement methods employed in both experimental and observational studies, particularly highlighting the importance of the use of precise contemporaneous measures of PA and glucose to allow the observation and/or detection of acute changes which may be clinically relevant. Common measures of activity such as self-report methods can be limited by bias and recall error (see chapter 2) leading to poor estimates of PA engagement and incorrect conclusions about links to health outcomes. When objective measures of PA such as activity monitors have been used to measure PA, often PA values are averaged over a period of time which prevents insight into inter and intra-daily variations in PA. Similarly measures of glucose such as HbA1c, although clinically meaningful have previously used have been criticised for not representing day-to-day changes which are known to be more important in determining diabetic health than static or average measures such as HbA1c and fasting glucose (Van Dijk et al., 2011).

As demonstrated in the current study, the ActicPAL activity monitor and iPro2 CGM were accepted by the participants as comfortable and having minimal impact on behaviour as demonstrated by the acceptability questionnaire. Furthermore, the wear time of both was high with no participants reporting removing devices during the measurement period. The findings from the acceptability questionnaire showed that most participants agreed that they did not mind wearing the monitor and five days wear time was acceptable, the majority also disagreed that wearing the monitors was a hassle, changed their behaviour, prevented them from doing certain activities and was uncomfortable. The continuing development of measurement methods such as accelerometers for PA and CGMs for glucose can be used to collect high quality data on concurrent PA and glucose such as the data collected in the current study which holds huge promise for the field.

5.3.2 Implications for Clinicians

Kime et al (2020) conducted a formative evaluation for how prepared clinicians and other healthcare professionals felt regarding supporting individuals with T2DM to be physically active. From the evaluation, it was understood that training and education for clinicians to deliver PA guidance is

vague and a limited understanding of clinical or public health guidance for PA in individuals with T2DM was reported. It was also found that clinicians who are active themselves in their personal lives were reportedly more likely to recommend PA or exercise as a therapy. This suggests that clinicians need to be provided with more specific education and training to effectively promote PA, and should be encouraged to discuss PA in consultations. Based on the present findings and others efforts to address these gaps in knowledge, and training should include the more recent insights that PA of any intensity is beneficial, not just higher intensity PA or structured exercise. Light intensity PA activity such as walking, is also beneficial in daily glucose as seen in the findings of the current study.

As PA is associated with improvements in glucose control and reduced risk of further health complications and mortality in individuals with T2DM (Batty et al, 2002; Tanasescu et al, 2003; Sadarangani et al, 2014; Loprinzi et al, 2015), it is important that PA monitoring is included as part of treatment and management regimes. Objective measures of PA such as accelerometers are more strongly associated with cardiometabolic biomarkers than self-report measured (Atienza et al, 2011); and now there are an increased number of accelerometers available which provide accurate measurement of PA, but are also small, user-friendly and mostly in-expensive (Arvidsson, Fridolfsson and Borjesson, 2019). Clinicians should be encouraged to objectively measure PA to be a part of clinical management and to incorporate it into treatment regimens (Arvidsson, Fridolfsson and Borjesson, 2019), this would also help with providing individualised advice for PA participation.

When making recommendations to patients based on current clinical PA guidelines, clinicians may be limited to recommending that individuals achieve 150minutes MVPA per week. As shown by the current study and previous research (Morrato et al, 2007), participation in MVPA is low in individuals with T2DM. This goal may be perceived as intimidating for some individuals and also does not provide enough support or guidance of how to increase their PA. Based on the current findings, recommending any increase in activity above sedentary can benefit glucose and should be encouraged. Individualised recommendations based on current PA participation may also increase the likelihood improving PA volumes in individuals with T2DM.

Based on the results of the current and previous studies, clinicians should aim to promote and discuss the benefits of participation in daily activity rather than simply accumulating an overall volume across the week. Those who are more regularly active have a better glucose profile than those who have inactive days separating active days. Focusing on daily activity rather than weekly averages could provide incremental improvements on a daily basis and lead to improved glucose control. In order to achieve this and the above recommendations, clinicians should be encouraged to incorporate PA discussions into consultation sessions with patients but should also be provided with more education and training in order to effectively promote PA which can have clinical benefits to glucose.

5.3.3 Implications for Policy makers

Much of the focus of both public health guidelines for PA (UK Chief Medical Officers' Physical Activity Guidelines, 2019) and clinical guidelines for PA in individuals with T2DM (Colberg et al, 2016) is on achieving a set standard of moderate to vigorous intensity PA. However it is clear from this study and others that there are benefits of light intensity PA (Dempsey et al, 2016). Increased total time stepping and total stepping at a light intensity improves glucose TIR in comparison to MVPA which no associations were found. This highlights the importance of including light intensity activity within the recommendations for individuals with T2DM. The current guidance is broad, clinicians report not knowing precisely which guidelines (e.g. NICE or ADA) and what information to refer to when consulting patients (Kime et al, 2020). There is a need to develop informative achievable and practical guidance and to work with clinicians to ensure communication with patients is clear and supported.

5.4 Possible application of these findings

5.4.1 Developing a points based PA monitoring system

The findings of the current study along with previous experimental and observational research collectively demonstrate that the effects of daily PA of any intensity, and the distribution of PA during each day is important for glycaemic control. This information could provide the basis for tools

to allow the self-monitoring of PA by individuals with T2DM to support their glucose management and to reinforce small beneficial and achievable changes in the PA patterns associated with improvements in glycaemic control. An approach which acknowledges the importance of such small changes in PA of any intensity could be a more acceptable than only targeting 150 minutes or more of MVPA, particularly in individuals who are currently inactive. Awarding 'points' in a scoring system for small beneficial changes in PA patterns can provide positive reinforcement and incremental success leading to sustainable behaviour change (Miche et al, 2008) in currently inactive individuals and optimise PA in those already active.

A possible direction for such a monitoring system would be to attribute points to different dimensions of daily PA which have been shown to benefit daily glucose. These could include achieving 150 minutes of MVPA over the course of the week, not allowing more than 2 days to pass without aerobic or resistance exercise, and interrupting prolonged bouts of sitting with light activity every 30 minutes, as described by the ADA recommendations for PA (Colberg et al 2016). However findings from the present study and others (Duviver et al, 2017; Dempsey et al, 2016) that accumulating light intensity activity throughout the day, but particularly after meals would elicit additional benefit for daily glucose control. Individuals with T2DM could monitor the PA points they accumulate for each week and target improvements based on weekly scores, rather than only counting time spent in higher intensity PA and exercise towards achieving volumes of activity that they may never before have undertaken.

If a system like this proved efficacious it could be used by individuals with T2DM to map incremental changes their daily PA patterns which associated with improvements in glycaemic control, and provide a framework for more long-term behaviour change.

5.5 Strengths and limitations of this study

To our knowledge this is the first study to investigate associations between both inter- and intra-daily variations in free-living activity and glucose using paired accelerometer and continuous glucose

monitoring in individuals with T2DM. In this way it makes a significant contribution to the literature on PA and glucose control in T2DM as existing literature has tended to focus only on higher intensity activity or on summary activity values which ignore potentially important variations in between and within day activity patterns.

In this study we employed well-validated and robust measures of PA and glucose. The activPAL accelerometer has been validated for measuring sedentary and activity time in adults; the accuracy of this monitor has been shown to be 96.2% for measuring time spent in varying intensities of PA, and produced a valid estimate of sedentary time such as prolonged sitting bouts (Lyden, 2017). This method of measuring PA has also shown to be relevant for measuring free-living PA and sedentary behaviours in clinical populations, such as individuals with T2DM (Paing et al, 2019). Continuous glucose monitors, such as the iPro CGM used in the current study, are routinely used in research (Taylor, Thompson and Brinkworth, 2018; Metcalf 2018) and clinical practice (Blevins, 2010) to collect data on glucose variability in individuals with T2DM. CGMs provide detailed measurement of interstitial glucose throughout the day including hypo- and hyperglycaemic (Rodbard, 2016) which is not possible to be measured using other methods such as HbA1c (Van Dijk et al, 2011). Compared to venous blood sampling, the mean absolute relative difference was found at 17.6% in healthy individuals showing a good agreement between venous and CGM readings (Akintola et al, 2015). This demonstrates the accuracy of the CGM method and a strength of the current study compared to others that have not used continuous glucose monitoring.

The responses from the acceptability questionnaire demonstrate that the attitudes towards the data collection were mostly positive. The activPAL device collected 5 full days of data for 31 participants and the CGM collected 5 full days which had no gaps more than 20minutes without a glucose readings for 29 participants; this demonstrates excellent compliance to wearing the devices. The instructions for finger-tip glucose samples were followed with 29 participants taking at least 1 finger-tip glucose reading every 12 hours. Two participants were excluded from the whole analysis due to insufficient finger-tip blood glucose readings per day. The high acceptability of device wear shows

that participants were comfortable to wear these particular devices which could be used in future research to improve the quality of research without dropout.

This study is not without limitation. There are a number of possible limiting factors for this study there are described below:

Although the findings from this study are novel and relevant, they should be interpreted with a degree of caution. The aim was to recruit at least 33 participants in order to achieve 123 complete days of paired glucose and activity data. Due to the closure of campus and cessation of all laboratory testing due to the COVID-19 pandemic, and in particular the impossibility of recruiting individuals with type II diabetes for laboratory visits (they were required to shield) data collection was stopped meaning the recruitment aim, in terms of the number of participants, was not achieved. However it should be noted that we did surpass the number of paired days of accelerometer and glucose data required by our sample size calculation. However issues of low statistical power may still have played a part in the presence of some of the null findings observed in this study. Our sample size calculation was based on achieving the magnitude of the observed association between minutes of MVPA and average glucose in the study by Kingsnorth et al (2018). While our allowance for drop out was very conservative, it may well have been the case that the observable effects for some of the PA exposures included in this study, particularly the novel examination of inter and intradaily variability, may well have been smaller than those expected for minutes of MVPA, and therefore a larger sample may have been required for these effects to be detectable in the present analyses. In addition the number of participants in each of the groups used in the interdaily variability analyses were low. This may well explain why although differences were reasonably large they did not reach statistical significance.

The use of accelerometers, although more accurate than self-report questionnaires (Murphy et al., 2009), has some limitations which it is appropriate to note. These devices are not able to accurately measure the intensity of all activities such as those which involving load baring or topographical transition in which acceleration patterns do not change but intensity does (Welk, 2002).

Furthermore, it is possible that the accelerometer intensity thresholds may incorrectly identify the intensity domain in which an individual was working depending on their fitness level. The threshold values for the accelerometers are based on the relationship between movement and energy expenditure which depends on fitness. If the fitness level of an individual is lower, then the relationship between movement and EE is different, hence the threshold for moderate intensity may need to be different. Without maximal oxygen uptake data from the current participants, we cannot confirm the intensity thresholds (>100 steps per minute for MVPA) were appropriate. However, there is evidence suggest that any misclassification in intensity domains in the current study may not be too severe (Serrano et al., 2017). Serrano et al (2017) found that in older adults with a lower fitness level moderate intensity activity threshold is reached at about 115 ± 10 steps per minute; a cadence just slightly quicker than the threshold in the current study (100 steps per minute).

Detail into meal composition (i.e. carbohydrate, fat and protein) and size were not recorded and collected. This decision was taken to reduce participant's burden, and due to the exploratory nature of this research and its focus on PA patterns and distributions. Therefore associations between PA and glucose in the present study may have been confounded by variations in consumption and composition. In addition no data was available on participants' socio-economic status. Both PA and metabolic health have been observed to be socially patterned. While it is unclear whether socioeconomic differences between participants would have impacted relationships between PA and glucose it is possible that socioeconomic differences may have impacted the types of physical activities that different study participants engaged with. Information on socioeconomic status may therefore have provided some useful explanatory information.

5.6 Future research

To improve our understanding of how PA, in particular the intensity and patterns, influences daily glucose in individuals with T2DM an investigation into intensity thresholds that can specific differentiate PA intensity in individuals with T2DM. The current PA intensity thresholds may not be suitable for individuals with T2DM; moderate intensity may be reached at a lower threshold than the

current one used leading to an underestimation of MVPA. As seen in the current study, MVPA was very low in the population based on the thresholds used. Understanding activity thresholds specific to individuals with T2DM could improve the ability for individualised prescriptions of activity may be more relevant based on the fitness and motivation levels of individuals.

Longer terms prospective studies with repeated contemporaneous measures of PA and glucose, such as those included here, could capture how patterns of PA influence glucose in the short term.Further investigating patterns of activity and which are associated with better glucose profiles and disease prognoses could help to improve the guidance given to individuals with T2DM. Improving such guidance and subsequently improving glucose control would not only influence disease progression, morbidity and mortality by also improve quality of life.

5.7 Conclusion

In conclusion, this is the first study to investigate the relationship between glucose and PA, including inter- and intra-daily variations in activity, through the use of paired continuous glucose monitoring and accelerometry in free-living. It contributes to the evidence that individuals with T2DM may have better glucose profiles if regularly engaging in PA than individuals who are less active, and on days individuals are more active their glucose levels are closer to optimal. The way in which PA is accumulated is an important determinant for daily glucose should be considered by researchers, clinicians and policy makers. In addition to this, light intensity walking is beneficial for glucose levels which should be included in PA guidelines for individuals with T2DM. The findings could be used in the development of system to ease monitoring glucose and activity, and to provide more specific guidance for individuals with T2DM on how activity can be used for glucose control.

6.0 Appendix

6.1 Participant information sheet

How does day-to-day movement effect glucose levels in Type II Diabetes?

Researchers: Dr Richard Pulsford, Miss Holly Mei Jones, Dr Sarah Jackman, Dr Brad Metcalf, Professor Rob Andrews

Project summary

We would like to invite you to take part in this research project which will investigate how people's day-to-day movement patterns may influence their glucose levels. This will involve monitoring your movement throughout your day-to-day activities, and your glucose levels using small wearable devices for a 5 day period. As this monitoring aims to capture your usual movement patterns this study involves no changes to your usual routine. Please take time to consider the information carefully, and to discuss it with any friends, relatives or your GP if you wish. After considering the information you can then decide whether or not you wish to participate. Please feel free to contact the research team to discuss any questions or concerns using the contact details at the end of this form.

Purpose of the research

We know that for individuals with Type II Diabetes having good control of glucose levels is important. We also know that moving the body during usual day-to-day tasks or during leisure can play an important role in regulating of glucose levels. Clinical guidelines recommend that individuals with Type II Diabetes aim to accumulate over 150 minutes of physical activity that is of a 'moderate intensity' (elevates breathing above resting levels) each week in bouts of 10 minutes or more. Recent research also suggests that even small light movements undertaken during day-to-day tasks (standing, walking, housework, commuting etc) may also help glucose regulation. However we know very little about how patterns of these day-to-day movements can influence glucose levels. This study will address this by measuring daily movement and glucose at the same time over 5 days to see how they change alongside each other. This information will allow us to improve guidance for individuals with Type II diabetes for managing glucose levels.

Why have I been approached?

We are inviting adult men and women who have been diagnosed with Type 2 Diabetes Mellitus to take part in this study. We hope to recruit individuals who are not currently pregnant and do not have any other underlying condition or injury that limits or prevents them from performing day-to-day tasks. Please contact the research team if you have any queries regarding your eligibility to take part.

What would taking part involve?

If you choose to volunteer for this project, you will be asked to visit us at the University of Exeter on two occasions separated by a five day measurement period.

At the first visit, we will discuss the study procedures and you will have the opportunity to ask any questions or share any concerns that you might have. If you are happy to participate in the study we will ask you to sign a consent form. We will then take measurements of your height, weight, blood pressure, waist circumference and body composition.

We will then provide you with the monitors which can track your movement and glucose levels during the measurement period. A continuous glucose monitor (CGM) will be fitted to your lower abdomen just forward of your hip on the side that you tend not to sleep on. This device is a small lightweight sensor (about the size of a 50p piece) which adheres to your abdomen. On the underside of this sensor is a very small fine probe which sits just under the surface of your skin. These devices are designed to be comfortable and to go relatively un-noticed while people go about their daily routines. Once the device is fitted comfortably and you will receive instruction on how to calibrate the monitor using a small finger-tip blood sample and a home glucose testing kit on three occasions each day. You will then be provided with two activity monitors (called accelerometers) to measure your daily movement patterns. One of these is worn on your wrist (like a small lightweight wrist-watch) and one is a small chip which will attach to your thigh using adhesive tape. You can undertake all your usual activities while wearing these monitors, although we do ask that you do not go swimming during the measurement period. We will also provide you with a study diary in which you will be asked to record what time you eat your meals and snacks, and when you go to bed and wake up during the five day measurement period. The entire visit will last no more than one hour in total. Over the following five days we ask that you go about your usual planned activities. You are not required to make any dietary or lifestyle changes or do anything that you wouldn't usually do during this period.

After the five days of measurement period, you will be asked to briefly return to the laboratory. We will remove the glucose monitor and both activity monitors, and collect the study diary from you. You will then have chance to discuss any further question or comments that you have with the research team. All costs associated with parking at St Lukes campus will be covered for your two visits.

What are the possible benefits of taking part?

After the study we would be very happy to provide you with a report detailing your movement and glucose patterns if you would like one. The wider benefits of this study will be in increasing our understanding of how our daily movement patterns effect glucose levels and how individuals with Type II Diabetes may be able to improve their glucose management in line with their clinical goals.

What are the possible disadvantages and risks of taking part?

Some individuals who have worn the glucose monitors have been concerned about covering the sensor with clothing. However we would like to reassure you that it is a small discrete device which is designed to go unnoticed when in use, and that it is very easy to cover up. Participants may experience a small scratch as the glucose monitor is fitted. However this is typically very slight and only lasts a second. We would not expect participants to experience any further discomfort when wearing the glucose monitor. You will be asked to provide finger-tip blood samples three times per day using a home glucose testing kit. This should take no-longer than one minute and any discomfort from the finger prick is typically minor and short lived. Full instruction, will be provided on how to do this at visit one and written instructions will also be provided for your reference. In very rare cases the adhesive tape used to fit the thigh-worn activity monitor and CGM may cause very mild skin irritation. If this occurs we simply ask that you contact researchers and remove the devices.

What will happen if I don't want to carry on with the study?

You are of course entitled to withdraw from the project at any time without giving a reason and without any disadvantage to yourself. Any data already collected up to the point at which you wish to withdraw can be destroyed.

How will my information be kept confidential?

The University of Exeter processes personal data for the purposes of carrying out research in the public interest. The University will endeavour to be transparent about its processing of your personal data and this information sheet should provide a clear explanation of this. If you do have any queries about the University's processing of your personal data that cannot be resolved by the research team, further information may be obtained from the University's Data Protection Officer by emailing dataprotection@exeter.ac.uk or at www.exeter.ac.uk/dataprotection

The data collected from the activity and glucose monitors will be stored on a password protected computer network, which will only be accessed by researchers involved in the project. All participants will be given a unique study identification number, no personal identifiable information (such names, dates of birth, etc) will be stored or used in the analysis or presentation of any of the study data. Data from the study diary will be entered into computer file and stored on the same password protected computer network. Hardcopy diaries will diaries will then be destroyed at the earliest possible opportunity.

What will happen to the results of this study?

Data from this study may be published in academic journals or presented at academic conferences. However this will only be anonymised data. No personal identifiable data will be used in any communication of the study findings.

Will I receive any payment for taking part?

There is no payment for taking part in this study. Charges for parking at St Luke's Campus will be covered by study investigators.

Who is organising and funding this study?

This project will be funded by the Sport and Health Science Department at the University of Exeter.

Who has reviewed this study?

This project has been reviewed by the Research Ethics Committee at the University of Exeter (Reference Number 190311/A/02),

Further information and contact details

Please contact the research team for further information and/or to take part.

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Thank you for your interest in this project.

6.2 Measurement Questionnaire

Measurement Questionnaire

Thank you for your participation in our research. We would like to ask you about your experience of wearing the activity monitors and the glucose monitor over the last 5 days.

Please respond to the statements in the table **by ticking one of the five response boxes**.

Participant ID _____

These statements refer to the **activity monitor which you wore on your wrist** (called a GENEActiv).

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
Wearing the activity monitor was a hassle					
I was able to wear the monitor all the time					
Wearing the activity monitor changed my behaviour					
I felt unable to do certain activities because I was wearing the activity monitor					
The activity monitor was uncomfortable to wear					
Wearing the activity monitor for 5 days is acceptable					
I did not mind wearing the activity monitor					

Please use the box below to provide any further comments that you have about your experience of wearing the physical activity monitors.

These statements refer to the **activity monitor which you wore on your thigh** (called an ActivPal).

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
Wearing the activity monitor was a hassle					

I was able to wear the monitor all the time					
Wearing the activity monitor changed my behaviour					
I felt unable to do certain activities because I was wearing the activity monitor					
The activity monitor was uncomfortable to wear					
Wearing the activity monitor for 5 days is acceptable					
I did not mind wearing the activity monitor					

Please use the box below to provide any further comments that you have about your experience of wearing the physical activity monitors.

These statements refer to the **glucose monitor**

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
Wearing the glucose monitor was a hassle					
Wearing the glucose monitor changed my behaviour					
I felt unable to do certain activities because I was wearing the glucose monitor					
The glucose monitor was uncomfortable to wear					

Wearing the glucose monitor for 5 days is acceptable					
I did not mind wearing the glucose monitor					

Please use the box below to provide any further comments that you have about your experience of wearing the physical activity monitors

6.3 Study Diary

Measurement period start date:

Participant ID:

How does day-to-day movement effect glucose levels in Type II Diabetes?

University of Exeter

Sport and Health Sciences

Contents

Study Overview **3**
Glucose Monitor instruction and FAQ **4**
Physical Activity Monitors information and FAQ **5**
Blood glucose meter instructions **6**
Food and Sleep diaries ..**7-12**
Contact Details **13**

Study Overview

Thank you very much for agreeing to participate in this study. This booklet provides you with information about the study's objectives, the measures we are making and about the glucose monitor and activity monitors. Importantly enclosed is also a section where you can record the time you eat your meals and snacks, and also when you go to bed and wake up. This information is very important for the study. Please take the time to read through and understand the details enclosed here and if you have any further questions, do not hesitate to contact the study team.

Background

We know that for individuals with Type II Diabetes mellitus having good control of glucose levels is important. We also know that moving the body, during day-to-day tasks or during leisure time activities can play an important role in regulating of glucose levels. However, much of this research has taken place in a laboratory setting and we know little about how low level day-to-day activities might also be of benefit. It is therefore important that we understand how routine movement, (including walking, day-to-day tasks and commuting) as well deliberate exercise are linked to glucose levels during a typical week.

Purpose

The purpose of this study is to measure movement and glucose at the same time over 5 days to see how they change alongside each other. This information will allow us to improve guidance for individuals with Type II diabetes for managing glucose levels.

Data collection

For this study we ask that you wear two activity monitors and a glucose monitor for 5 days. You do not need to change anything about your daily routine, as the purpose of the study is to observe how your usual daily activities might influence your blood glucose.

Wearing a Glucose Monitor

We ask that you wear a glucose monitor, which sticks to your abdomen. On the underside is a very fine probe which sits just under your skin to measure your glucose levels.

Here are some frequently asked questions regarding the glucose monitor:

Q: What do I need to do?

A: The glucose monitors collect information automatically. However we ask that you calibrate the device 3 times per day (ideally at the times specified within the diary) using a finger-tip blood sample. You will have received instructions on how to do this but if you are uncertain please contact the study team. If you forget to do this prior to a meal don't worry, please just do so as soon as possible.

Q: Can it be worn in the shower?

A: Yes. It can be worn in the shower, but we ask that you do not swim or bath during the 5 day measurement period until the device has been removed. Importantly, we would ask that you do **not** removed the device at any point by yourself.

Q; Can the device let me see my glucose levels?

A: No, the monitors do not provide feedback straight away. However we would be happy to provide you with your results after the study.

Wearing an Activity Monitor

We ask that you wear two activity monitors. One on your wrist like a wrist watch, and a small sensor which is attached to your thigh.

Here are some frequently asked questions about the activity monitors:

Q: What do I need to do?

A: Nothing at all! The activity monitors collect information automatically. Please just carry on with your normal daily routines. We are interested in your usual day-to-day behavior.

Q: Can it be worn in the shower?

A: Yes. It can be worn in the shower, but we ask that you do not swim or bath during the 5 day measurement period until the device has been removed.

Q: Does the monitor know my location?

A: No, the activity monitors only measure the movements you make. They do not collect any information on where you are

Q: Can the devices let me see my movement patterns

A: No, the monitors do not provide feedback straight away. However we would be happy to provide you with your results after the study.

Image 2. GENEActiv Physical Activity monitor (worn on the wrist).

Image 3. ActivePAL Physical activity monitor (worn on the thigh).

Finger-tip sampling steps

We ask that you record 3 glucose values on each day using the home glucose testing pack that we have provided. This includes:

Home glucose meter

Test strip

Alcohol wipe

Lancet

Cotton wool

Plasters

Instructions:

Clean the finger-tip from which you will be using with the alcohol wipe.

Insert a new test strip into the home glucose meter, ensuring that the strip is inserted as far as it can go. The meter will turn on once the strip has been fully inserted.

Twist the disposable cap off the lancet and place the lancet lightly against the side of your finger tip. Please note that you do not need to press vary hard. Press the button on the top of the lancet down, this will prick your finger.

Wipe away the first drop of blood using the cotton wool and allow another small droplet to form. Apply this to the test strip until the device bleeps. It will then count down and display your glucose reading on the screen. Record this in the study diary.

If the sample is unsuccessful or there is an error reading, discard the strip and try again with a new strip.

Apply light pressure to the site until the skin has healed (approximately 2-3 minutes). Apply a plaster if necessary.

Image 4. Home glucose meter.

Food intake and sleep timing

In this section, please record what time you wake up and what time you go to bed, and what time you consume all meal/snacks that you consume during each day. There is no need to

describe precisely what you eat, a brief description such as ‘breakfast’ or ‘snack’ is fine. See the example below.

Example:

Time of waking: *08:00*

Time in bed: *22:45*

Meal/snack	Start Time	Finish Time
Breakfast	***08:30am***	***08:40am***
Tea	***9:45am***	***10:00am***
Snack	***10:20am***	***10:30am***
Lunch	***12:30pm***	***12:50pm***
Snack	***04:20pm***	***04:30pm***
Dinner	***07:15pm***	***07:35pm***

Time of monitor reading	Reading on monitor
Morning: ***08:25am***	***6.4***
Afternoon: ***04:00pm***	***7.4***
Before bed: ***10:45pm***	***7.2***

Other comments:

Slept well last night, feeling energized today. Walked to work today.

……

Date of visit 1:

Time of monitor reading	Reading on monitor
2 hours after visit:	
Before bed:	

Day 1

Date:

Time of waking:

Time in bed:

Meal/snack	Start Time	Finish Time

Time of monitor reading	Reading on monitor
Morning:	
Afternoon:	
Before bed:	

Other comments:

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Day 2

Date:

Time of waking:

Time in bed:

Meal/snack	Start Time	Finish Time

Time of monitor reading	Reading on monitor
Morning:	
Afternoon:	
Before bed:	

Other comments:

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Day 3

Date:

Time of waking:

Time in bed:

Meal/snack	Start Time	Finish Time

Time of monitor reading	Reading on monitor
Morning:	
Afternoon:	
Before bed:	

Other comments:

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Day 4

Date:

Time of waking:

Time in bed:

Meal/snack	Start Time	Finish Time

Time of monitor reading	Reading on monitor
Morning:	
Afternoon:	
Before bed:	

Other comments:

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Day 5

Date:

Time of waking:

Time in bed:

Meal/snack	Start Time	Finish Time

Time of monitor reading	Reading on monitor
Morning:	
Afternoon:	
Before bed:	

Other comments:

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Contact

If you have any questions about the study or any of the measurements, please contact Holly Mei Jones using the email address below.

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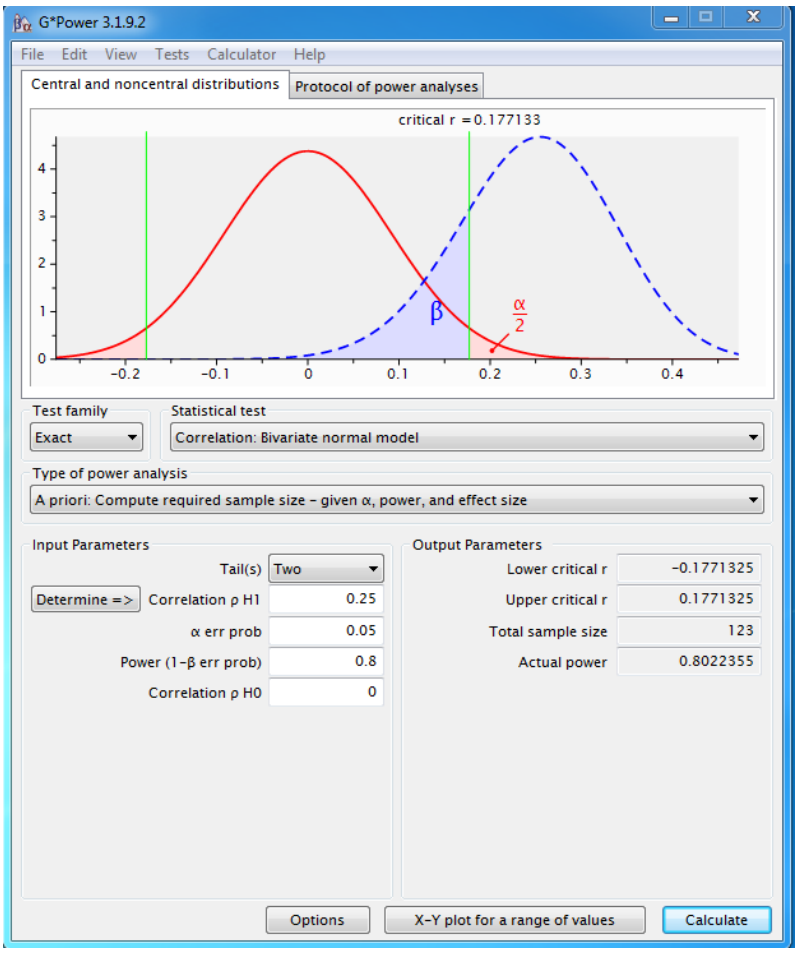
6.4 Calculation of sample size based on Kingsnorth et al (2018)

The sample size for this study was based on the effect size observed by Kingsnorth et al (2018) in a study examining links between accelerometer defined physical activity and continuously measured glucose in healthy adults. As this study is in individuals with T2DM who will have poorer glycaemic control we expect the effect of PA on glucose to be larger, however we wish to be conservative in our estimates. Accordingly if we assume (as in Kingsnorth et al) a mean correlation between minutes of daily MVPA and glucose of r=0.25 (R^2 =6%) we would require 123 days of complete data (PA and glucose) across all participants to observe a significant

association with 80% power (note the ‘sample size I the screenshot below is for days of paired glucose and PA data – not individual participants). We will measure each participants for 5 days. We will account for some data loss (due to device non-wear or failure or participant drop out) by assuming that we will obtain on average 4 days of data from each participants. Therefore to gain 123 days of data we require 31 participant. To further ensure the study is sufficiently powered we will aim to recruit 33 participants.



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